Neuromuscular Stimulation of the Leg

Thesis submitted to Imperial College London for the degree of Doctor of Philosophy

Miss Katherine J. Williams

SUPERVISOR

Professor Alun H. Davies

Professor of Vascular Surgery and Honorary Consultant Vascular Surgeon

Section of Surgery and Cancer

Faculty of Medicine

Imperial College London

Word Count: 104621 (including appendices, 26133)

12 April 2017

Statement of Originality

The material presented in this thesis is the original work of the author. Where figures or results have been reproduced from the work of others, the source is clearly stated.

Signed: 

Date: 12 April 2017

Copyright Declaration

The copyright of this thesis rests with the author, and is made available through the Creative Commons Attribution Non-Commercial No Derivatives licence.

Researchers are free to copy, distribute or transmit the thesis on the condition that they attribute it appropriately. They are prohibited from usage for commercial purposes, and from altering, transforming, or build upon it. For any reuse or redistribution, researchers must make clear to others the licence terms of this work.

Table of Contents

1 Abstract 24

2 Background 26

2.1 Definition of vascular disease and patient populations 26

2.1.1 Chronic venous disease 26

2.1.2 Peripheral arterial disease 29

2.1.3 Diabetes 31

2.1.4 Summary of key points 36

2.2 Neuromuscular Electrical Stimulation 37

2.2.1 Definition 37

2.2.2 Nerve structure and function 37

2.2.3 Structure of muscle 38

2.2.4 Skeletal muscle innervation 39

2.2.5 The neuromuscular junction 40

2.2.6 Plasticity of the neuromuscular junction 41

2.3 Implementation of medical devices in the United Kingdom 45

2.3.1 Inspiration 45

2.3.2 Development 46

2.3.3 Regulation 46

2.3.4 Implications for healthcare 47

3 Electrical therapy for the treatment of disease 49

3.1 The precedent for medical treatment with electricity 49

3.2 Electrical terms, and the action of electricity in animal and human models .. 53

3.2.1 Current 53

3.2.2 Resistance and impedance 54

3.2.3 Frequency 54

3.2.4 Electricity perception 55

3.2.5 Fatigue 56

3.2.6 Electrode Placement 57
3.2.7 Muscle fibre recruitment 58
3.2.8 Electrical safety parameters 59
3.2.9 Stimulation dose and protocol 65
3.3 Modern Electrical Stimulation Devices 67
3.3.1 Neuromuscular Electrical Stimulation 67
3.3.2 Transcutaneous Electrical Nerve Stimulation 71
3.4 Known limitations of electrical stimulation 72
3.5 Summary of important points 75
4 NMES and vascular disease 76
4.1 Literature review: Calf muscle pump function and clinical venous disease 76
4.1.1 Method 78
4.1.2 Results 81
4.1.3 Discussion 85
4.2 Literature review: Venous disease and NMES 88
4.2.1 Methods 89
4.2.2 Results 89
4.2.3 Discussion 101
4.3 Literature Review: Peripheral arterial disease and NMES 105
4.3.1 Methods 105
4.3.2 Results 107
4.3.3 Discussion 111
4.4 Literature review: Diabetes and NMES 115
4.4.1 Methods 115
4.4.2 Results 116
4.4.3 Discussion 121
5 End-point Measurement 124
5.1 History and examination 125

5.2 Quality of life questionnaires 125
5.2.1 Generic 125
5.2.2 Depression 127
5.2.3 Venous disease 127
5.2.4 Arterial disease 129
5.2.5 Diabetes 129
5.3 Functional assessment 131
5.3.1 Treadmill distances 131
5.3.2 Nerve function 133
5.3.3 Pain 140
5.4 Tissue oxygenation 142
5.4.1 Laser doppler fluximetry 142
5.4.3 Near-infrared spectroscopy 149
5.5 Wound healing 151
5.5.1 Planimetry and 3D cameras 152
5.6 Blood-flow assessment 160
5.6.1 Haemodynamic Ultrasound 160
5.7 Lower limb oedema 170
5.7.1 Water displacement 170
5.7.2 Tape measure 171
5.7.3 Perometer 173
6 Bench testing and small preliminary trials using NMES in healthy subjects 177
6.1 Femoral blood flow and laser doppler fluximetry changes with electrical stimulation of selected muscles in the leg 178
6.1.1 Methods 178
6.1.2 Results 185
6.1.3 Discussion 192
6.2 The effect of common peroneal nerve stimulation on blood flow measurements 196

6.2.1 Method 196
6.2.2 Results 197
6.2.3 Discussion 199
6.3 The effect of electrical footplate stimulation on blood flow measurements 201
6.3.1 Method 201
6.3.2 Results 203
6.3.3 Discussion 206
6.4 Concomitant use of common peroneal nerve stimulation and graduated compression stockings 208
6.4.1 Method 208
6.4.2 Results 210
6.4.3 Discussion 213
6.5 Comparison of NMES with intermittent pneumatic compression 217
6.5.1 Intermittent pneumatic compression 217
6.5.2 Methods 219
6.5.3 Results 223
6.5.4 Discussion 228
6.6 NMES compared to graduated compression stockings for management of oedema 232
6.6.1 Methods 232
6.6.2 Results 235
6.6.3 Discussion 238
6.7 Changes in autonomic activation with NMES of the legs 242
6.7.1 Methods 242
6.7.2 Results 246
6.7.3 Discussion 254
6.8 Summary of findings from bench testing and small trials 257
7 Pilot trials of clinical efficacy 258
7.1 NMES in the management of chronic venous disease (VeINS) 258

7.1.1 Methods 259
7.1.2 Results 261
7.1.3 Discussion 267
7.2 Adjuvant NMES in the management of peripheral arterial disease (PAD) 270
7.2.1 Methods 270
7.2.2 Results 274
7.2.3 Discussion 286
7.3 NMES in the management of diabetic foot ulcers (ULCERS) 289
7.3.1 Methods 289
7.3.2 Results 292
7.3.3 Discussion 300
7.4 NMES in the management of diabetic peripheral neuropathy (NERVES) 303
7.4.1 Methods 303
7.4.2 Results 308
7.4.3 Discussion 316
8 Summary ..... **Error! Bookmark not defined.**
8.1 Devices and anatomical application 319
8.1.1 Muscle target 319
8.2 Adjuvant compression garments 320
8.3 Dosing regimen 321
8.4 Populations of interest 321
8.4.1 Prevention of venous thromboembolic events 321
8.4.2 The treatment of venous disease 322
8.4.3 Peripheral arterial disease 322
8.4.4 Diabetes (ulcers and neuropathy) 322
8.4.5 Oedema, and comparison of geko, Revitive, and graduated compression stockings 324
8.4.6 Other possible applications 325
8.5 Method of effect 325

8.5.1 Systemic effect 326
8.5.2 NMES compared to intermittent pneumatic compression..... 327
8.6 Limitations of trials..... 327
8.6.1 Measurements 327
8.6.2 Populations..... 328
8.6.3 Device failure..... 329
8.6.4 Skin reaction..... 329
8.6.5 Adherence 330
8.6.6 Long term effects 330
8.6.7 The “ideal” device 330
9 References 332
10 Appendices..... 360
10.1 Tables relating to NMES in venous disease..... 361
10.2 Tables relating to NMES in peripheral arterial disease..... 383
10.3 Tables pertaining to Exercise and Electrical Stimulation in Diabetes 390
10.4 Results tables pertaining to small trials and bench testing of NMES devices 397
10.4.1 Haemodynamic changes with common peroneal stimulation 398
10.4.2 Haemodynamic changes with footplate NMES..... 399
10.4.3 Comparison of footplate and common peroneal stimulation 399
10.4.4 NMES versus IPC in healthy subjects 400
10.4.5 PAD trial..... 404
10.4.6 NERVES trial..... 405
10.5 Trial subject information sheets 408
10.6 Academic achievements 2012-2015 442
10.6.1 Publications 442
10.6.2 Presentations..... 443
10.6.3 Grants 447
10.7 Published Papers in Full..... **Error! Bookmark not defined.**

Tables

Table 1 - Selection criteria for literature search exploring involvement of the calf muscle pump in symptomatic venous disease 78

Table 2 - Ejection fraction and venous filling index compared between healthy controls and patients with chronic venous disease 82

Table 3 - Impact of calf muscle pump exercise treatment on calf ejection fraction and venous filling index 84

Table 4 - Co-existence of chronic venous disease (CVD) and calf muscle pump (CMP) failure 85

Table 5 - Eligibility criteria for literature search evaluating neuromuscular stimulation in the management of diabetes 115

Table 6 - Significance of colour of ulcer model analysed using one-way ANOVA, compared to planimetry. 158

Table 7 - Sources of error in measuring limb volume flow using haemodynamic ultrasound 166

Table 8 - Inclusion and exclusion criteria for trial subjects in muscle pump trial 179

Table 9 - Allocation order of muscle stimulation for trial protocol 182

Table 10 - Current intensity applied for protocol with subject tolerability scores 191

Table 11 - Inclusion and exclusion criteria for small trial assessing haemodynamic effect of geko device 196

Table 12 - A comparison of changes to blood flow in the femoral vessels with two different types of NMES stimulation 206

Table 13 - Eligibility criteria for adjuvant effect of graduated compression stockings with use of geko 208

Table 14 - Percentage change to arterial and venous haemodynamic parameters with NMES stimulation, both with and without compression hosiery 211

Table 15 - Inclusion and exclusion criteria for trial of NMES versus IPC 219

Table 16 - Laser doppler readings expressed as a change from baseline 227

Table 17 - Eligibility criteria for inclusion in trial comparing NMES to graduated compression in the management of occupational oedema 233

Table 18 - Order of device application in occupational oedema trial 235

Table 19 - Summed subject preference scores for the devices.....238

Table 20 - Inclusion and exclusion criteria for "Autonomic Activation" trial 243

Table 21 - Physiological changes with neuromuscular stimulation.....247

Table 22 - Demographic data of recruited subjects to VeINS trial 261

Table 23 - Anatomical segments affected by venous disease in VeINS trial patients.....262

Table 24 - Haemodynamic changes in the femoral vein, with use of NMES in VeINS trial, “device off” compared to “device on”.....264

Table 25 - Laser Doppler fluximetry changes in the hand and foot with use of NMES in VeINS trial, “device off” compared to “device on” 264

Table 26 - Changes in unilateral leg volume in VeINS trial 265

Table 27- Quality of life scores over time in VeINS trial 266

Table 28 - Eligibility criteria for PAD trial.....270

Table 29 – Table of subject descriptors for PAD trial 276

Table 30 - Walking distances for subjects at the beginning and end of PAD protocol 276

Table 31 - Change to walking distances with PAD trial over 6 weeks 276

Table 32 - Absolute and percentage changes to haemodynamic variables in PAD subjects 280

Table 33 - Percentage change from baseline with NMES in PAD subjects (arterial).....280

Table 34 - Percentage change from baseline with NMES in PAD subjects (venous).....280

Table 35 - Disease severity and quality of life questionnaires for subjects in the PAD trial .283

Table 36 - Eligibility criteria for ULCERS trial 289

Table 37 - Endpoints used in the ULCER trial.....290

Table 38 - Subject characteristics for ULCERS trial 293

Table 39 - Questionnaire scores for subjects in ULCER trial, between beginning and end of trial (6 weeks) 297

Table 40 - Arterial haemodynamic changes with geko device activation (ULCERS trial) 299

Table 41 - Venous haemodynamic changes with the geko device (Ulcers trial) 300

Table 42 - Eligibility criteria for NERVES trial 304

Table 43 - NERVES population characteristics 308

Table 44 - NERVES nerve conduction studies paired samples, comparing “device” to “no device” 310

Table 45 – Subject questionnaire scores at weeks 0 and 10 during NERVES trial.....312

Table 46 - Absolute and percentage changes in haemodynamic variables from baseline with use of NMES 315

Table 47 - Effect of electrical stimulation on venous haemodynamics of healthy individuals 362

Table 48 - Venous Haemodynamics in perioperative patients.....369

Table 49 - Venous haemodynamics in patients with chronic venous disease 372

Table 50 - The effect of electrical stimulation on oedema.....373

Table 51 - Electrical stimulation as a method of thromboprophylaxis 375

Table 52 - Comparing Intermittent Pneumatic Compression/ Sequential Compression Device and Electrical Muscle Stimulation.....380
Table 53 - Effect of electrical stimulation in patients with impaired calf muscle pump.....382
Table 54 - Results of systematic review evaluating the use of non-invasive medical devices in the management of peripheral arterial disease, grouped according to indication 384
Table 55 - The use of electricity in the management of diabetic peripheral neuropathy 391
Table 56 – Summary table of trials using an exercise protocol in the management of diabetes mellitus.....392
Table 57 - Changes from baseline arterial haemodynamic values with ipsilateral common peroneal nerve stimulation 398
Table 58 - Changes from baseline venous haemodynamic values with ipsilateral common peroneal nerve stimulation 398
Table 59 - Haemodynamic changes with footplate stimulation measured in the femoral vein and superficial femoral artery using ultrasound 399
Table 60 - Data used for analysis in section 6.3.2, comparing common peroneal and footplate stimulation 399
Table 61 – Haemodynamic ultrasound measurements from the femoral vessels, in the protocol comparing intermittent pneumatic compression to neuromuscular stimulation of the leg via the common peroneal nerve.....400
Table 62 - Temperature and fluximetry changes with use of the geko in the PAD trial 404
Table 63 - Nerve conduction study results (sensory) 405
Table 64 - Nerve conduction study results (motor).....406
Table 65 - Nerve conduction study results (H-reflex latency) 407

Figures

Figure 1 - The neuromuscular junction.....41

Figure 2 - The torpedo fish.....50

Figure 3 - Illustration of copper wire holding experiment, with frequency increased until subject perceives heat in the palm of their hand.....56

Figure 4 - "Let-go" currents plotted against frequency.....61

Figure 5 - Graph illustrating the relationship between minimum fibrillation current and body weight, for different animal species.....63

Figure 6 - The geko-T1 neuromuscular stimulation device, with application as per manufacturer instructions.....68

Figure 7 – The Revitive neuromuscular electrical stimulation footplate device.....70

Figure 8 - PRISMA diagram of systematic review evaluating muscle pump dysfunction in venous disease.....80

Figure 9 - Calf muscle pump (CMP) failure probability given patients with chronic venous disease (CVI).....85

Figure 10 – PRISMA diagram illustrating literature search evaluating the effect of neuromuscular stimulation in the prevention and management of venous symptoms.....90

Figure 11 - Forest plot comparing neuromuscular electrical stimulation (NMES) to control (no thromboprophylaxis) in the prevention of deep vein thrombosis.....97

Figure 12 - Forest plot comparing neuromuscular electrical stimulation (NMES) to unfractionated heparin in the prevention of deep vein thrombosis.....98

Figure 13 - Forest plot comparing neuromuscular electrical stimulation (NMES) to NMES in combination with heparin in the prevention of deep vein thrombosis.....99

Figure 14 - PRISMA diagram illustrating literature search investigating the use of electrical stimulation in peripheral arterial disease 106
Figure 15 - PRISMA diagram of systematic review for the management of diabetic peripheral neuropathy with exercise or electricity 116
Figure 16 - Normal motor (median) nerve conduction waveform 135
Figure 17 - Representative recording of the human sympathetic skin response 138
Figure 18 - Graph showing Bland-Altman analysis between repeat laser Doppler measurements taken from the dorsum of a foot of a healthy subject. 145
Figure 19 - Fluximetry trace over time, first phase at rest, and second phase during heel-toe exercises 146
Figure 20 - Fluximetry readings taken at rest, and after exercise 147
Figure 21 - Graph showing Bland-Altman analysis between laser doppler measurement percentage difference measurements taken 24 hours apart 148
Figure 22 - The adsorption spectra of haemoglobin and water 149
Figure 23 - Cured modelling clay ulcer models 153
Figure 24 - Three-dimensional cameras licensed for clinical use 154
Figure 25 - Bland-Altman plots of measurement system comparisons for both surface area and wound volume. 156
Figure 26 - Graphs showing correlations between digital planimetry and 3D cameras, for both surface area (A) and wound volume (B) measurements. 158
Figure 27 - Method for measuring volume flow in a vessel using doppler ultrasound 163
Figure 28 - Bland-Altman charts of measurements taken with ultrasound from the right femoral vein and superficial femoral artery of a healthy subject. 169

Figure 29 - Diagram of location of repeat measurements taken from the leg of a healthy subject.....171
Figure 30 - Bland-Altman graphs of measurements taken by observers X and Y 172
Figure 31 - A perometer, used for assessing leg volume.....173
Figure 32 - Bland-Altman chart of repeated measures taken from a volunteers leg.....176
Figure 33 – Subject positioning for MUSCLE PUMP protocol.....181
Figure 34 - Venous vasculature draining lower limb with stimulated muscles. 182
Figure 35 - Outline of the MUSCLE PUMP study protocol. 183
Figure 36 - Duplex ultrasound recordings taken from the femoral vein during MUSCLE PUMP protocol.....186
Figure 37 - The percentage change to haemodynamic parameters with electrical stimulation of tibialis anterior, to illustrate between-subject variability.....187
Figure 38 - Box-plots depicting percentage change in femoral vein haemodynamic parameters from baseline with stimulation. 188
Figure 39 - Laser Doppler changes in blood flow during rest and stimulation in the right and left foot. 189
Figure 40 - Graphs showing correlation between discomfort score and current applied with electrical myostimulation during MUSCLE PUMP 191
Figure 41 – Haemodynamic parameter percentage changes from baseline over time with common peroneal nerve stimulation 198
Figure 42 - Protocol for measuring the effect of footplate stimulation on blood flow changes 202
Figure 43 - Haemodynamic ultrasound from the femoral vessels with use of a footplate NMES device 204

Figure 44 - Percentage changes from baseline in ultrasound parameters with use of NMES footplate device. 205

Figure 45 - Protocol comparing haemodynamic effect of the geko, with and without concomitant compression 210

Figure 46 - Percentage changes to arterial and venous haemodynamic parameters with geko usage, with and without compression.....212

Figure 47 - Diagrammatic representation of an intermittent pneumatic compression device, applied to the lower limbs 218

Figure 48 - Diagrammatic representation of intermittent pneumatic compression cycle for measurement equation 221

Figure 49 - Trial protocol for the comparison of neuromuscular stimulation and intermittent pneumatic compression.....222

Figure 50 - CONSORT diagram for trial comparing intermittent pneumatic compression and neuromuscular stimulation.....223

Figure 51- Graphs depicting arterial haemodynamic changes with the application of intermittent pneumatic compression and neuromuscular stimulation 225

Figure 52 - Graphs depicting venous haemodynamic changes with the application of intermittent pneumatic compression and neuromuscular stimulation 227

Figure 53 - Changes in leg volume over testing protocol, comparing graduated compression stockings and NMES devices 237

Figure 54 - Percentage changes in great saphenous vein diameter plotted against percentage changes in leg volume.....238

Figure 55 - Protocol for assessment of “Autonomic Activation” outcome measures.....245

Figure 56 – Equipment application to subject during “Autonomic Activation” protocol 245

Figure 57 - Physiological changes over time with neuromuscular stimulation 247
Figure 58 - Changes in sympathetic skin response with “Autonomic Activation” protocol..249
Figure 59 - Changes in superficial femoral artery haemodynamic parameters with “Autonomic Activation” protocol 251
Figure 60 - Changes in femoral vein haemodynamic parameters with “Autonomic Activation” protocol 252
Figure 61 - Changes in fluximetry parameters of the dorsum of the hand and foot with “Autonomic Activation” protocol 253
Figure 62 – VeINS trial diagram showing recruitment and follow-up numbers 262
Figure 63 - Haemodynamic changes in the femoral vein with neuromuscular electrical stimulation in VeINS trial, taken at weeks 0 and 6 265
Figure 64 – Protocol for the use of neuromuscular stimulation in peripheral arterial disease 273
Figure 65 - CONSORT diagram for the PAD trial 274
Figure 66 - Mean walking distances for subjects in the PAD trial 277
Figure 67 - Absolute changes in walking distances during PAD protocol 278
Figure 68 - Percentage changes in walking distances during the PAD protocol 279
Figure 69 - Temperature and fluximetry changes from baseline, with activation of the neuromuscular stimulation device during PAD protocol 281
Figure 70 - Graph showing individual responses to NMES activation in PAD trial 282
Figure 71 - Scores over time for intermittent claudication questionnaire in PAD trial 284
Figure 72 - Scores over time for EQ-5D questionnaire in PAD trial 285
Figure 73 - Visual analogue scores over time for PAD trial 285
Figure 74 - Protocol for ULCERS trial 292

Figure 75 - Graphs illustrating results for wound surface area healing in ULCERS trial.....295

Figure 76 - Graphs illustrating results for wound volume healing in ULCERS trial.....296

Figure 77- Histograms of questionnaire responses for ULCER trial.....298

Figure 78 - Protocol for NERVES trial.....307

Figure 79 - Nerve conduction velocities for specific nerves during the NERVES protocol....311

Figure 80 - Diabetes specific quality of life (morbidity) questionnaire results, with changes over 10 weeks.....313

Figure 81 - Generic quality of life questionnaire results, with changes over 10 weeks.....314

Abbreviations

3D	Three dimensional
ABPI	Ankle brachial pressure index
ACh	Acetyl choline
AChE	Acetyl cholinesterase
AChR	Acetyl choline receptor
ANOVA	Analysis of variance
ARR	Absolute risk reduction
AVVQ	Aberdeen Varicose Vein Questionnaire
AWD	Absolute walking distance
BMI	Body Mass Index
BMT	Best medical therapy
CEAP	Classification system used in venous disease(Eklöf *et al.* 2004)
CES-D	Centre for Epidemiological Studies Depression Scale
CFA	Common femoral artery
CHD	Coronary heart disease
CLI	Critical limb ischaemia
CMP	Calf muscle pump
CONSORT	Consolidated Standards of Reporting Trials
COV	Coefficient of variation
CRF	Case report form
CVD	Chronic venous disease
DPN	Diabetic peripheral neuropathy

DQOL	Diabetes Quality of Life (questionnaire)
DVT	Deep venous thrombosis
EF	Ejection fraction
EQ5D	EuroQol-5D
FV	Femoral vein (previously known as superficial femoral vein)
GCS	Graduated compression stockings
GSV	Great saphenous vein
ICC	Intra-class correlation
ICD	Intermittent claudication distance
ICREC	Imperial College London Research Ethics Committee
IMC	Intermittent mechanical compression
IPC	Intermittent pneumatic compression
NIH	National Institute of Health (USA)
NIHR	National Institute of Health Research (UK)
NMES	Neuromuscular electrical stimulation
NMJ	Neuromuscular junction
NRES	National Research Ethics Committee
ns	Not significant
MHRA	Medicines and Healthcare Products Regulatory Agency
MMP	Matrix metalloproteinase
mRNA	Messenger ribonucleic acid
MyoD	Protein playing a major role in muscle differentiation
PAD	Peripheral arterial disease
PAID	Problem Areas in Diabetes (questionnaire)

PE	Pulmonary embolism
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PTS	Post-thrombotic syndrome
PV	Peak velocity
RNA	Ribonucleic acid
ROM	Range of movement
RRR	Relative risk reduction
SD	Standard deviation
SF-12	Short Form 12
SF-36	Short Form 36
SFA	Superficial femoral artery
SSR	Sympathetic skin response
SSV	Short saphenous vein
TAMV	Time averaged maximum velocity
TENS	Transcutaneous electrical nerve stimulation
TIMP	Tissue inhibitor of metalloproteinase
TM	Trademark
US	Ultrasound
USA	United States of America
USS	Ultrasound scan
VAS	Visual Analogue Score
VCSS	Venous Clinical Severity Score
VDS	Venous Disability Score
VF	Volume flow

VFI	Venous filling index
VSGBI	Vascular Society of Great Britain and Ireland
VTE	Venous thromboembolism
WHO	World Health Organisation

1 Abstract

Disorders of the circulation of the leg are a problem in both the developed and developing world, and are a major cause of morbidity and mortality. The incidence of diabetes is increasing, the population is ageing, and resources to treat vascular disease are finite.

Exercise can augment the circulation, and has many beneficial effects on the cardiovascular system as a whole. Electrical stimulation of the muscles of the leg may work in a similar fashion to both voluntary contraction and external muscle compression, in that venous return is increased, and symptoms of vascular disease are reduced.

This body of work aims to delineate the clinical problems to which this solution should be applied, carry out preliminary testing and pilot trials to assess feasibility and effect size of electrical therapy, and to design a device which best addresses the needs of the identified population.

Hypothesis

Neuromuscular electrical stimulation (NMES) of the leg increases the circulatory parameters of the leg, and treatment with NMES over time will alleviate clinical symptoms of vascular disease and improve quality of life.

Methods

The incidence of medical morbidity related to vascular disease was explored, and target populations requiring development of current inadequate management strategies were identified. Neuromuscular electrical stimulation has been defined, and the history of the use

of electrical therapies has been reviewed. Bench testing and small trials were conducted to evaluate the physiological impact of neuromuscular electrical stimulation device usage, and how they compare to current standards of non-pharmaceutical and non-operative medical care (e.g. graduated compression, intermittent pneumatic compression).

Clinical trials in patient groups of interest were instituted, looking specifically at chronic venous disease, peripheral arterial disease, and diabetic vascular complications.

Results

Flow rates and speed of blood in the femoral vessels increased in both healthy subjects and those with vascular disease. Those patients with severe diabetic foot complications were more resistant to flow changes. Fluximetry measurements taken from the foot increased with NMES device use. Improvements in functional outcomes were shown, particularly in the management of claudication and wound healing in the diabetic foot. Quality of life measures improved but on the whole were not statistically significant.

Conclusions

The judicious use of NMES in patients with vascular disease could prove useful, especially in the setting of multiple comorbidities and polypharmacy. The risk profile is very low, especially when compared to anticoagulants or surgery. The health economic benefits are not known, but future studies could use the data in this thesis to power future trials. The biological effects of device usage, both long and short term have not been explored, and may be amenable to testing in further trials in humans.

2 Background

2.1 Definition of vascular disease and patient populations

Blood moves around the human body under pressure, and is distributed according to the configuration of interconnected arteries, veins and capillaries. Pressure is provided by the effective contraction of the heart on fluid filled ventricles, and the action of gravity. Return of blood to the heart is challenged by upright posture, and can be augmented during ambulation by the compressive action of the leg muscle on the deep veins, and through the action of valves to prevent blood backflow. Problems with the cardiovascular system can present with clinical symptoms of disease, and those pertaining to the peripheral vascular system can be termed "vascular disease".

The worldwide burden of vascular disease is vast, and whilst there exist excellent treatment options for venous, arterial and diabetic limb pathology, large cohorts of patients remain recalcitrant. This is not only due to geographical differences in funding, physician and nursing skill sets and access to care, but also because some treatments are unsuitable or contra-indicated.

2.1.1 Chronic venous disease

Worldwide both superficial and deep venous disease are common, with increasing levels of prevalence observed (White 2003; Beebe-Dimmer *et al.* 2005). The incidence of superficial

venous disease is roughly 40-55% in Europe, and around half of these will have symptomatic varicose veins (Callam 1994). The Bonn Vein Study estimates prevalence of C2-6 disease (CEAP classification (Eklöf *et al.* 2004)) to be approximately 32% (Maurins *et al.* 2008). Female sex, increased age, pregnancy, geographical site and race are risk factors for varicose veins(Maurins *et al.* 2008). Clinical manifestations include telangiectasia, varicose veins, peripheral oedema and ulceration.

Traditionally varicose veins and superficial venous disease have been treated by saphenectomy, or "high ligation with saphenous stripping". This can cure symptoms, but causes pain, bleeding, carries a significant risk of nerve damage, and has a recurrence rate of 20-40% at 5 years (Stonebridge *et al.* 1995). Endovenous ablation treatments using laser or radiofrequency, or foam sclerotherapy can be done in an office-based setting, and are now held to be more acceptable for patients as regards treatment tolerability and cosmetic result(NICE 2013). The literature is now also reporting equivalent or higher technical success rates over stripping (85-95%), however failure rates of up to 28% at 1 year are still seen (van den Bos *et al.* 2009; Rasmussen *et al.* 2011) .

Superficial venous disease is now thought to be undertreated across Europe, with disparity from country to country (Moore *et al.* 2013). Factors such as primary care provision, patient and clinician prioritisation, national guidelines, and reimbursement pathways are likely to play a role (Neglen *et al.* 2010; Marsden *et al.* 2013). If the incidence of symptomatic superficial disease is taken at 20% of the European population (731 million people), assuming all symptomatic patients are treated, with a treatment failure rate of 30%, then the estimated burden of recalcitrant superficial venous disease is 43.9 million people.

Deep venous disease is less prevalent than superficial venous disease, however morbidity and mortality from deep venous thrombosis (DVT), venous thromboembolism (VTE), and post

thrombotic syndrome (PTS) are significant (Baldwin *et al.* 2013). DVT is recognised as an increasingly important and frequent cause of venous disease, has an incidence of 0.2% in the general population, and up to 25% in the hospital population (Clagett *et al.* 1988). Risks are increased by immobility, dehydration, sepsis and cancer, and are commonly seen after long haul flights and hospital inpatient episodes. Reversal of these risk factors is thought to be protective against venous thromboembolism, and underlies government anti-VTE initiatives in hospitals. Despite this, 1000 people in the UK are diagnosed with DVT every week, and roughly 50% of these will go on to develop pulmonary embolism, a potentially life-threatening condition (2010/11) (Howard *et al.* 2013). In 2007 nearly 17000 recorded deaths in England and Wales recorded DVT or PE as either the primary cause of death or a contributory factor (ONS 2007). Below knee DVT is classically treated with anticoagulation and compression stockings, whilst patency following iliocaval DVT may be enhanced through the use of interventions such as pharmacomechanical thrombectomy (Enden *et al.* 2011).

PTS is a chronic and frequent complication of acute DVT, affecting approximately 50% of patients within the first two years despite adequate anticoagulation (Brandjes *et al.* 1997; Dharmarajah *et al.* 2014). Symptoms of PTS include leg pain and heaviness, oedema, hyperpigmentation, lipodermatosclerosis, and venous ulceration, occurring to differing levels of severity, and its pathogenesis is poorly understood (Soosainathan *et al.* 2013). Severe PTS occurs in 5–10% of all symptomatic DVTs, and may lead to severe life-changing venous ulceration. With an incidence of 0.2% of DVT in the general population, and assuming 50% develop PTS, there will be 73,100 Europeans with severe, life-changing symptoms. PTS results in significant disability (Kahn *et al.* 2005; Baldwin *et al.* 2013), and has a substantial healthcare cost (MacDougall *et al.* 2006). It has been demonstrated that patients with PTS have a poorer quality of life than patients with chronic lung disease, diabetes, or arthritis (Kahn *et al.* 2008),

and that those with severe PTS have a quality of life comparable to that of patients with congestive heart failure or cancer (Kahn *et al.* 2005). There is currently no cure for PTS, although compression or intermittent pneumatic compression may help manage symptoms.

2.1.2 Peripheral arterial disease

Peripheral arterial disease (PAD) can be defined as a narrowing of the arteries distal to the aortic arch, having a deleterious effect on distal blood flow. Its incidence is estimated at 7-14% in the general population (Shammas 2007; Alzamora *et al.* 2010), increasing with age to ~20% in the over-sixties, and ~40%in the over-seventies (Gregg *et al.* 2004; Peach *et al.* 2012). It is associated with progressive and profound effects on mobility, skin integrity and quality of life (Dumville *et al.* 2004). Significant clinical manifestations include intermittent claudication, rest pain, gangrene, and limb loss. It is most commonly due to atherosclerosis, and has associations with cardio-respiratory disease, stroke, and diabetes (Murabito *et al.* 2003; Dumville *et al.* 2004; McDermott *et al.* 2004; Shammas 2007).

The severity of PAD can be classified based on severity, the most common of which is the Fontaine classification system. Stage 1 is largely asymptomatic, 2 experiences limb intermittent claudication, 3 is where rest pain is experienced, and at stage 4 necrosis and gangrene occur. Critical limb ischaemia (Fontaine 3 and above) equates to limb threatening disease, and represents a management watershed where more risky strategies are employed for limb salvage. The natural history of lower extremity PAD has been assessed in a variety of studies, both with regard to progression of disease in the leg as well as long-term morbidity from concurrent generalised atherosclerotic disease. An estimated 5% of the population suffer with intermittent claudication, 1.2% with critical limb ischaemia (Meijer *et al.* 1998; Sigvant *et al.* 2007) . Radiographic progression of PAD may be documented in 63% of patients

after 5 years (Da Silva *et al.* 1979), 5 year amputation rates for claudicants are reported to be 2-7% (Bloor 1961; Dormandy *et al.* 2000). Limb loss is much more frequent once rest pain is experienced, 12·2% at 3 months in an Italian prospective study (1997). Those with PAD have an age- and sex-adjusted relative risk of dying of 3.1 when compared to non-PAD subjects, 5.9 for deaths from cardiovascular disease, and 6.6 for death from coronary heart disease (Criqui *et al.* 1992). There is a 15-fold increase in risk of cardiovascular mortality among those with symptomatic severe PAD(Criqui *et al.* 1992). 30% of PAD patients can be expected to die in 5 years (Kröger *et al.* 2006).

Management of PAD is based on encouraging aerobic exercise, and modification of cardiovascular risk factors such as smoking, hypertension, hyperlipidaemia, and diabetes (Hiatt *et al.* 1990; Layden *et al.* 2012; Peach *et al.* 2012). Six months of supervised exercise classes have been shown to be of additional benefit, over and above unsupervised exercise, the effect significant at 6 months, and maintained at 9 and twelve months (Cheetham *et al.* 2004). Exercise tolerance is often limited by poor performance status due to other concurrent morbidities. Provision of supervised exercise is poor and sporadic across the UK (Babber *et al.* 2014). A survey of the Vascular Society of Great Britain and Ireland reported 24% of vascular clinics had access to a suitable exercise clinic, with only 14% of VSGBI members referring all eligible patients (Shalhoub *et al.* 2009). Severe symptomatic disease can be treated via revascularisation therapies (angioplasty or surgical bypass), but these procedures carry with them a significant risk of morbidity and mortality (Moxey *et al.* 2013). Patency rates of angioplasty are 60-80% at 3 years, depending on the anatomical level treated, whilst mortality rates are less than 1% (Bosch *et al.* 1997). Localised short segment disease may be treated by endarterectomy, whilst surgical bypass with autologous vein can achieve 5 year patency of 70-85%, with mortality rates of 2-4% (Taylor *et al.* 1990; Belkin *et al.* 1996; Chiu *et al.* 2010).

There then remains a percentage of patients with diffuse disease who are not suitable for revascularisation, or those who have had failed revascularisation, and have few options save amputation available to them. The estimated European burden of non-reconstructable critical limb ischaemia (assuming 20% have failed angioplasty, and a further 20% of these have failed surgical reconstruction) is 350,900 people.

Some trials have shown that increasing the blood flow in the legs over time using medical devices (intermittent pneumatic compression, muscle stimulators), in addition to maximal medical and surgical therapy, can increase claudication distance, absolute walking distance, decrease rest pain, and improve limb salvage (Morris *et al.* 2002; Delis 2005; Delis *et al.* 2005; de Haro *et al.* 2010; Sultan *et al.* 2011; Abraham *et al.* 2013). It is here that there may be scope for further work to create new management strategies for non-reconstructable disease.

2.1.3 Diabetes

Diabetes affects approximately 347 million people worldwide, and by 2030 the WHO projects that complications of diabetes will be the 7th leading cause of death (WHO 2013).

Diabetics have a substantially reduced life expectancy, with age specific mortality rates approximately twice that of non-diabetic population in developed countries (Panzram 1987; Finch C 1988). Rates of diabetes vary between major ethnic groups, reflecting underlying behavioural, environmental and social risk factors, such as diet, level of obesity, and physical activity (Amos *et al.* 1997). People with diabetes are more likely to suffer from ischaemic heart disease, stroke, nephropathy, retinopathy, foot ulcers, and peripheral neuropathy (Amos *et al.* 1997; Currie *et al.* 1998; Donnelly *et al.* 2000; Stratton *et al.* 2000). Diabetics have a 2-8 fold excess cardiovascular mortality compared to non-diabetics (Tuomilehto 1997).

2.1.3.1 Diabetic neuropathy

Peripheral neuropathy is a dysfunction of nerves, most commonly a distal symmetric polyneuropathy involving both small and large fibres in the arms and legs (Boulton *et al.* 2005). Diabetes is the leading cause of neuropathy in the Western world, and is the most common diabetic problem, affecting between 20-50% of diabetic people (estimated at 30% of 347 million diabetics, 121.5 million people) (Amos *et al.* 1997; Vinik *et al.* 2000; Tesfaye *et al.* 2012). Of all diabetic complications, peripheral neuropathy has the greatest detrimental effect on quality of life (Venkataraman *et al.* 2013). Diabetic neuropathy is implicated in 50-75% of non-traumatic amputations (Vinik *et al.* 2000) .

An interplay of metabolic and vascular factors seem to be involved in the pathogenesis of DPN. Small nerve fibre neuropathies occur early in the course of diabetes, and frequently develop with no objective signs or electrophysiological evidence of nerve damage (Vinik *et al.* 2000). At the time of diagnosis DPN is often well established.

Several studies have shown that intensive therapy and optimal glycaemic control may affect the natural history of DPN (Dahl-Jorgensen *et al.* 1986; Azad *et al.* 1999). Preventative metabolic treatments, autoimmune therapies, and nerve growth factors have been trialled with varying levels of success(Vinik 1999). The Veteran study however showed that 2 years of intensive glycaemic control leads to some, albeit little, improvement in clinically evident neuropathy, in the upper limbs only (Abraira *et al.* 1995). The Diabetes Control and Complications Trial neurophysiology data, with ten times the recruited number of patients, reported at 5 years that intensive diabetes management in patients with insulin dependent diabetes mellitus markedly reduces the risk for developing clinically overt, objectively confirmed diabetic polyneuropathy (Group 1995). The UK Prospective Diabetes Study, the Stockholm Diabetes Intervention Group, and the Steno Study Group did not demonstrate any

neurological benefit from 10 years of intensive glycaemic control compared to a standard arm (Lauritzen *et al.* 1985; Reichard *et al.* 1993). Recent studies suggested that that aerobic physical activities, alone or in combination with resistance exercises, may be an effective therapeutic modality for type 2 diabetes (Castaneda *et al.* 2002; Maiorana *et al.* 2002). Regular exercise has beneficial effects on glycaemic control, insulin sensitivity, lipid abnormalities, and hypertension in diabetics, (Goldhaber-Fiebert *et al.* 2003; Balducci *et al.* 2004), and trials looking at neurological outcome modification in diabetics have had mixed success (Tesfaye *et al.* 1992; Richardson *et al.* 2001; Balducci *et al.* 2006).

2.1.3.2 Diabetic foot ulcers

Leg ulcers are open wounds on the lower limb, typically secondary to vascular disease. The estimated incidence rate in the general population is approximately 0.3%, incidence increasing with age, and prevalence rising in an aging population (Callam *et al.* 1987; Jull *et al.* 2004; Gonzalez-Consuegra *et al.* 2011). They are often unpleasant for the subject, a potential portal for infection, and treatment represents a large percentage of community nursing care and tertiary clinic time (Baker *et al.* 1991; Maddox 2012). Diabetic foot ulcers are estimated to occur in 15% of all patients with diabetes (approximately 52.1 million people globally) (Reiber 1995; Reiber 2001; Brem *et al.* 2007), often co-existing with neuropathy and peripheral vascular disease which compromise the limb's ability to heal. Foot infections in this cohort are common, and diabetic foot ulcers serve as a portal for infective organisms to enter the body. Unchecked, infection can spread contiguously to involve underlying tissues, including bone. A diabetic foot infection is often the pivotal event leading to gangrene and lower extremity amputation. Diabetes accounts for over one million leg amputations every year, and represents 60% of all amputations in developed countries (Turns 2012; Caravaggi *et*

al. 2013; Peters *et al.* 2013). Individuals with chronic leg ulceration may have significantly impaired health-related quality of life due to pain, impaired mobility, poor sleep, restricted work capacity, depression, and social isolation (Jull *et al.* 2004; Moffatt *et al.* 2009; Gonzalez-Consuegra *et al.* 2011; Maddox 2012). Leg ulcers appear to reduce quality of life to an extent that is similar to other common chronic conditions, such as arthritis, diabetes, renal failure, heart failure, and multiple sclerosis (Jull *et al.* 2004; Hopman *et al.* 2009; Hopman *et al.* 2014). The psychological component of the SF-36, a generic quality of life assessment tool, is strongly correlated with ulcer duration: those who had an ulcer for more than twelve weeks were three times more likely to score below population mean for age and gender (Hopman *et al.* 2014).

Effective treatment strategies for diabetic foot ulcers are still lacking, as the underlying pathogenesis is thought to be calcification of microcirculation, coexisting with renal and neuronal dysfunction. If coexisting peripheral arterial disease can be treated with angioplasty or surgical revascularisation, outcomes may be improved. Infections are commonly gram positive bacteria (staphylococci and streptococci), but the isolation of gram-negative and antibiotic resistant strains is not unusual in chronic ulcers (Gardner *et al.* 2013). Clinical signs and symptoms of infection cannot be reliably used in chronic wounds to direct antibiotic treatment, making medical management of these patients seem an art rather than a science (Gardner *et al.* 2001; Caravaggi *et al.* 2013). Systemic and topical antimicrobial treatments are commonly used in ulcer management, however, their efficacy is not supported by trial data (Howell-Jones *et al.* 2005). If healing occurs, it is slow, often over many months. The tendency for ulcers to form over weight bearing prominences means granulation tissue is prone to pressure damage (Veves *et al.* 1992). When healed, close to two-thirds of individuals experience ulcer recurrence.

There are large swathes of society that would benefit from circulatory support for vascular disorders. The needs identified here are large, clinically relevant, and worth investigating further.

2.1.4 Summary of key points

1. European burden of symptomatic superficial venous disease recalcitrant to treatment = 43.9million
2. 1000 DVTs per week in the UK despite preventative national guidelines
3. Estimated number of Europeans with severe life-changing PTS = 73,100
4. Estimated European burden of intermittent claudication = 36.6million
5. Availability and sustained attendance at supervised exercise classes – Poor, but unknown
6. Estimated European burden of non-reconstructable critical limb ischaemia = 350,900 people
7. There is no effective current treatment for established diabetic neuropathy, and patients face a lifetime of pain-killers, leg ulcers, and ultimately amputation.
8. Estimated global burden of symptomatic diabetic peripheral neuropathy = 121.5million
9. Estimated global prevalence of diabetic foot ulcers ~ 52.1 million.
   Two thirds of these will recur.

2.2 Neuromuscular Electrical Stimulation

2.2.1 Definition

Over the passage of time, electrical stimulation has been used by many people for varied purposes. It has come to mean very different things to different people. For the purposes of this thesis, a modern definition of NMES may be described as: "the transcutaneous passage of electricity, used to depolarise nerves and/or muscles for the purpose of muscle contraction".

2.2.2 Nerve structure and function

Basic nerve structure consists of a cell body and an axon. The nucleus resides in the cell body and is the site of protein synthesis. When needed, these are transported along microtubules in the axon distally. The axon is usually solitary, and can vary in size. They are specialised conductors and propagators of single electrical impulses (action potential), which travel in one direction only. Unstimulated, a nerve cells resting membrane potential is -60mV relative to the extracellular matrix. Depolarisation by a stimulus selectively opens and closes voltage gated ion channels in the membrane, perpetuates the action potential down the axon, and prevents signal degradation. A refractory period prevents retrograde signal. Signals are delivered to the terminal branches of the axon where a neurochemical signal is created, and passed across a synapse to a nerve or muscle.

Nerves can be described as being of three main types: motor, sensory, and interneurons. Sensory neurons take information to the central nervous system for processing, whilst motor nerves effect motor signals to muscles. Nerves can also be classified as somatic (voluntary) or autonomic (not under conscious control). Autonomic nerves innervate muscles and glands to

facilitate passive processes such as circulatory tone and digestion. Sympathetic and parasympathetic autonomic neurons are usually antagonistic, and exist in a dynamic balance of firing patterns. Interneurons facilitate communication between nerves.

2.2.3 Structure of muscle

Skeletal muscle is a specialised contractile tissue. Changes in muscle length facilitate and control skeletal movement. This is under both conscious and unconscious control via complex networks of nerves.

Each muscle contains thousands of sarcomere units, organised into rows and columns in a highly stereotyped and reproducible fashion. Functional groups of muscle fibres are termed motor units. This regimentation gives a microscopically striated appearance to the cell. It is the specialised properties of the sarcomere units which allow muscle cells to contract and shorten. The mechanism whereby this occurs was first described in 1954 by Huxley et al (Huxley *et al.* 1954; Huxley *et al.* 1954). The sarcomere contains many parallel fibres of two types of protein: actin and myosin. In a relaxed state, actin and myosin are unbound to each other, binding sites blocked by regulatory proteins troponin and tropomyosin (Lehman *et al.* 1994). High concentrations of intracellular calcium change the conformation of these regulatory proteins, exposing the actin-myosin binding sites. Binding of the myosin globular head to actin forms an actin-myosin "cross-bridge", and effects a contractile force which shortens the sarcomere. Hydrolysis of intracellular ATP allows this bond to break, and the sarcomere to relax. The forming and breaking of actin and myosin cross-bridges continues cycling for as long as there is sufficient calcium and ATP. Intracellular calcium levels are strictly controlled by the cell, stored inside the sarcoplasmic reticulum.

Skeletal muscle cells have a specialised cell membrane called the sarcolemma. It consists of a bi-layer lipid membrane, the outer layer of which is anchored to the basement membrane. The sarcolemma contains numerous specialised scaffolding proteins and ion channels. At each end of the muscle fibre, the sarcolemma surface fuses to form tendon fibres. These inelastic binding structures adhere to the surface of the bony skeleton. A special feature of the sarcolemma is that it invaginates into the cytoplasm of the muscle cell, forming membranous tubules radially and longitudinally within the fibre (T-tubules). These T-tubules are arranged in close proximity to the sarcoplasmic reticulum. Electrical activity of the sarcolemma travels down the T-tubules, causing the sarcoplasmic reticulum to release stores of intracellular calcium, facilitating quick and coordinated activation and contraction of the muscle cell.

2.2.4 Skeletal muscle innervation

Skeletal muscle is innervated by motor neurons, whose cell bodies lie in the central nervous system. The nerve cell sends a myelinated axon through a plexus of peripheral nerves to a single motor unit, where it branches to innervate many muscle fibres. The myelin sheath enhances electrical transmission down the axon via the nodes of Ranvier. As the axon branches, the myelin sheath becomes thinner and eventually dissipates, whilst the repeated branching of the axon forms a fine spray of terminal branches. These lie in grooves (junctional folds) on the surface of the muscle fibre sarcolemma, and in mammals it has been noted that one terminal branch innervates one muscle fibre (Hall *et al.* 1993). Specialised myelinating 'terminal' Schwann cells form caps over the neurosynaptic cleft. These cells have the ability to induce nerve growth, and serve as substrates to guide this growth. Schwann cells appear to participate actively in the maintenance and repair of neuromuscular synapses"(Son *et al.*

1996).

2.2.5 The neuromuscular junction

Motor nerves and muscles are beautifully designed as an integrated unit, separated into pre- and post-synaptic elements, with the space between termed the synaptic clefts. These are collectively named the neuromuscular junction (NMJ). Pre-synaptic neurotransmitters, such as acetyl choline (ACh), are stored in intracellular pockets termed synaptic vesicles. The arrival of an action potential at the terminus of a motor neuron triggers voltage dependent calcium channels to open, and calcium enters the neuron (Figure 1). Calcium binds to sensor proteins on synaptic vesicles, triggering vesicle fusion with the plasma membrane and release of neurotransmitter into the synaptic cleft. This diffuses across the cleft and binds to post-synaptic acetyl choline receptors, which causes activation of ligand-gated ion channels in the sarcolemma. The selective opening and closing of these ion channels changes the electrical properties of the post synaptic muscle cell. Depolarisation occurs and a post-synaptic action potential is created and propagated. This is synchronously delivered to the other sarcomere units in the muscle fibre via the sarcolemma. Muscle contraction occurs in an orchestrated fashion in response to the electrical stimulation. The neurotransmitter dissociates from its receptor and is removed by enzymatic inactivation by acetylcholinesterase (AChE).

These transmissions from nerve to muscle occur over milliseconds.

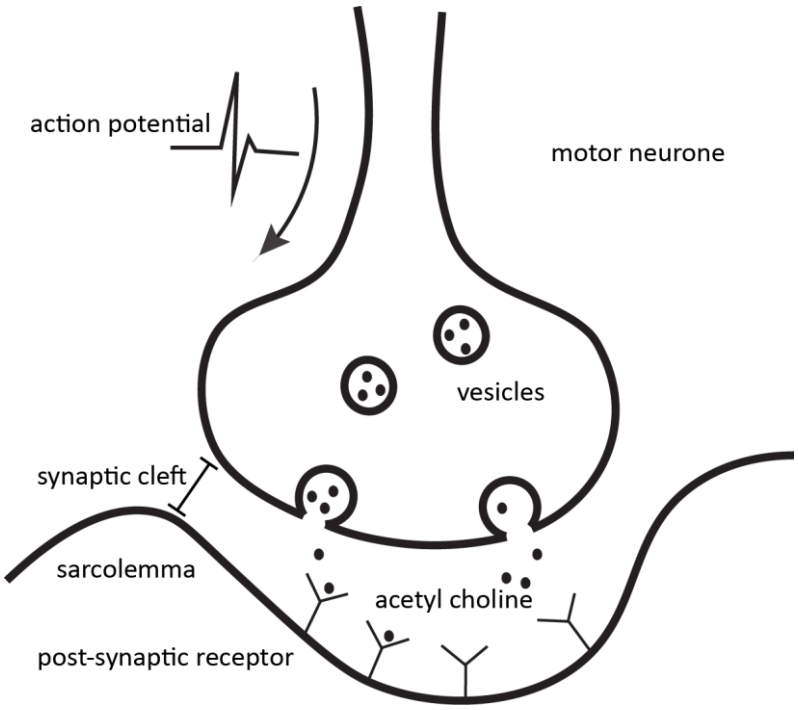


Figure 1 - The neuromuscular junction

Chemical transmission offers several advantages over direct electrical communication (e.g. gap junctions). It is possible to amplify the signal via modulation of the synaptic transmissions system (e.g. quantity of neurotransmitter, post-synaptic receptors, or differing degradation of the neurotransmitter). Action potentials can be converted to an inhibitory signal. Regulation of the signal at multiple levels allows for the modulation presumed to underpin learning and memory.

2.2.6 Plasticity of the neuromuscular junction

The interplay between synaptic nerve and muscle is dynamic and complex. Organisation of the NMJ during embryonic development, growth, and maintenance is coordinated by signals exchanged between motor neurons and their innervated muscle fibres (Hall *et al.* 1993). Extensive remodelling of the NMJ occurs within five days of skeletal muscle denervation, and

can be demonstrated microscopically (Fahim *et al.* 1986).

Expression of acetylcholine receptors (AChR) at the muscle fibre surface is neuronally controlled. The post synaptic cleft is littered with high densities of AChR, whilst extra-synaptic AChR are much smaller in number. Denervation of the fibre causes up-regulation of AChR subunit RNA and protein, and increased expression of extra-synaptic ACh receptors (Brockes *et al.* 1975). Repeated electrical activity of denervated muscles reduces AChR levels, whereas blockade of synaptic transmission in innervated muscle leads to a rise in extra synaptic AChR levels (Lomo *et al.* 1972; Berg *et al.* 1975; Lomo *et al.* 1975). Transcription factors controlling expression of AChR have been identified in transfected cells and transgenic mice - MyoD and myogenin mRNA are regulated with electrical activity in parallel with levels of the AChR subunit mRNAs (Eftimie *et al.* 1991; Witzemann *et al.* 1991; Neville *et al.* 1992; Piette *et al.* 1992). The linkage of neuronal activity to post-synaptic gene expression is an important one, as it allows for a model of plasticity, repair, and NMJ remodelling.

Other cell surface molecules concentrated at the synaptic cleft are N-CAM and AChE. They exhibit a similar pattern of expression in both developing and denervated muscle as AChR. They are expressed embryonically across the whole cell surface, are lost from the extra-synaptic membrane during development, but remain concentrated at synaptic sites. Levels increase after denervation, and decrease after re-innervation. This spatiotemporal pattern of expression, and its similarity to the expression of AChR may support a role for them in muscle plasticity and NMJ remodelling.

Several messenger candidates have been identified, both neurotrophic and myotrophic: brain derived neurotrophic factor, neurotrophin-3, neurotrophin-4, glial derived neurotrophic factor, insulin-like growth factor, and fibroblast growth factor, matrix degrading metalloproteinase MMP-2 and MMP-9, and tissue inhibitors of MMPs (TIMPs) (Kherif *et al.*

1998; Carmeli *et al.* 2004; Jang *et al.* 2011). Sustained electrical stimulation of denervated muscle increases levels of calcium and inositol triphosphate. Activity levels of protein C are also increased, although whether this plays a part in these transcription pathways is not clear. Castration and hormone replacement experiments in mice have suggested that androgens modulate muscle fibre volume and surface area (Balice-Gordon *et al.* 1990). The postsynaptic component of the NMJ shrink or expand with the surface area of the muscle fibre. Castration-induced muscle atrophy was associated with clustering of AChR in junctional folds, and less densely packed as muscle fibres hypertrophied following testosterone replacement.

Retrograde signalling from the muscle component must also play a part in NMJ stability and maintenance. Immobilisation of murine soleus muscle fibres unilaterally, for 5 days, produced results amenable to scanning electron microscopy (Fahim 1989). Muscle fibres atrophied. The NMJ of disused muscle fibres exhibited greater amounts of degeneration than either contralateral limbs or control subjects (nerve terminal disruption, exposed junctional folds with lack of terminal Schwann caps, depleted numbers of post-junctional folds). Signs of regeneration and remodelling could also be seen (expansion of junctional folds, several small terminals occurring within the same primary synaptic cleft, and several axons wrapped by the same Schwann cell).

Damage can occur to the neuromuscular system for many reasons. The literature suggests that maintenance of the NMJ is dynamic, with several modulatory systems that can interplay to produce plasticity and remodelling both in health and disease.

It has been known for a long time that chronic disease such as peripheral arterial disease and diabetes can induce changes in nerve conduction, muscle mass, and musculoskeletal fine control (Burnstock *et al.* 1994; Flynn *et al.* 1995; Fahim *et al.* 2000; Ochi *et al.* 2010; Allen *et al.* 2014). This often coexists and interacts with those changes occurring with natural ageing.

Age-related loss of muscle mass and function (sarcopaenia) greatly affects quality of life in the elderly population (Rizzoli *et al.* 2013). Several hypotheses have been proposed, but key changes seem to be linked to functional denervation, muscle wasting, and weakness. Age-associated degeneration of the NMJ and its components have been well documented (Wernig *et al.* 1986; Smith *et al.* 1987; Balice-Gordon 1997; Luff 1998; Deschenes *et al.* 2010). With advancing age, pre-terminal portions of motor axons exhibit regions of abnormal thinning, distension, and sprouting. Post-synaptic endplates decrease in number and size, post-synaptic folds decrease in length and density. Oxidative stress may play a crucial role in NMJ degeneration and progression of sarcopenia. Homozygous deletion of Cu,Zn superoxide dismutase (CuZnSOD, SOD1 – an antioxidant enzyme) leads to acceleration of age-dependent muscle atrophy, with significant NMJ degeneration similar to that seen in elderly controls(Jang *et al.* 2011). Literature evidence for disease-specific changes to nerves, muscles and synaptic elements are harder to elucidate.

Pain may also have a modulatory effect on both nerve and muscle. Pain is often a feature of vascular disease, whether it be the calf pain of the claudicant, or peripheral neuropathy in diabetic foot disease, or the heavy dragging sensation at the end of the day for those with chronic venous disease. Chronic musculoskeletal pain is commonly accompanied by muscle weakness, however it is not clear if this is due to voluntary or involuntary processes (Graven-Nielsen *et al.* 2002). Experimental pain reduces maximal voluntary contraction, but inherent contractile mechanisms remained intact. This leads one to believe that central pain processes mediate the development of weakness. Literature search has not revealed any histological studies looking at chronic pain and neuromuscular health.

The ability of the NMJ to adapt in a plastic fashion in response to environmental burden may lend itself to artificial manipulation. Clinical trials looking at neuromuscular changes with

electrical stimulation must take these factors into account when interpreting results, particularly changes in nerve function.

2.3 Implementation of medical devices in the United Kingdom

2.3.1 Inspiration

The development of a medical device requires not only inspiration and engineering skill, it also requires financial and intellectual knowledge, and authorisation by the appropriate governmental bodies.

The concept of “intellectual property” is the underpinning principle that an idea is unique and belongs to someone. This ownership can be transferred, and unauthorised use equates to theft. The regulation of intellectual property can be through copyright, trademark, and patents, all of which have their positive and negative aspects. Failure to observe this type of intellectual property is prosecutable in a court of law. A trade secret is intellectual property that is only protected by secrecy, much like the recipe for Coca Cola. Theft of this information is non-prosecutable.

Devices are usually protected by patents, which constitutes a contract with the government. To qualify for a patent the device must be novel, non-obvious, and useful. The inventor discloses information in exchange for the limited right to exclude others from making, selling, and offering the product for sale. Patents are issued by the Intellectual Property Office, and typically have a fixed term of 20 years from the date of patent application. Patents typically take up to four years to be granted or refused.

2.3.2 Development

Development of a device can be a long arduous process, and is usually shrouded in secrecy with pending patents limiting collaboration with outside agencies. Materials and mechanisms have to be tested to a level acceptable to authorising bodies (e.g. MHRA, discussed later), to show that they are safe to use, free of contamination, risks from malfunction are minimal, and that ageing materials are not hazardous. In vitro and in vivo trials are typically performed according to risk profile, and the extent of testing will depend on many factors:

- Population
- Indication
- Outcome measures
- Ethics
- Sponsor
- Funding
- Safety, adverse event reporting

2.3.3 Regulation

Non-pharmaceutical products used as medical devices in healthcare are regulated by Notifiable Bodies. In the United Kingdom this is done by the Medicines and Healthcare Products Regulatory Agency (MHRA, part of the Department of Health), and is advised by an independent advisory panel, the Committee on the Safety of Devices(MHRA).

Device Authorisation

Authorisation of a medical device is signified by the awarding of a “CE mark”, which confirms that the product meets regulatory requirements, works properly, and is acceptably safe.

Manufacturers have to support any performance claims with evidence. The level of evidence is usually proportional to the perceived risk to the public posed by the device. Devices are always tested for mechanical and electrical safety, but are not always subjected to a clinical trial if it is impractical to do so. Novelty and risk are major factors in this decision.

Monitoring of safety and quality standards

The MHRA conducts several processes to monitor standards in licensed products. This can take the form of inspection, audit, intelligence gathering on counterfeit products, and managing the General Practice Research Database. Information leaflets, product labelling, and advertising are all subject to scrutiny, and unsubstantiated claims are challenged.

Prosecution

The legal framework for action by the MHRA is provided by the Medicines Act of 1968, and the MHRA has the power to take action in one of three ways:

1. To issue a medical device alert
2. To withdraw a product from the market, or suspend production of a product
3. To prosecute a manufacturer or distributer in the crown court, if the law is broken.

The action taken is determined by the scale of the threat posed to public safety.

2.3.4 Implications for healthcare

The systems for medicine and device approval and monitoring have been developed over many years, and have been shaped by many health crises. The Tuskegee syphilis experiment (1932), Nazi experiments on prisoners of war during the second world war, and the outcry over Thalidomide-related birth defects have all been used as examples of human rights

abuses by medical research. Most recently, the scandals surrounding metal-on-metal hip implants, and PIP silicone breast implants, led to the international recall of product, and caused significant patient harm and redefinition of standards. Scientific researchers are obliged to follow the principles of the Declaration of Helsinki (World Medical Association), and are tightly monitored by overseeing bodies(Association 2013). Regulation is tight, but is also stifling.

The difficulty lies in the translation of innovation to wide-spread adoption for saving life, re-establishing health or alleviating suffering. Development and authorisation processes can be lengthy, expensive, and prohibitive. Most entrepreneurs prefer to see a return on financial investment, and therefore ideas, concepts and advances are kept secret. This is in opposition to the current movement towards transparency in clinical trials, where the publication of both positive and negative data can accelerate research towards better patient management decisions and more productive use of research resources(Altman *et al.* 2013). Pre-registration of clinical trials methods is now largely mandatory for national funding applications, and consideration for publication by peer-reviewed journals. Research networks such as EQUATOR (www.equator-network.org) provide best practice guidelines and reporting resources as an international initiative to improve research transparency and reporting. The dichotomy between private ownership and public need will always lead to conflicts of interest in reporting, marketing, and research funding opportunities. It is the responsibility of the academic clinician, and the Department of Health, to both find and interpret the evidence to form best-practice.

3 Electrical therapy for the treatment of disease

3.1 The precedent for medical treatment with electricity

The electrical properties of nerves and muscle make them ideal substrates for manipulation by the passage of exogenous electrical current. The normal process of conversion of chemical to mechanical energy can be bypassed through electrical depolarisation of the cell membranes. Throughout history this type of therapy has fallen in and out of fashion, and both natural and man-made delivery systems have been utilised. A short history of electrical stimulation in humans is detailed here.

The first identified publication detailing the use of electrical therapy in humans is attributed to a Roman physician, Scribonius Largus. In his formulary he prescribes the analgesic electrical discharges of the fish Torpedinidae (Largus).

> *"Headache even if it is chronic and unbearable is taken away and remedied forever by a live black torpedo placed on the spot which is in pain, until the pain ceases..... For any type of gout a live black torpedo should, when the pain begins, be placed under the feet. The patient must stand on a moist shore washed by the sea and he should stay like this until his whole foot and leg up to the knee is numb."*

The torpedo fish is now known to generate electricity for the use of hunting its prey. In the dissections of the great anatomist, John Hunter, he exposes the internal organ responsible for the production of electricity, and its nervous connections (Figure 2)(Hunter 1773).

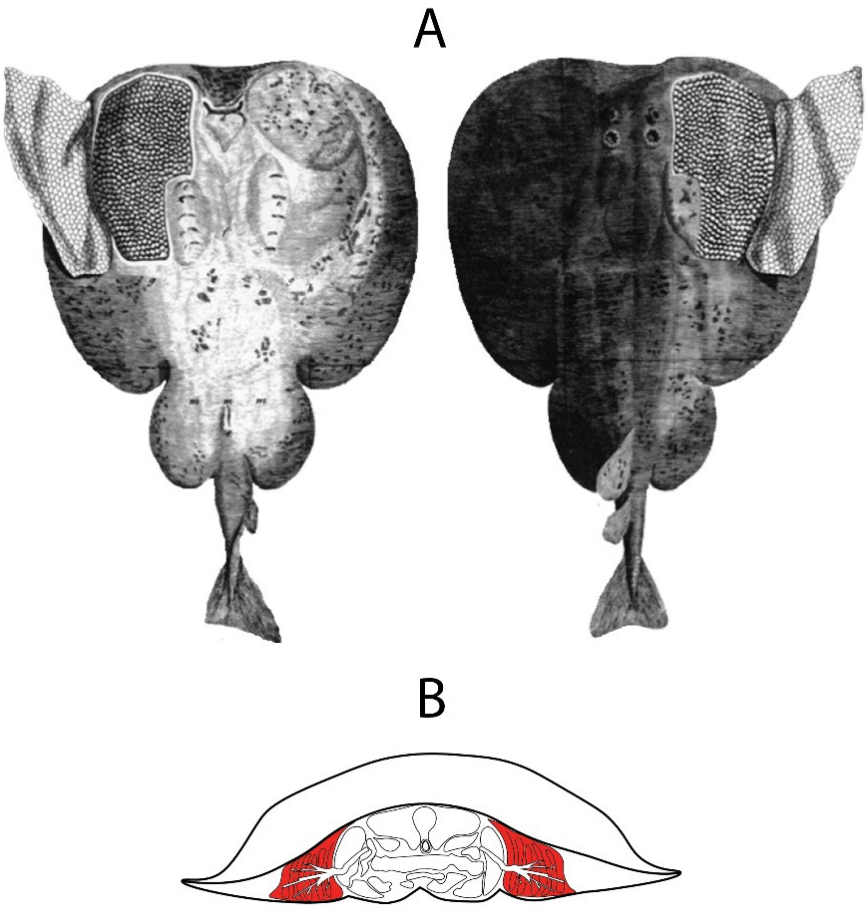


Figure 2 - The torpedo fish.

A - Hunter's dissection with one electrical organ exposed (superior and inferior views).
B - Coronal section with electrical organs shown in red

Picture adapted from Wu et al 1984(Wu 1984)

Galvani's observations of artificial contractions produced in cadaveric frog legs were transcribed in the early 1700's (Ganot 1859). He believed that metallic contact with tissue completed a circuit, causing muscle contraction by short circuiting the natural "animal electricity". Therapy with metal implements, underscored by a fundamental

misunderstanding of charge and current, became known as "galvanism". This was popular with the less salubrious characters of the time, where metallic objects were uselessly stroked over the surface of the skin in an attempt to cure various ailments.

Theories of electricity were strange and abundant, ranging from ethers, invisible liquids, or magical forces. The ability to produce static electricity by rubbing glass was realised in the time of Newton and Hooke, and its invisible action on feathers, threads and small pieces of paper were communicated to the Royal Society (Hackman 1978). In 1660, Otto von Guerricke is credited with inventing the first continuous electrostatic generator. This was followed by the first capacitor in 1745, which could store electrical energy, and discharge it on command. The twinning of the two together in a simple hand operated machine allowed many scientists to experiment with this force privately. Benjamin Franklin, the famous scientist and statesman, used and popularised an electrical machine such as this to deliver shock treatments to his patients. This type of therapy came to be known as "Franklinism", and was adopted for a short time by large London Hospitals such as Guys, and Kings College. So popular were the machines that philanthropist John Wesley, the English Methodist, procured his own machine for treatment of the "poor": in treatments for blindness, cramp, gout, headache, palsy, rheumatism, musculoskeletal sprains, and toothache. His personal machine can still be viewed in the Wesley Museum, London.

Electrical enlightenment made leaps and bounds forward in the 1800's. Allesandro Volta created the first battery from stacking alternate silver and zinc discs, each separated by water-soaked card, obviating the need for a generator. Michael Faraday created a unified theory of electricity in the 1830's, that "static", "battery" and "animal" energy were the same, caused by the movement of charged particles. The discovery of electromagnetic induction and field

theory allowed him to develop the transformer and electric motor. His equipment allowed stimulation of nerves to create active and reproducible movement. "Faradism" caught favour with many medical institutions throughout Europe. A Faraday apparatus consisted of one electrode of a sheet of copper, upon which the patient placed their feet, the other electrode a wet sponge applied to the appropriate patient body part. Electrophysiologist and neurologist Guillaume-Benjamin-Amand Duchenne applied Faraday's methods to formalise diagnostic principles in neurology and neurobiology (Duchenne 1871). Experimentation with nerve and muscle biopsy, nerve conduction tests and clinical photography formed the basis for explanation of neurological diseases pertaining to the brain, brain stem, and peripheral nerve lesions, which are still taught today.

Modern medicine utilises electrical devices in both diagnostic and therapeutic medicine. The properties of electrical and magnetic radiation are put to use in diagnostic imaging (X-ray, computational tomography, magnetic resonance), nuclear medicine, and electrophysiological testing. Devices that deliver electricity directly to the patient, such as diathermy, endovascular ablation, cardiac defibrillation machines and pacemakers are now commonplace in most hospitals in the western world. The safe delivery of electricity to the tissues of the body makes it a versatile tool for medical use, and the application to the treatment of vascular disease will be further explored later in this manuscript.

3.2 Electrical terms, and the action of electricity in animal and human models

Neuromuscular electrical stimulation (NMES), is typically provided with an alternating current at frequencies of 20-60 Hz, expressly to produce muscle contraction. Typically stimulation will be delivered anatomically via two electrodes to create the desired physiological effect. Consideration should be given to the current intensity, duty cycle, pulse pattern, program duration, program frequency, and muscle group activated to produce the desired effect. .

3.2.1 Current

Current refers to the flow rate of charge units (electrons or ions) and is measured in amperes (A). Current cannot flow without a circuit, where charge moves between two electrodes with a potential difference. Potential difference (voltage) is the "pushing" power per unit current. The heat energy delivered to tissues is proportional to the square of the voltage. The mathematical relationship between current (I), potential difference (V) and resistance to flow (R) in an electrical circuit is defined by Ohm's Law (V=IR).

In general, the higher the current amplitude, the stronger the depolarising effect on the structures underneath the electrodes, and the greater the number of motor units recruited. Increasing intensity can cause increases in contraction strength, however, antidromic transmission along the peripheral nerves can occur. Transcutaneous devices tend to use amplitudes of 15-30mA.

3.2.2 Resistance and impedance

Human tissue resists current flow. Internal body resistance is about 300Ω, as salt-water saturated tissues conduct easily. More than 99% of the body's resistance is at the skin (RM Fish 2009). Calloused dry skin may have more than 100,0000Ω per square centimetre due to the poorly conductive stratum corneum, but when wet this can be reduced by 99% (Dalziel 1972). Cuts, abrasions and other skin lesions alter transcutaneous conduction properties. Due to the skin's high resistance, it can act as a capacitor. This is why the action of direct current across the skin only has effect at the onset and offset of power application. Build-up of charge in the electric field prevents further charge movement. Alternating current does not encounter this problem, as the charge changes direction with the applied frequency. It has been noted in biological specimen that skin ceases to obey Ohm's Law above 1kHz, and becomes non-linear. Resistance rapidly decreases above these levels, and is unpredictable (Dalziel 1972). When applying alternating current, tissue is therefore described as having impedance rather than resistance.

3.2.3 Frequency

This refers to the number of times an electrical wave cycles in a minute. In an analogue continuous wave (e.g. sine wave) this is a simple concept, and easily measured on a graph of amplitude against time. For discrete digital waveforms such as those produced by computers, energy waves obey Boolean logic. The wave has a baseline, a rise-time to a higher energy, a fall time, and then the wave repeats according to parameters applied. In this case, simple frequency is not useful, as one needs more information on how long the wave is at high energy (pulse width), the pulse repetition interval (PRI; time from the beginning of one pulse to the beginning of the next) or pulse repetition frequency (PRF; number of pulses per

second). The duty cycle describes the “on-time” for a pulsed signal. It is usually described as a fraction or percentage.

The pulse width can be physiologically matched to the action potential firing pattern of the neural network or NMJ selected. The setting chosen affects the recruitment of muscle fibres at any given voltage/frequency. A rest period in between pulses also may allow recovery of tissue, especially when contraction of muscle obliterates nutritive vessels. This may be especially important in subjects with compromised blood vessels (e.g. claudicants).

In a rectangular pulse, the relationship between threshold amplitude I (mA), pulse width d (μs) is:

$$(I - r)d = k$$

where k is a constant and r is the rheobase or "threshold value" that results in target membrane depolarisation. This relationship indicates that that as pulse width increases a lower stimulation intensity is needed to activate the motor nerves. A study comparing incremental increases in pulse width, (50-1000μs) delivered to soleus muscle at a constant frequency (20 Hz), found that wider pulse widths produced stronger contractions and penetrated more deeply into subcutaneous tissues (Baldwin *et al.* 2006).

3.2.4 Electricity perception

Perception of electrical activity in the human body changes with parameter settings. Threshold perception of current varies between individuals, and follows a normal distribution(Ferris *et al.* 1936).

Altering applied frequency has an effect on perception current, illustrated by shifting the curve in Figure 3. Note that at 60Hz, the perception current is 1.1mA (50^{th} percentile), and as

frequency increases, the perception current increases. At 5kHz the threshold of perception is 7mA. Above 100kHz, electrical sensations change from feelings of “tingling” to heat.

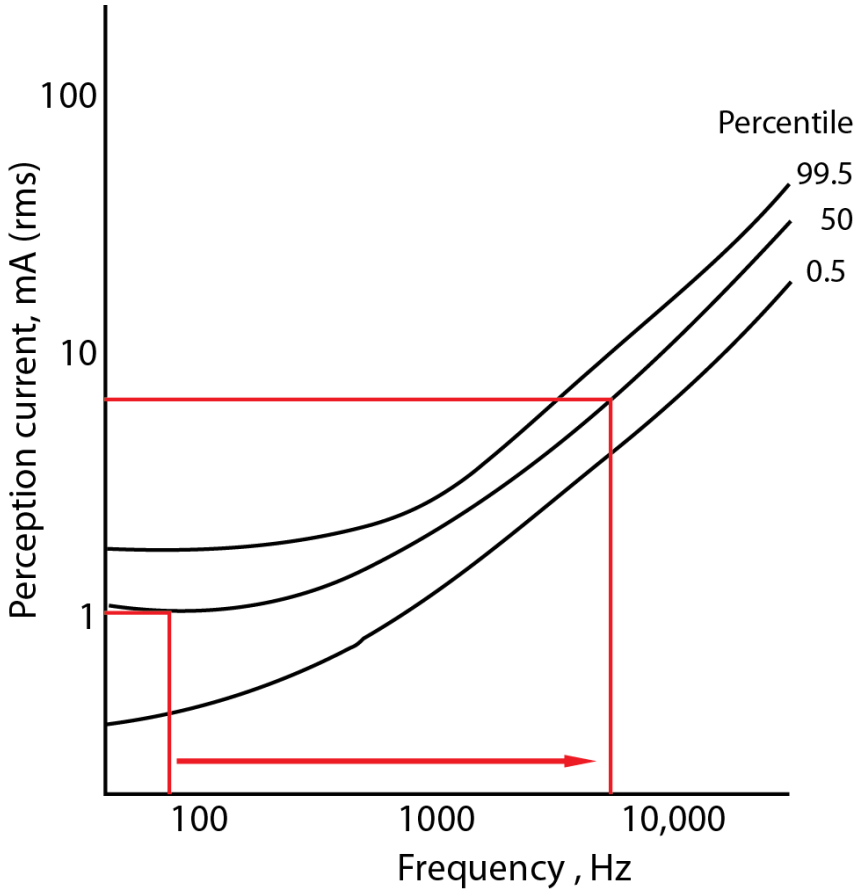


Figure 3 - Illustration of copper wire holding experiment, with frequency increased until subject perceives heat in the palm of their hand.

Lines shown at frequencies 60Hz and 5kHz. At higher frequencies, a greater current is needed to perceptibly heat the wire. Adapted from Ferris (Ferris *et al.* 1936)

3.2.5 Fatigue

The sustained ability of a nerve or muscle fibre to fire declines over time of stimulation. This is termed fatigue. This is important in terms of neuromuscular stimulation, as fatigue limits clinical efficacy and subject adherence.

The rate of fatigue is directly related to stimulation frequency (Binder-Macleod *et al.* 1991). The greater the frequency of sustained stimulation, the greater the force of contraction generated, and the faster the rate of fatigue (Bickel *et al.* 2011). This may be due to increased conduction velocities in the nerve or sarcolemma due to accumulation of extracellular cations (Na^+ and K^+), or exhaustion of neurotransmitter at the NMJ (Mortimer *et al.* 1970). Typical voluntary motor unit recruitment patterns attempt to recruit a large number of motor units at low frequencies in an effort to maintain force output and avoid fatigue.

Stimulation settings can vary widely, but most clinical regimens stimulating muscle use 20-60Hz patterns (Bigland-Ritchie *et al.* 1979; Hultman *et al.* 1983; Baker *et al.* 1988). Experiments performed on anaesthetised humans showed direct stimulation of quadriceps muscle at 20Hz produced up to 70% of the maximum voluntary force, whilst increasing the frequency to 50Hz could reach up to 100% (Hultman *et al.* 1983). Bigland-Ritchie et al showed that higher frequencies of stimulation (50 Hz and 80 Hz) have been shown to result in a rapid decline in force after approximately 20 seconds (Bigland-Ritchie *et al.* 2000). Stefanovska and Vodovnik found that after repeated 10 second bursts at 50Hz, "negligible fatigue" occurred, as compared to stimulation at 2.5kHz using 10 second trains of stimulation, where a rapid decline in force was seen (Stefanovska *et al.* 1985).

3.2.6 Electrode Placement

The anatomical placement and size of electrodes will affect the nature of the electrical current applied. Systems usually adopt one of two electrode configurations – either monopolar or bipolar. In a monopolar configuration, electrodes of different dimensions are used. The smaller electrode is used for active stimulation. Current density underneath this will be high, and when placed over a motor nerve or muscle will cause depolarisation and muscle

contraction when threshold has been attained. A larger dispersive or return electrode is placed nearby to complete the circuit, where current is relatively dispersed and produces no local effect. In a bipolar configuration the electrodes are of similar dimensions. Current density is more uniform across the current path, allowing for focal concentration of current with less chance of stimulation crossover into nearby muscles, but dense current increases the chance for discomfort or pain.

The pathway that the current takes will dictate its physical effect (e.g. over quadriceps, soleus, peroneal nerve), and care must be taken when electrodes are placed centrally that normal physiological processes may be adversely affected.

3.2.7 Muscle fibre recruitment

Although electrical stimulation has the capacity to produce movement in muscles, it is inherently less efficient than human movement. Hennemann's "Size Principle" dictates that in order to move a load, motor units are recruited from smallest to largest (Jabre *et al.* 1996; Conwit *et al.* 1999). Small motor units are innervated by small diameter axons, supplying slow twitch muscle which is relatively fatigue resistant (Bickel *et al.* 2011). Large motor units have large diameter axons, supplying larger numbers of fast twitch fibres. As more force is needed, units are recruited in an ascending size order, according to the magnitude of their force output. This permits a fine level of control, and conserves energy.

In electrical stimulation of muscle, the motor unit recruitment is non-selective, compromising the natural rate of fatigue resistance. This may be due to a slightly lower excitation threshold of the larger motor units (Bickel *et al.* 2011). Neuromuscular stimulation (where a main nerve trunk is stimulated) avoids some of these problems by mimicking neural messages from the

central nervous system, and effecting a more normal size dependent recruitment strategy (Baldwin *et al.* 2006; Bergquist *et al.* 2011). NMES applied over the tibial nerve as compared to the triceps surae muscle belly observed that contractions were more forceful, activated spinal neurons for increased central nervous system input, and tended to follow the normal physiological motor recruitment size principle (Bergquist *et al.* 2011). Studies with spinal injuries and NMES indicated that a motor recruitment order similar to that which occurs in voluntary muscle contractions could be seen (Doucet *et al.* 2012). Kots and Xvilon noticed that muscle hardness (as measured by an indentation device) was always greater when electronic contractions were used rather than voluntary ones, whereas the force evoked was always greater for voluntary contractions (Kots YM *et al.* 1971). From this they conclude that electric stimulation produces greater force in the excited muscle than when recruited voluntarily, but voluntary contractions reflect the contribution of all synergistic muscles contributing to a contraction, not just the isolated muscles targeted by NMES. They recommend training with both voluntary resistance training and a synergistic program of NMES.

3.2.8 Electrical safety parameters

Electricity is a powerful physical force, and has accompanying safety concerns. Adverse events can be related to the direct interaction with body tissues (pain, burns, cardiac dysrhythmias), and through its action on muscles causing involuntary movement.

3.2.8.1 Biological effects of electricity

Alternating and direct current will have different effects on biological tissue. With alternating current, at 60Hz, 60 cycles of voltage change will occur per second. If the current is high

enough, there will be a feeling of electric shock for as long as electrical contact is made. In contrast, with direct current there is a feeling of shock only when the current circuit is connected and disconnected. The higher the voltage, the greater the risk of burns. At 240V and above, the voltage punctures the skin instantly, leaving a deep localised burn (RM Fish 2009).

The effect of electricity will depend on the anatomical location of applied electrodes. Those applied across the torso (and heart) will have different effects to those applied across a limb or joint. When holding an electrode with the hand, increasing sensation and discomfort develop with increasing current levels. If this is continued past the point at which muscle contraction occurs, a point at which voluntary movement can no longer overcome the contractive effect of the electricity is reached – the so called "let-go" current. The size and shape of the conductor, size of the electrodes, or presence or absence of moisture at the interface have no effect on this threshold. Again, threshold levels vary with a normal distribution, and is approximately 16mA (Figure 4). Engineers take the 0.5 percentile as the maximum uninterrupted and reasonably safe levels of current for adults, approximately 10mA. This is important when considering the use of electrical devices in hospitals, out-patient clinics and during home use. If the subject is unable to let go of the device, they may be unable to inactivate it should the device malfunction. Devices which have maximum stimulation currents below 16mA would be favourable. Pulsed currents are even more favourable, as muscle relaxation can occur between pulses.

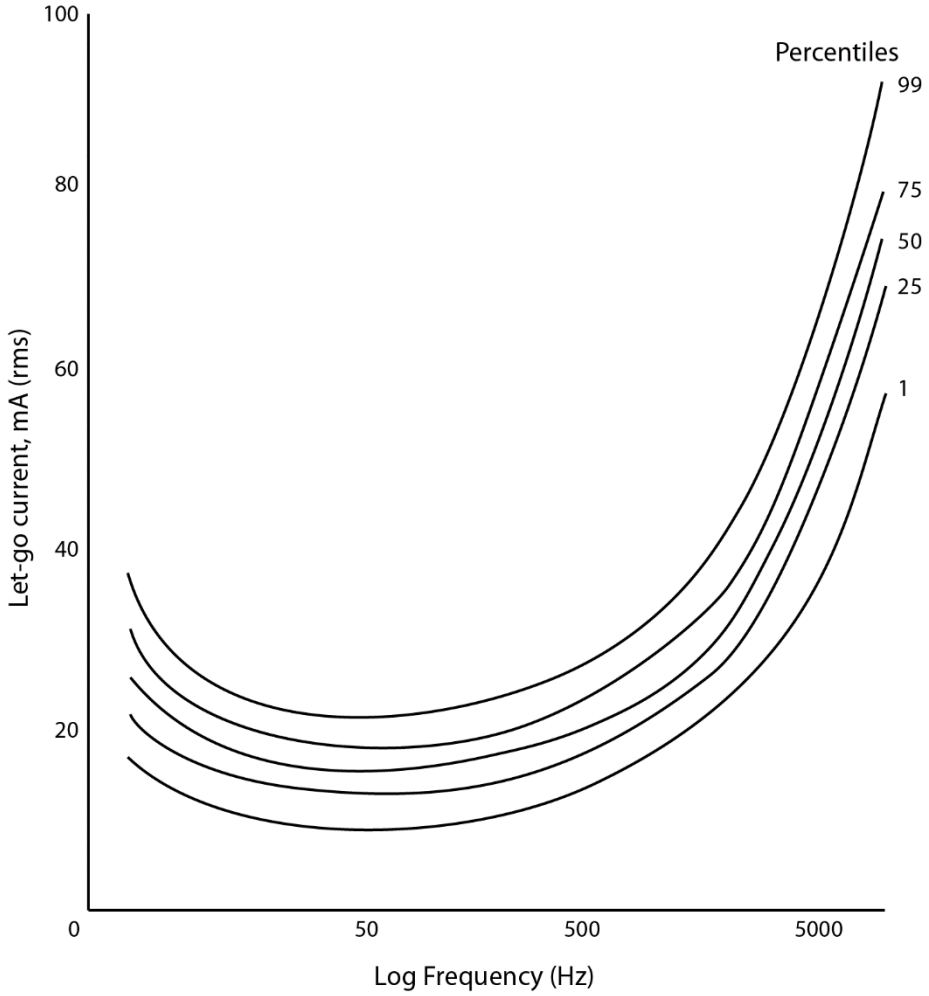


Figure 4 - "Let-go" currents plotted against frequency.

Threshold of cardiac fibrillation

Currents applied to the chest in excess of 18mA contract the intercostal muscles so that respiratory arrest is induced. However normal breathing resumes upon interruption of the current (Dalziel 1972). No human trials have been performed looking at cardiac events in response to experimental electrical stimulation for obvious ethical reasons. Animal studies have been able to quantify "minimum ventricular fibrillating current" in calves, dogs, sheep, and pigs, and can be calculated using body mass, current, and shock duration values (Dalziel 1972). When the minimum fibrillating current is compared between guinea pigs, rabbits,

dogs, sheep, calves and pigs, the lines of best fit demonstrate that the average is proportional to body mass (Figure 5)(Ferris *et al.* 1936). Reference conditions were 60Hz for 3 seconds, with electrodes on forepaw and hind foot. If these assumptions are applied to an average weight man, then the minimum current needed to produce fibrillation is approximately 77mA. The current applied during cardiac defibrillation during resuscitation varies from model to model, but is approximately 25A.

Higher frequencies reduce the current needed to produce ventricular fibrillation, although the effect in humans has not been explored (Ferris *et al.* 1936).

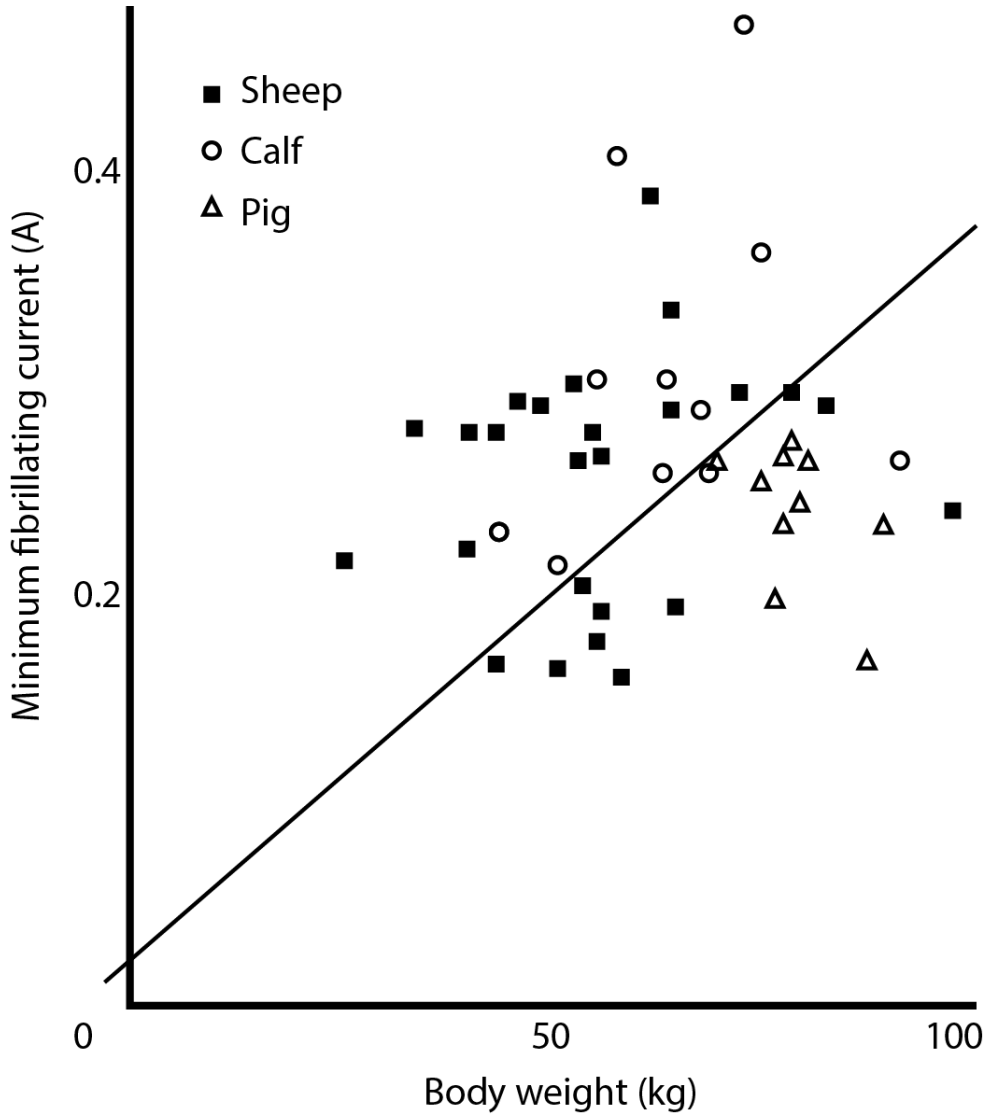


Figure 5 - Graph illustrating the relationship between minimum fibrillation current and body weight, for different animal species

Adapted from - (Ferris *et al.* 1936)

3.2.8.2 Clinical safety testing of neuromuscular stimulation

Electrical stimulation of the quadriceps is thought to be beneficial in heart failure, but NMES has been previously suspected to be dangerous in the context of cardiac devices such as pacemakers, implanted defibrillators, and external pacing machines. There is also concern regarding using circulatory devices in the context of recent myocardial infarction, cardiac

procedure, or heart failure. The perceived risk is serious, and precludes large-scale clinical safety trials. However case series have reported safe use in patients with cardiac pathology.

In a small series of seven subjects with severe chronic heart failure awaiting cardiac transplant, NMES was applied to their lower limbs for 20 minutes (Wiesinger *et al.* 2001; Crevenna *et al.* 2003). All had bipolar dual-chamber implanted pacemakers, and were monitored continuously with ECG. The NMES protocol consisted of 0.7ms pulses at a frequency of 8-50Hz, 100mA, at constant voltage (unspecified). The minimum distance between applied NMES skin electrodes and implanted pacemaker electrodes was 50cm. No subjective or objective negative clinical changes in the patients occurred over the protocol, including telemetry. Examination of the pacemakers post-protocol by a cardiologist revealed no damage to the device or programmed software.

NMES has also been trialled following elective cardiovascular surgery in the chest, in 68 patients admitted for routine post-operative intensive care (Iwatsu *et al.* 2015). 90% completed bilateral NMES sessions to the quadriceps and biceps surae from post-operative day 1 to 5. Electrical protocol used was a variable-frequency train (20-200Hz) of direct current, 0.4s on and 0.6s off, in pulse groups of 10. Current intensities were set at 10% and 20% of maximal voluntary contraction (pre-operative levels), in cycles of 10-10-20%. Session durations were 30 minutes, extended to 60 minutes if tolerated by the patient. Cardiovascular parameters remained within acceptable parameters for all participants. There were no adverse events associated with NMES reported in those with temporary cardiac pacing. No ventricular arrhythmias occurred during NMES. Two patients who underwent coronary artery bypass grafting had episodes of atrial fibrillation during NMES, which were controlled with

beta-blockers, and self-terminated within 24 hours. The background incidence of atrial fibrillation episode in this cohort was 18-27%.

Data such as this suggests that not only might NMES be safe to use in patients with cardiac pathology or implanted cardiac devices, but that it may also be beneficial. There is not enough evidence to exclude safety concerns completely, but future trials looking at the cardiac cohort may glean interesting and potentially beneficial results.

3.2.9 Stimulation dose and protocol

Dosing of NMES/EMS programs can vary greatly, and is one of the factors limiting the comparison of different studies.

Kots and Xvilon performed a series of trials, stimulating bilateral biceps brachii, using a combination of protocols. Using Russian stimulation protocols (10s on/50s off/10s on) over 9 and 19 days, they either applied sessions once a day or once every two days (Kots YM *et al.* 1971). They showed an increase in muscle torque response over time to NMES, from day 0 to day 19, whereas there was no difference between those who performed daily sessions compared to once every two days. Gains were similar to a control group who performed similar voluntary contraction mediated exercises.

This was replicated by Andrianova et al in a study using young wrestlers as trial subjects. An 18 day stimulation protocol of biceps surae and quadriceps was performed (Andrianova GG *et al.* 1971). Russian stimulation was applied (10s on/10s off) either to the muscle bulk, or to the motor nerve. After 18 days, a substantial and significant increase in calf muscle maximum voluntary contraction (45%), limb circumference (3%) and jumping height (15%) was seen. Stimulation via the nerve trunk appeared to result in faster contractile force gains, but by 18

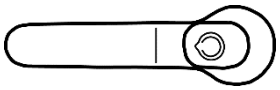
days this was not statistically different to direct stimulation of the muscle. Gains increased in a linear fashion, and had not reached plateau by day 18.

Dosage regimens must reflect the purpose for which they are being employed. It is logical to use daily training for athletic strength/endurance training as this replicates or compliments their normal training. Whether this will be the effective in the treatment of intermittent claudication remains to be seen. Where NMES is being used in wound healing or treatment of leg oedema, optimal regimens and clinical effectiveness are yet to be established. Devices such as the Revitive™ are limited to a 30 minute stimulation program, whereas devices such as the geko™ and Veinoplus™ can be applied according to user preference. Protocols with maximum efficacy must also be tempered to incorporate tolerability and activities of daily living.

3.3 Modern Electrical Stimulation Devices

The delivery of electricity to the body must occur in a measured, safe and effective manner, with minimisation of risk to patient and people in the immediate environment. An ideal device would be easy to operate (especially important in those with reduced dexterity and eyesight), portable and comfortable. Historically there have been problems with electrical safety, and pain during application. There are several devices which are now licensed for use in humans and available on the open market. They have had measured success in some areas, but remain largely untested in vascular cohorts. Here follows a short description of the devices to be discussed in this thesis. Section 4 explores neuromuscular electrical stimulation in the context of vascular disease management in more depth.

3.3.1 Neuromuscular Electrical Stimulation



3.3.1.1 Common peroneal nerve stimulation

There is one commercially available NMES device that acts transcutaneously via the common peroneal nerve. The geko™ T-1 device (Firstkind Ltd, UK) is small and battery-powered, single-use, and disposable. It is CE marked and licensed for use in post-exercise recovery, to improve circulation, and to prevent DVT. It is available online through the company website or by post, and clients with circulatory problems are required to use the device under the instruction of a medical practitioner.

The device is fitted to the lateral aspect of the knee joint just below the central knee crease as described by the manufacturer. The stimulation electrodes are thus positioned over the common peroneal nerve as it winds around the head of the fibula (Figure 6). By stimulating the nerve before its bifurcation into anterior and posterior branches, simultaneous activation of the tibialis, peroneus longus and lateral gastrocnemius muscle provides a semi-isometric compression of the calf vessels, possibly more efficiently than gastrocnemius compression alone. An adhesive hydrogel holds the device in place once positioned, and is loosened again either with gentle traction from one end of the device off the skin, or through the use of a wet cloth to dissolve the adhesive. The device pulse widths can be adjusted, and are set to the minimum level that can achieve slight outward twitching of the foot when raised from the ground.

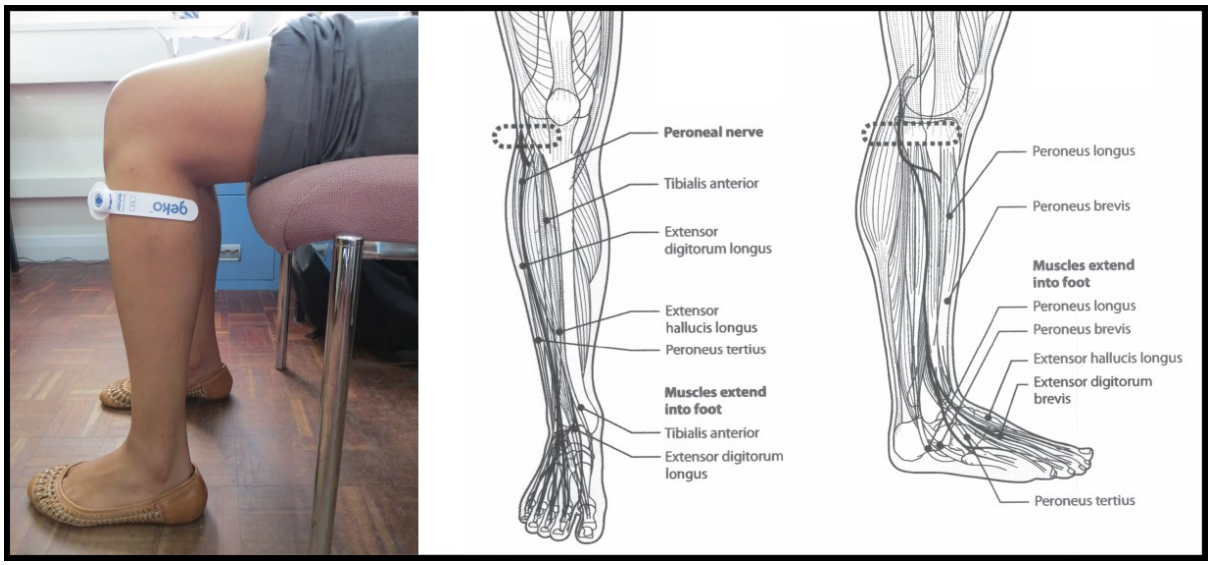
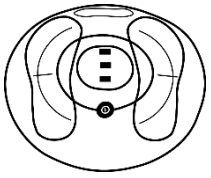


Figure 6 - The geko-T1 neuromuscular stimulation device, with application as per manufacturer instructions

Small clinical studies using this particular device in healthy people have shown enhancement of peripheral circulation, during and after one stimulation protocol (Tucker *et al.* 2010). Fifteen sequential electrical stimulations were applied for 5 minutes each, followed by a 10 minute recovery phase. During stimulation significant increases in venous blood flow volume and velocity and capillary blood flow were found. Foot skin oxygen levels were maintained. No changes to systemic parameters (blood pressure, heart rate, pulse oximetry) were observed. All subjects tolerated the device well, and rated the experience of discomfort as "minimum sensation".



3.3.1.2 Footplate stimulation

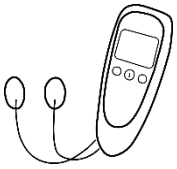
The Revitive™ device (Actegy Ltd, UK) consists of a set of electrical footplate electrodes, combined with a rocking platform. Stimulation via the device causes contraction of foot and calf muscles, and the rocker facilitates ankle movement (shown in Figure 7).

It operates a cycle of programmed stimuli protocols, each in turn. Operating parameters are 1-50Hz, 15mA, 5W. At the present time, the device is only licenced for use in healthy individuals.

There is no available published data in peer reviewed journals on the clinical efficacy of this device, although the company literature refers to unpublished studies in the UK (Revitive). They claim that over 20 minutes, the device can alleviate symptoms of inactivity, such as cold or painful limbs, swollen ankles, feet and lower legs, comparative to heel toe raises.



Figure 7 – The Revitive neuromuscular electrical stimulation footplate device



3.3.1.3 Calf muscle stimulation

The Veinoplus™ (Ad Rem Technology, France) is a device that was brought to the market in 2004. It is an electrostimulator for direct transcutaneous application to the gastrocnemius muscles bilaterally. It is designed to be portable, with self-adhesive electrodes which connect to a battery operated generator. It produces bursts of square wave stimuli of 50ms duration, pulse width 240µs, maximum voltage 5V, and frequency of 1-250Hz. When operated correctly, it causes calf contraction and dorsiflexion at a rate of 60-100 per minute.

Studies have shown the Veinoplus™ can augment peripheral circulation (Griffin *et al.* 2010), with reported ten- to twelve-fold increases in baseline popliteal venous velocity and flow when operated over the lower half of the frequency range. In both healthy and claudicating limbs, diminishing returns in haemodynamic parameters were seen when stimulating muscle contractions more frequently than 75 per minute (Abraham *et al.* 2013). Studies in chronic

venous disease, peripheral arterial disease (claudicants only), and pregnancy, have shown it to be safe, and efficacious in reducing clinical symptoms over a maximum of one month(Le Tohic *et al.* 2009; Bogachev *et al.* 2011; Lobastov *et al.* 2014). No long-term studies have been published.

3.3.1.4 Functional electrical stimulation

Functional electrical stimulation (FES) is a treatment that uses electricity specifically to improve mobility. It is used extensively in physiotherapy, and in the treatment of solitary nerve palsies (e.g. foot drop). In FES, neuromuscular stimulation augments functional movement. Individuals must be able to initiate muscle contraction, and the electronic device supplements the electrical impulses to make the movement more purposeful and effective. As a specialised form of neuromuscular stimulation, it is not discussed further in this thesis.

3.3.2 Transcutaneous Electrical Nerve Stimulation

Transcutaneous electrical nerve stimulation (TENS) is historically a form of electrical stimulation that is administered at frequencies that cause sensation only. True TENS, not to be confused with NMES, should not cause muscular contraction. Misnomers occur frequently, and is a common cause of confusion in this field.

The use of TENS in the management of pain is based on gate theory (Wall 1978). Nerves carrying pain messages are modulated by both descending signals from the central nervous system, and facilitated or inhibited by other nerve fibres carrying sensory information. TENS causes activation of peripheral nerves, and can modulate subject experience of noxious

insults. TENS is used in many clinical settings (e.g. obstetrics, physiotherapy), and complimentary therapies.

3.4 Known limitations of electrical stimulation

Excessive subcutaneous tissue and fluid

As the effect of electrical stimulation is composed of stimulating the membranes of nerves and muscle fibres, any materials lying between the electrode and the target will cause impedance and degradation of current. Subcutaneous fluid and adipose tissue limit the efficacy of transcutaneous devices. Skin surface-stimulating electrodes typically reach superficial motor units up to 10-12 mm to the electrode face (Fuglevand *et al.* 1992). Penetrating thick layers of insulating fat or dissipating water, or activation of deeper structures, is usually not possible with standard surface stimulation.

Percutaneous stimulation uses electrodes that are inserted through the skin into the nerve or muscle of choice, are thought to be superior to transcutaneous surface electrodes when specificity of stimulation is paramount. The leads of the electrodes exit the skin and connect to an external stimulator, bypassing most sensory nerve endings and reducing discomfort. They usually target specific deeper muscle locations without the consequence of unintentionally activating surrounding tissues. Electrodes normally have a limited life span, prone to trauma fracture and may induce a local inflammatory reaction. They have been used successfully in the context of faecal incontinence via sacral nerve stimulation, and limb

salvage for non-reconstructable critical limb ischaemia by placement in and around the spinal cord (Vaizey *et al.* 1999; Spincemaille *et al.* 2001).

Lack of placebo

Clinical effectiveness of NMES has yet to be established in many pathologies, and one of the major drawbacks to establishing this is the inability to perform randomised controlled trials. There is no adequate placebo for an electric shock – the real life experience of which is binary, either off or on. Some studies have attempted to use sham devices with vague explanations to patients. TENS has been used as a placebo in that it delivers palpable energy pulses but does not cause contraction. This assumes that the beneficial effects of NMES are purely due to repetitive muscle contraction, and that there is no neuromodulatory effect. Trials of non-superiority do show some promise in leading research forward, but present difficulties when exploring and optimising protocol durations and parameters.

Long-term use

Long-term effectiveness following discontinuation of stimulation is uncertain, and may be pathology related. For short term indications such as VTE prophylaxis, protocols may be more straightforward: use of the device in times of high risk (such as the peri-operative period), or during times of immobility. However where the therapy is used for muscle enhancement training, or circulatory support, evidence for length of use is unclear. There are reports of decreasing perceived benefit after withdrawal of therapy. For example, whilst stimulation can cause reduction of spasticity in children with cerebral palsy, cessation of treatment causes return to baseline values (Ozer *et al.* 2006). The same can be said of small trials looking at functional hand-use after stroke, and joint stability after shoulder subluxation (Knutson *et al.*

2007; Koyuncu *et al.* 2010). The long-term effect on vascular circulation has not been evaluated.

3.5 Summary of important points

1. The use of electricity for the diagnosis and treatment of medical problems is now more safe and tenable than it has ever been.
2. There are multiple devices available for use by patients in the comfort of their own homes. They have been shown to change blood velocities in the legs.
3. Intensity of stimulation can be changed by varying pulse width or current. Higher intensities cause recruitment of more motor units, affecting the magnitude of force output.
4. The effects of electricity on humans varies from subject to subject, but in terms of safety is often expressed as the 0.5 percentile values.
5. Constant current values greater than 77mA should be avoided, as risk of cardiac arrhythmia increases above this approximate value (based on animal testing). This value decreases proportionally with the duration of stimulus.
6. Frequencies of 20-60Hz are physiologically realistic, and produce near-maximal muscle contraction in the legs.
7. Small studies to date have shown that NMES devices are safe when used with cardiac pacemakers, temporary cardiac pacing devices, and immediately following major cardiac surgery.
8. NMES and TENS devices are fundamentally different in their physiological and clinical effects, and care must be taken to use the correct nomenclature.
9. There are significant limitations to our knowledge of the long-term effects of NMES.

4 NMES and vascular disease

4.1 Literature review: Calf muscle pump function and clinical venous disease

The venous anatomy of the lower extremity consists of a complex network of thin walled, high capacitance veins, in which the maintenance of appropriate venous return depends on the interaction of an effective pumping mechanism, a pressure gradient and competent venous valves (Meissner *et al.* 2007).

Venous return from the lower extremities is vitally dependent on the action of the foot, calf and thigh muscle pumps, with approximately 90% of venous return attributed to these muscular structures during ambulation (Meissner 2005). Amongst these, the calf muscle pump plays the most pivotal role, reflected in the fact that it has the largest capacitance and generates the highest pressure, with an ejection fraction (EF) of approximately 65% in healthy subjects, in comparison to the thigh muscle pump which has a significantly lower EF of approximately 15% (Meissner 2005). During ambulation Alimi et al showed a pressure increase of 92% in the deep posterior compartment of the calf, 104% in the superficial posterior compartment, and 18% in the anterior tibial compartment. This was associated with a rise in venous pressures of 63% in the popliteal vein and 32% in the great saphenous vein (Alimi *et al.* 1994). Eberhart et al showed that on contraction of the calf, an increase in pressure of up to 250mmHg is observed in the posterior fascial compartment (Eberhardt *et al.* 2005) showed that on contraction of the calf, an increase in pressure of up to 250 mmHg

is observed in the posterior fascial compartment. Upon calf relaxation the resting venous pressure falls to between 15 and 30mmHg at which point the function of the bicuspid valves become vital in order to prevent retrograde flow (Eberhardt *et al.* 2005).

Dysfunction or impairment of either the valvular or musculoskeletal components of the calf muscle pump may be a major mechanism for the development of venous incompetence, and can lead to several manifestations of chronic venous disease (CVD) (Eberhardt *et al.* 2005).

CVD defines the spectrum of manifestation of venous pathology that results from ambulatory venous hypertension. Venous reflux due to valvular incompetence, venous obstruction and calf muscle pump function determine ambulatory venous pressure, therefore dysfunction of one or more of these can lead to CVD with varying degrees of severity. An effective calf muscle pump in the presence of valvular dysfunction or obstruction plays a compensatory role and may thereby go some way to offset CVD (Eberhardt *et al.* 2005). On the other hand, failure of the peripheral pump caused by musculofascial weakness, loss of joint motion, valvular failure or outflow obstruction has been reported to be associated with dysfunction of the peripheral venous system (Kan *et al.* 2001). Valvular incompetence and venous reflux are associated with a rapid refill time after muscular contraction; this is due to refill occurring not only via inflow from the superficial system but also via pathological retrograde venous flow (Meissner 2005). Reflux generates abnormal pressure characteristics, and as there is no reduction in pressure after ambulation this further potentiates the accelerated refill time.

Venous obstruction results in resistance to the outflow of blood that in turn causes elevated venous pressures during calf muscle contraction, as well as minimal (if any) reduction in resting pressure post contraction. This has the potential to result in ambulatory venous hypertension and thus the pathogenesis and potentiation of CVD. The pathological effects of

chronic venous hypertension are observed in the skin and subcutaneous tissues and manifest in the form of oedema, pigmentation, fibrosis and ulceration (Eberhardt *et al.* 2005). The annual cost of venous ulcers in the United Kingdom estimated to be between £400 and £600 million (Nicolaides *et al.* 2000). Conventionally, severity of CVD is evaluated using both the CEAP classification, and venous severity or quality of life scores (Eklöf *et al.* 2004)

The primary aim of this study is to evaluate the function of the leg muscle pump. In order to investigate the function of the leg muscle pump, evidence available in the literature will be evaluated regarding:

- The extent to which calf muscle pump failure contributes to CVD.

- A consensus of the point at which the calf muscle pump becomes haemodynamically compromised and thereby unable to pump adequately, thus having a detrimental impact.

4.1.1 Method

Using the OVID portal to gain access to the archives of MEDLINE and Embase, articles from 1946 to present were searched using the keywords: "calf" AND "muscle" AND "pump" AND" venous" AND "insufficiency" AND ("lower limb*" OR "leg*"). Selection criteria for this systematic review are tabulated in Table 1.

Table 1 - Selection criteria for literature search exploring involvement of the calf muscle pump in symptomatic venous disease

Inclusion	Cross sectional and longitudinal studies in humans relating to chronic venous insufficiency, highlighting the role of the calf muscle pump in CVD, and the extent to which the calf muscle pump is impaired in cases of CVD.
Exclusion	Animal models Case studies, reviews and letters Endpoints relating to surgery Studies that aimed to highlight calf muscle pump dysfunction or CVD potentiation as a result of syndromes.

	Any studies in which the main focus was the relationship between calf muscle pump dysfunction and lymphoedema.

No filters, limits or language exclusions were applied to the search.

Titles were screened for clinical relevance in relation to CVD and calf muscle pump involvement. The abstracts of titles that appeared to bear significance were then read in full to ensure that the studies were in accordance with the inclusion criteria. The full articles of studies that appeared to meet the demands of the inclusion criteria were then independently assessed using the STROBE statement to verify the methodological quality of the studies (von Elm *et al.* 2007). A PRISMA diagram is shown in Figure 8.

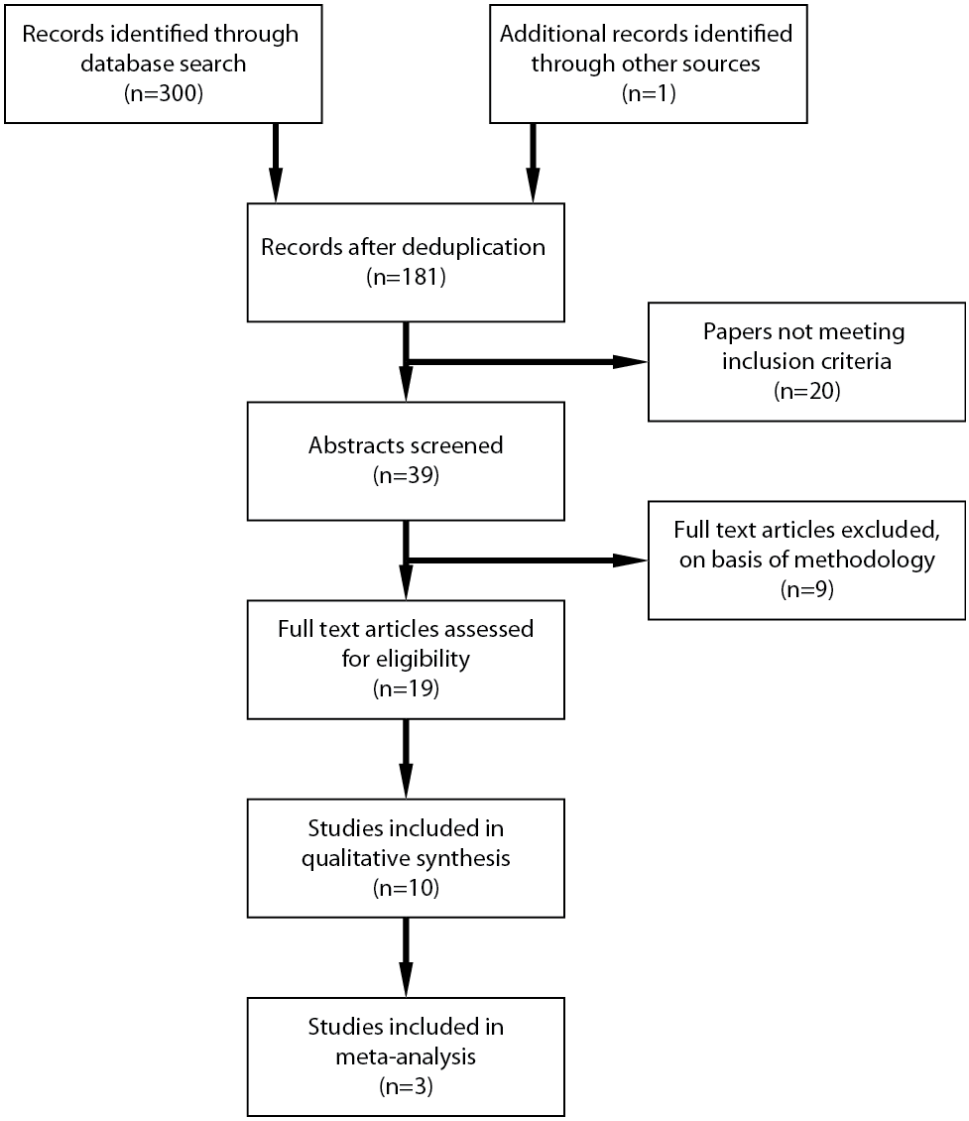


Figure 8 - PRISMA diagram of systematic review evaluating muscle pump dysfunction in venous disease

Excluded studies

- Studies that lacked information relating to certain parameters that measure/quantify the efficacy of the calf muscle pump (i.e. ejection fraction or venous filling index)(Bundens 1995; Dix *et al.* 2003; Shiman *et al.* 2009; Crisostomo *et al.* 2014).

- Studies that focused solely on calf muscle pump function in healthy subjects and therefore did not contain sufficient information with regards to calf muscle pump function and its impact on CVD(Barendsen *et al.* 1984; Nadland *et al.* 2009).

4.1.2 Results

The ability of the calf muscle pump to eject blood is measured using an ejection fraction (EF). It represents a measure of calf muscle pump efficiency and efficacy. It is calculated as the volume of blood ejected with one tiptoe manoeuvre divided by the venous volume of the calf at rest (Welkie *et al.* 1992). Complications of CVD, such as ulceration, have been shown to correlate with the severity of reflux assessed with ejection capacity (Nicolaides *et al.* 2000). Studies with EF data comparing healthy to CVD patients have been tabulated in Table 2 (Cordts *et al.* 1992; Welkie *et al.* 1992; van Bemmelen *et al.* 1993; Araki *et al.* 1994; Back *et al.* 1995). A total of 605 limbs were analysed (203 healthy, 402 CVD).The mean calf ejection fraction was 62.1% in healthy limbs, and 46.2% in CVD limbs ($p<0.001$). The mean percentage reduction in CVD limbs compared to healthy was 25.6%.

Table 2 - Ejection fraction and venous filling index compared between healthy controls and patients with chronic venous disease

Year	Authors		Patients	Limbs	Limbs with CVD	Mean EF in control limbs (%)	Mean EF in CVD limbs (%)	P value	% reduction of EF in CVD limbs	Mean VFI of control limbs (ml/s)	Mean VFI of CVD limbs (ml/s)	P value	% increase of VFI in CVD limbs
1992	Cordts		88	142	39	58.6	51.9	-	11.4	1.0	6.8	-	580
1992	Welkie		177	330	236	65.6	49.1	<0.05 between C0 and C1*	25.2	1.52	5.6	<0.05*	268
1993	Van Bemmelen		28	32	32	-	47.9	-	-	-	4.5	-	-
1994	Araki		55	69	69	-	41	-	-	-	7.9	-	-
1994	Back		26	32	26	66	49.3	<0.001†	25.3	1.3	7.4	<0.001†	469.2
Total			374	605	402	62.1	46.2	Z=3.32 (p<0.001)	25.6	1.25	5.91	Z=81.48 (p<0.001)	372.8

(CVD – chronic venous disease; * - Wilcoxon rank test; † - ANOVA)

The venous filling index (VFI) is a direct measure of venous filling upon standing. It is calculated by measuring 90% of the venous volume and dividing this by the time required to fill 90% of the venous volume after resuming an upright position. Valvular pathology would cause filling not only from arterial inflow but also pathological venous reflux, thus the greater the VFI the greater the level of valvular incompetence. A normal venous filling index is <2 ml/s, whereas higher levels (>4 ml/s) have been found to correlate with the severity of CVD (Christopoulos *et al.* 1988). Studies with venous filling data comparing healthy to CVD patients are shown in Table 1 and Figure 2. The mean VFI of healthy limbs is 1.25ml/s compared to 5.91ml/s in CVD which was statistically significant ($p<0.001$).

Table 3 shows the studies in which the aim was to assess the benefit of exercise treatment programs on calf muscle pump function. Yang et al (Yang *et al.* 1999) recruited patients with healed venous ulcers, screened them for the absence of arterial disease and ankle movement problems. Venous obstruction was not an exclusion criteria but none were included in the trial. They used a six week supervised exercise programme of heel raise exercise specially tailored to each patient, and measured the changes in torque, power, EF and VFI compared to healthy subjects baseline values (Yang *et al.* 1999). Kan et al used a much shorter 7 day programme of plantar flexion against resistance whilst seated, and used a population of patients with active perimalleolar ulcers that had been present for more than two months, comparing them with a control group treated with best medical therapy only (Kan *et al.* 2001). Cases of venous obstruction were excluded, as were limitations to ankle motion. The calf EF was seen to rise from pre-treatment levels in both groups, from 57.8 to 69.5% and 40 to 65% ($p<0.01$ for both respectively, Wilcoxon signed-rank test). Venous filling index did not change significantly in either study.

Table 3 - Impact of calf muscle pump exercise treatment on calf ejection fraction and venous filling index

Author	Patients	Limbs with CVD	Mean %EF in pre-treatment CVD limbs	Mean %EF in post-treatment CVD limbs	P value (Wilcoxon signed rank)	Mean VFI(ml/s) in pre-treatment limbs	Mean VFI(ml/s) in post-treatment limbs	P value (Wilcoxon signed rank)
Yang 1999	20	20	57.8	69.5	0.001	5.9	6.3	0.351
Kan 2001	21	10	40	65	0.006	(Difference 0.22)		0.72

Table 4 highlights the studies in which data regarding EF and/or VFI were absent, however the authors included the percentage value of CVD patients that also had calf muscle pump failure (Haenen *et al.* 2000; Simka 2004; Simka 2007). Failure of the calf muscle pump in these studies was identified via air-plethysmographic methods in which the calf muscle pump was regarded as dysfunctional if the drop of cuff pressure during calf flexion was lower than 1mmHg (Simka 2004). A meta-analysis was performed (Figure 9). A mean of 55% of CVD patients were identified as having calf muscle pump failure, with an odds ratio of 1.37 (95% confidence interval 0.34-5.60, p=0.66). In only a proportion of CVD patients is calf muscle pump dysfunction present.

Table 4 - Co-existence of chronic venous disease (CVD) and calf muscle pump (CMP) failure

Authors	Patients	Limbs	Patients with CVD	Limbs with CVD	% of patients with CMP failure
Haenen 2000	81	-	60	-	77
Simka 2004	48	59	48	59	49
Simka 2007	129	-	129	-	42.6
Total	258	59	237	59	55

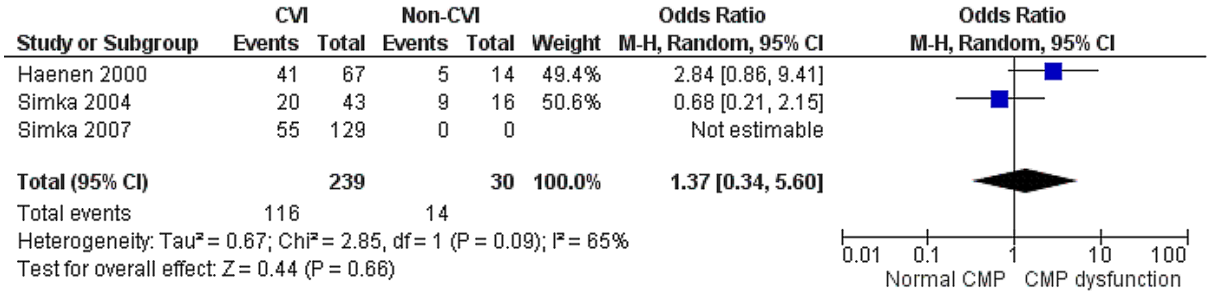


Figure 9 - Calf muscle pump (CMP) failure probability given patients with chronic venous disease (CVI)

4.1.3 Discussion

The study performed by Welkie et al reported a correlation between calf muscle pump dysfunction and CVD (Welkie *et al.* 1992), with data implicating calf muscle pump impairment as a clinical manifestation associated with symptomatic disease. The available literature is consistent with a linear relationship between the clinical manifestations of CVD with EF, but is not conclusive. The authors of this paper suggest that the threshold EF value at which the

calf muscle pump may be described as going from functional to dysfunctional lies between 42-62%, however, this range includes healthy controls in the studies and cannot be held as absolute. It must also be considered that a threshold value that implies a poorly functioning calf muscle pump may not be clinically helpful or informative.

Summation analysis comparing calf muscle pump dysfunction with the presence of CVD failed to show statistical significance. Impairment of the calf muscle pump is not a requirement for the development of CVD however patients with symptomatic venous disease are 1.37 times more likely to have calf muscle pump dysfunction. It may be implied that when calf muscle pump dysfunction is present it may potentiate the progression of CVD. Welkie et al (Welkie *et al.* 1992) observed that calf muscle pump ejection fraction decreased significantly when moving from CEAP class C0 to C1, a decrease which then plateaued with progressive clinical deterioration, leading the authors to conclude that calf muscle pump dysfunction is associated with the manifestation of CVD from asymptomatic to symptomatic in the earlier stages. The muscle pumps of the leg may perhaps delay the onset of clinical symptoms until compromised.

The literature supports the association between calf muscle pump dysfunction and objective measures of CVD severity. In the study conducted by Araki et al (Araki *et al.* 1994) it was observed that EF was significantly reduced in cases of CVD in patients with CEAP class C6 disease, that is those in which active ulcers were present. The study conducted by Back et al (Back *et al.* 1995) echoed this finding, and further highlighted the role of ankle range of motion (ROM). The study showed that poor ROM was significantly associated with calf muscle pump dysfunction and greater clinical venous disease severity. The significantly positive effect of isotonic training programmes on EF, and its failure to influence VFI, support the idea that

the calf muscle pump has its effect by modifying the pump action, rather than on any intrinsic venous modifications.

Care must be taken when interpreting the data from studies examined in this review. Small studies may result in a type two error, and in some cases where control groups were used, there was a limited effort to control for patient factors such as age, sex, body mass index, and this may have skewed the data. There is a lack of information regarding the foot and thigh muscle pump, therefore any impact that these structures have on the calf muscle pump, venous return and CVD have not been accounted for.

With regards to clinical implications, this review emphasises the requirement for more attention to be placed on the treatment of calf muscle pump failure in cases of venous disease using exercise treatment programs or other methods, which may be of clinical importance in managing symptomatic disease. To establish this in routine clinical practice these results would need to be replicated in appropriate clinical trials. It would also be logical to look at other modifiable muscle pumps, such as the thigh and foot, and to explore the potential benefit of electrical devices acting on the leg (e.g. electrical muscular or neuromuscular stimulation), especially for those patients in whom exercise capacity is limited.

Other vascular disorders may benefit from increased or improved muscle pumps of the lower limb, as increased venous return may be beneficial in peripheral arterial disease, diabetes, and lymphoedema.

4.2 Literature review: Venous disease and NMES

The muscular pumps of the lower limb (foot, calf and thigh) consist of deep venous plexuses surrounded by muscle groups in tight ensheathing fascia, and are credited with providing approximately 90% of venous return during ambulation (Meissner 2005). The calf muscle pump, first described by William Harvey in 1628, has an ejection fraction of approximately 65% in healthy individuals (Harvey 1628; Williams *et al.* 2014). In a normal individual, resting standing venous pressure is 80-90mmHg, which drops by more than 50% with calf exercise (Eberhardt *et al.* 2005). This pumping effect is blunted in patients with venous reflux (Eberhardt *et al.* 2005). An effective calf muscle pump in the presence of valvular dysfunction or obstruction plays a compensatory role, and may offset symptoms in chronic venous disease (Davies J 2008). Therefore it is not surprising that stimulation of the calf muscle pump has been the target for a number of potential clinical applications in the prevention and management of venous disease. The calf muscle pump can be augmented passively or actively. Applying external pressure to the calf results in passive augmentation of the calf muscle pump. This pressure can be applied continuously (compression stockings) or intermittently (intermittent pneumatic compression – IPC; or calf squeezes). Alternatively, active stimulation can be achieved by voluntary calf muscle exercises.

Electrical muscle stimulation (NMES) utilises transcutaneous electrodes to cause muscle contraction either by activating the muscle itself (direct), or the nerve supplying a muscle group (indirect). A wide variety of commercial and medical products are emerging in the market with applications such as strength training, exercise recovery tools, rehabilitation in

immobilised patients, and in the management of acute pain (e.g. child birth). The aim of this review is to provide a summary of the available literature on the use of electrical stimulation in the management of venous disease, and its relevance in the modern era.

4.2.1 Methods

The Medline and Embase databases were searched to identify all articles relating to application of electrical muscle stimulation in treating venous disease on 15th August 2014. The following search string was applied:

(Electric$) AND ("calf" OR "foot" OR "thigh" OR "buttock" OR "gluteal) AND ("vein" OR "venous" OR "oedema" OR "edema"). English language and human limitations were applied.

Studies were included if they contained venous haemodynamic or clinical data for the use of electrical muscle stimulation (regardless of device or protocol involved). The full articles of studies that appeared to meet the demands of the inclusion criteria were then independently assessed using the STROBE statement to verify the methodological quality of the studies (von Elm *et al.* 2007). Studies were excluded if results did not relate to electrical muscle stimulation and venous flow. Wound healing studies with methodology not using muscle contraction were excluded.

4.2.2 Results

The search strategy returned a total of 265 articles. Articles were screened by title, abstracts and full text (RR and KW). Following screening 44 articles met the inclusion criteria and were included in the review. The search strategy is illustrated in the PRISMA diagram in Figure 10.

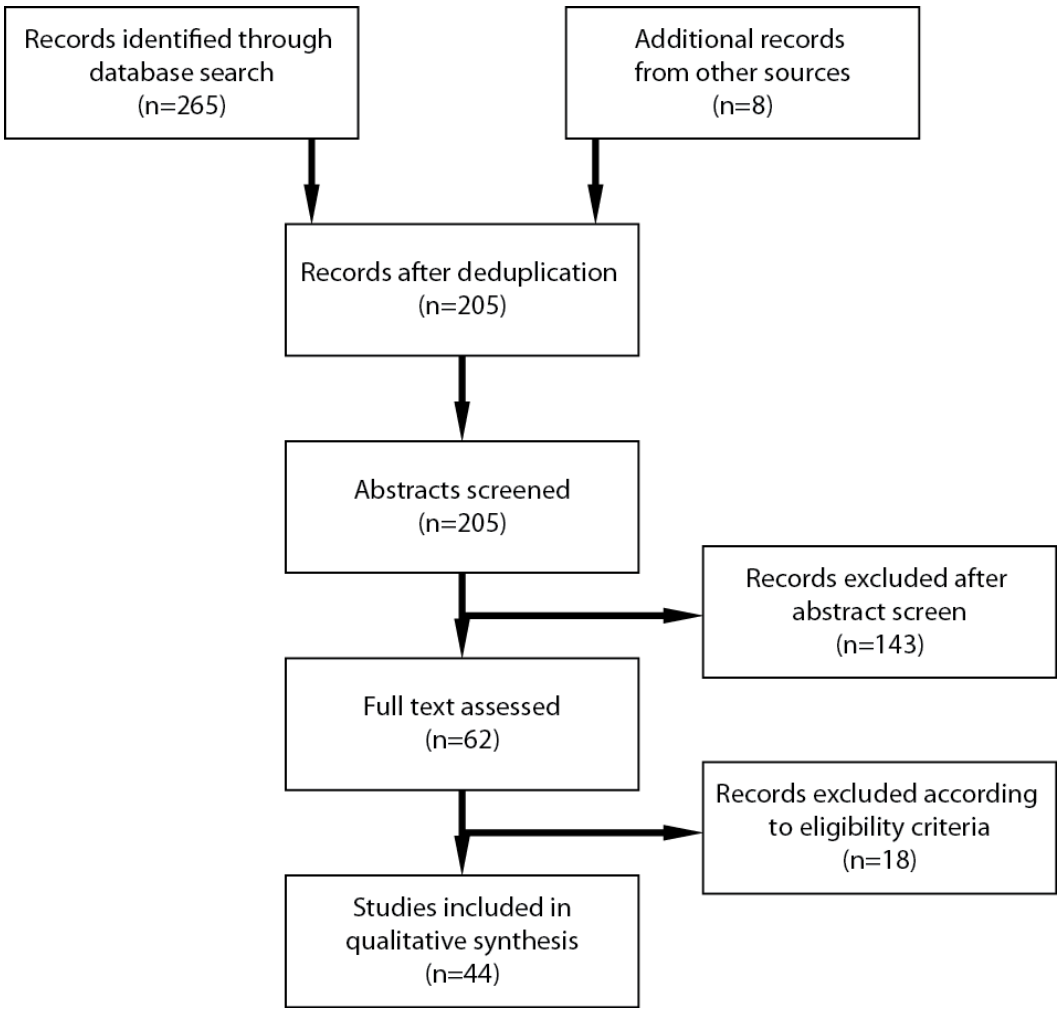


Figure 10 – PRISMA diagram illustrating literature search evaluating the effect of neuromuscular stimulation in the prevention and management of venous symptoms

Although comparison between trials is hindered by heterogeneity of protocols and outcome measures, some investigators have made comparisons varying parameters of electrical stimulation. All studies showed an improvement in venous haemodynamic parameters with stimulation of the calf muscle pump compared to rest:

- Volume flow calf increased 50-719% from baseline(Heath *et al.* 1992)(venous occlusion plethysmography), 60-614% (ultrasound) (Broderick *et al.* 2009; Broderick *et al.* 2010; Broderick *et al.* 2011; Broderick *et al.* 2013; Izumi *et al.* 2014).

- Femoral and popliteal peak velocity increased by 25-650% (Kaplan *et al.* 2002; Lyons *et al.* 2002; Broderick *et al.* 2009; Broderick *et al.* 2010; Broderick *et al.* 2010; Tucker *et al.* 2010; Broderick *et al.* 2013; Izumi *et al.* 2014) (ultrasound).
- Time averaged maximum velocity (TAMV) increased by 178-354 (Broderick *et al.* 2013).
- Ejection volume due to electrical stimulation was 60-100% of that elicited by voluntary contraction (Faghri *et al.* 1998; Miller *et al.* 2000) and popliteal venous velocity "strongly correlated" to force of plantar flexion (Corley *et al.* 2009).
- Calf vascular resistance was significantly reduced after electrical stimulation (Miller *et al.* 2000), equivalent to post-exercise (voluntary calf contractions to a metronome).
- Kaplan et al found that stimulation of the foot and calf are equivocal in their effect on popliteal flow (Kaplan *et al.* 2002).

A full table of results is available in the appendix (Section 10.1).

4.2.2.1 Effect of electrical parameters on venous haemodynamics

Direct stimulation of the muscle

Nicolaides et al report that pulse width 50-100ms and frequency 25mHz (15/min) produced maximal venous velocity without discomfort (Nicolaides *et al.* 1972). Settings above this were untenable. Griffin et al increased the frequency of stimulation between 2-120 stimulations per minute and found that the popliteal vein peak velocity increased for stimulations up to 10mHz, but decreased towards 2Hz (Griffin *et al.* 2010). Ejected calf volume per minute increased from 20ml/min to 120 ml/min with increasing stimulation frequency.

Indirect stimulation (neuromuscular)

Lyons et al found that pulse duration 300µs and frequency of 35Hz applied to the tibial nerve produced the greatest increase in popliteal peak venous velocity with a pulse width 200-300µs, and frequency 24-35Hz (Lyons *et al.* 2002). Tucker et al found that increasing stimulation frequency of the common peroneal nerve, from 1-5Hz and pulse amplitude from 1-40mA, had a positive correlation with increasing venous volume flow peak venous velocity and microcirculatory flux (Tucker *et al.* 2010).

Izumi et al (Izumi *et al.* 2010) compared two different electrical stimulation frequencies (10Hz vs 50Hz) using the same stimulator and found that the lower frequency produced a higher peak velocity compared to 50Hz stimulation, however the findings seem to be confounded by a mix of direct and indirect stimulation.

4.2.2.2 Comparison of NMES to other medical devices

Four studies compared the effect of electrical stimulation to IPC (Laverick *et al.* 1990; Jorgensen *et al.* 1994; Czyrny *et al.* 2010; Williams *et al.* 2015). Both indirect stimulation via the common peroneal nerve, and direct stimulation of calf muscles have been shown to be non-inferior to calf and/or foot IPC.

On comparing calf IPC to electrical stimulation via the common peroneal nerve, Williams et al (Williams *et al.* 2015) demonstrated that whilst peak venous velocity increased significantly with both methods, only NMES had a significant effect on time averaged mean velocity (TAMV) and volume flow. On comparing foot IPC to calf NMES, Laverick et al demonstrated a greater increase mean venous velocity and peak venous velocity with NMES (Laverick *et al.* 1990).

Faghri et al demonstrated increased cardiac stroke volume (24%), cardiac output (26%) and a reduced total peripheral resistance (21%) with electrical stimulation when compared to calf/thigh IPC (Faghri *et al.* 1997).

Lyons et al demonstrated that the effect of electrical stimulation is augmented by a factor of two with the addition of graduated compression stockings (Lyons *et al.* 2002).

Nicolaides reported an increased incidence of peri-laparotomy DVT with NMES when compared to a combination of graduated compression stockings and calf/thigh IPC (18% vs 4%)(Nicolaides *et al.* 1983).

4.2.2.3 Efficacy of clinical application

4.2.2.3.1 Electrical stimulation as a method of thromboprophylaxis

Prior to the development of heparin, NMES was utilised as a mechanical mode of thromboprophylaxis was investigated with enthusiasm. However, NMES devices in the 1960s caused painful contractions, skin irritation and burns, which did not permit its use in non-anaesthetised patients. Initial trials investigated the effect of NMES as a mode of thromboprophylaxis intraoperatively (Pollock ; Doran *et al.* ; Browse *et al.* 1970; Doran *et al.* 1970; Nicolaides *et al.* 1972; Becker *et al.* 1973; Dejode *et al.* 1973; Rosenberg *et al.* 1975; Lindstrom *et al.* 1982; Nicolaides *et al.* 1983; Bostrom *et al.* 1986; Izumi *et al.* 2014), whereas later studies investigated its effect on patients with spinal cord injury (Merli *et al.* 1988), partially-ambulatory patients undergoing stroke rehabilitation (Pambianco *et al.* 1995) and, more recently, postoperative patients (Velmahos *et al.* ; Lobastov *et al.* 2014). Trials involved general surgical (Pollock ; Doran *et al.* ; Browse *et al.* 1970; Doran *et al.* 1970; Nicolaides *et al.* 1972; Becker *et al.* 1973; Dejode *et al.* 1973; Rosenberg *et al.* 1975; Lindstrom *et al.* 1982; Nicolaides *et al.* 1983; Lobastov *et al.* 2014), neurosurgical (Bostrom *et al.* 1986; Lobastov *et*

al. 2014), orthopaedic(Izumi *et al.* 2014), trauma (Velmahos *et al.*), and non-surgical patients (Merli *et al.* 1988; Pambianco *et al.* 1995).

Trial protocols and NMES devices varied considerably. The study characteristics and outcome measures included in the qualitative and quantitative analysis are summarised in the appendix (section 10.1). The methodology of initial trials was poor, resulting in several studies being excluded from the quantitative analysis. For example, initial trials applied unilateral NMES, comparing the incidence of DVT to the contralateral (control) limb (Doran *et al.* ; Browse *et al.* 1970; Doran *et al.* 1970; Dejode *et al.* 1973).

The trials compared the incidence of DVT in patients receiving NMES to no prophylaxis (Pollock ; Nicolaides *et al.* 1972; Becker *et al.* 1973; Rosenberg *et al.* 1975; Lindstrom *et al.* 1982; Nicolaides *et al.* 1983), heparin (Nicolaides *et al.* 1983; Bostrom *et al.* 1986), dextran (Becker *et al.* 1973; Lindstrom *et al.* 1982), IPC (Nicolaides *et al.* 1983), and as an adjunct to standard therapy. Standard therapy was variable (both groups received heparin+IPC (Velmahos *et al.* ; Izumi *et al.* 2014) or GCS+heparin (Lobastov *et al.* 2014)). In non-surgical patients, NMES was used as an adjunct to heparin, compared to heparin and no prophylaxis (Merli *et al.* 1988) in patients with acute spinal cord injury. Pambianco et al (Pambianco *et al.* 1995) compared the effect of NMES only to heparin and IPC in patients undergoing rehabilitation following stroke, but the NMES arm of the study was terminated early due to poor tolerance to the NMES device. Although there was no outcome data for the NMES limb of this trial, we considered it an important trial in the qualitative analysis due to the failure of NMES therapy.

The diagnosis of DVT was based upon clinical symptoms (Doran *et al.* ; Doran *et al.* 1970), 125I–fibrinogen uptake test (125I-FUT) (Pollock ; Browse *et al.* 1970; Nicolaides *et al.* 1972;

Becker *et al.* 1973; Dejode *et al.* 1973; Rosenberg *et al.* 1975; Lindstrom *et al.* 1982; Nicolaides *et al.* 1983; Bostrom *et al.* 1986; Merli *et al.* 1988), impedance plethysmography(Merli *et al.* 1988), phlebography(Velmahos *et al.* ; Becker *et al.* 1973; Lindstrom *et al.* 1982; Lobastov *et al.* 2014) and ultrasound(Velmahos *et al.* ; Pambianco *et al.* 1995; Izumi *et al.* 2014; Lobastov *et al.* 2014). Following the introduction of 125I-FUT by Negus in 1968, studies used this diagnostic test, but the protocols were variable. 125I-FUT was conducted prior to NMES in some studies (Browse *et al.* 1970; Nicolaides *et al.* 1972; Becker *et al.* 1973; Dejode *et al.* 1973; Nicolaides *et al.* 1983) , but not others(Pollock ; Rosenberg *et al.* 1975; Lindstrom *et al.* 1982; Bostrom *et al.* 1986). The diagnostic criteria and number of measurements per limb were also variable. Phlebography (Velmahos *et al.* ; Becker *et al.* 1973; Lindstrom *et al.* 1982; Merli *et al.* 1988; Lobastov *et al.* 2014) was used to confirm the diagnosis, but this was not performed in all patients due to technical reasons, patient choice and the greater sensitivity of 125I-FUT to detecting thrombi. Pre-treatment ultrasound was routinely performed in three (Velmahos *et al.* ; Pambianco *et al.* 1995; Lobastov *et al.* 2014) of four studies (Izumi *et al.* 2014). Only two trials used diagnostic imaging for PE (Lindstrom *et al.* 1982; Lobastov *et al.* 2014).

The follow up duration of the studies varied between 1 (Izumi *et al.* 2014) to 28 (Merli *et al.* 1988) post-operative days (median 10 days). DVT was most frequently detected in the first day postoperatively (53.2%), decreasing to 27.7% and 18.9% over the first 72 hours and nine days, respectively.

Reporting of electrical parameters was not standardised precluding any meaningful comparison between the devices. However, early trials utilised galvanic (direct) current, whereas newer devices used alternating current.

Seven studies were excluded from quantitative analysis due to non-randomisation (Lobastov *et al.* 2014), absence of diagnostic imaging (Doran *et al.*), lack of appropriate control (Browse *et al.* 1970; Dejode *et al.* 1973), inability to complete the NMES limb of trial (Pambianco *et al.* 1995) and absence of accurate data (results reported in percentages of benign and malignant disease)(Pollock). Despite having diagnostic imaging for DVT, studies by Browse et al and de Jode et al were excluded as all patients received unilateral NMES and the unstimulated limb was used as a control (Browse *et al.* 1970; Dejode *et al.* 1973). Although Nicolaides et al (Nicolaides *et al.* 1972) also used unilateral electrical stimulation, a separate group that did not receive any electrical stimulation acted as the control group.

Thromboprophylaxis: NMES compared to control

Four RCTs compared the effect of NMES to negative controls (no thromboprophylaxis). Trial methodology was weak in these early trials, with randomisation techniques using alternate limbs and date of birth. As NMES was only administered intraoperatively, this was considered blinding of participant and personnel. However, these limitations were compensated for by the large number of patients recruited in the early trials.

A total of 517 patients were included in the analysis; 209 in the NMES group and 308 in the control group. The imbalance in groups is attributed to two factors; the control group for the trial by Nicolaides et al(Nicolaides *et al.* 1972) was reported as number of limbs affected (56 patients, 112 limbs) as the intervention group received unilateral NMES, and the method of randomisation by Rosenberg et al(Rosenberg *et al.* 1975) was by month of birth, leading to poorly matched groups.

The pooled incidence of DVT in the NMES group (14.3%) was significantly lower than the control group (31.8%), with a corresponding odds ratio (OR) of 0.26 [95% confidence interval (CI) 0.10-0.72; p=0.009; Figure 11). However, there was no statistically significant heterogeneity across the trials (I2 =63%; p=0.05).

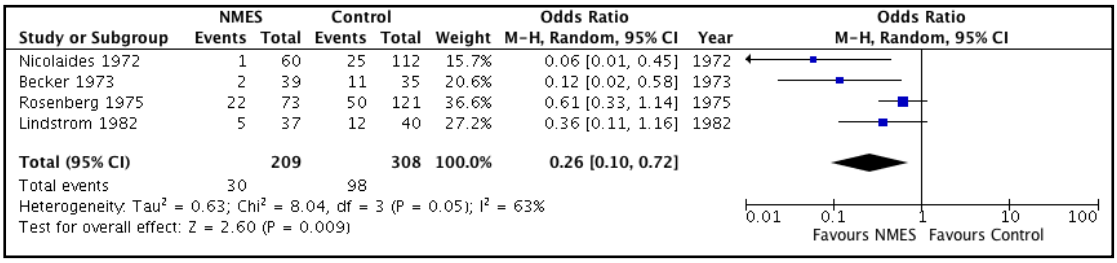


Figure 11 - Forest plot comparing neuromuscular electrical stimulation (NMES) to control (no thromboprophylaxis) in the prevention of deep vein thrombosis

(CI confidence interval; df degrees of freedom;M-H Mantel-Haenszel)

Thromboprophylaxis: NMES compared to Heparin

Three RCTs compared the effect of NMES to unfractionated heparin (UFH; 5000iu BD), with 163 patients in the NMES group and 178 patients in the heparin group. Once again, NMES was only administered intraoperatively and the quality of trials was a limiting factor due to randomisation techniques using the date (Bostrom *et al.* 1986) and month (Rosenberg *et al.* 1975) of birth. Other sources of bias include failure to observe protocol (Rosenberg *et al.* 1975) and patient exclusion following randomisation due to complications (Bostrom *et al.* 1986) (reason given).

The odds ratio of developing DVT with NMES was non-inferior to unfractionated heparin (NMES 23.9% vs UFH 13.4%; OR 2.00, 95% CI 1.13-3.52; p=0.02) as shown in Figure 12. There was low statistical heterogeneity between these trials (I2= 0%; P=0.71).

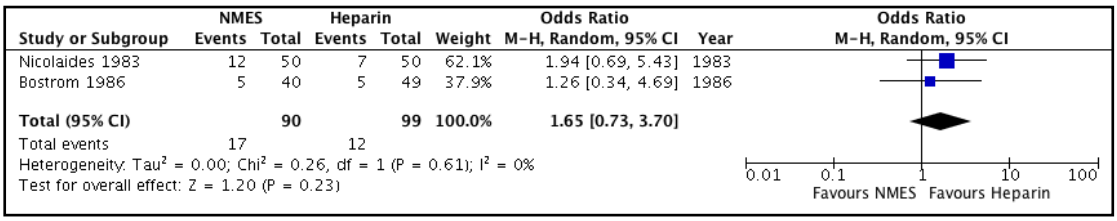


Figure 12 - Forest plot comparing neuromuscular electrical stimulation (NMES) to unfractionated heparin in the prevention of deep vein thrombosis

(CI confidence interval; df degrees of freedom; M-H Mantel-Haenszel)

Thromboprophylaxis: NMES + heparin compared to Heparin

Three studies compared the effect of NMES as an adjunct to heparin to heparin only. Devices used in the trials were modern with no reported complications. NMES was administered in patients with acute spinal cord injury (Merli *et al.* 1988), following severe trauma surgery (Velmahos *et al.* 2005) and intraoperatively during total knee arthroplasty (Izumi *et al.* 2014). Authors used subcutaneous UFH only (Merli *et al.* 1988) or either UFH or low molecular weight heparin (LMWH) (Velmahos *et al.* ; Izumi *et al.* 2014).

Two trials underwent a priori power calculations (power 0.8, α 0.05) to predict the sample size for their respective patient populations. However, one trial was discontinued prematurely due to lack of funding and clinically important trends.

Pooling data resulted in the inclusion of 168 patients, 86 in the NMES + heparin group and 82 in the heparin only group. The odds ratio of developing DVT were lower with combination

therapy than with heparin alone (15.1% vs 34.1%; OR 0.33, 95%CI 0.10-1.14; p=0.08; Figure 13). There was no statistically significant heterogeneity between the three trials (I2 =53%; P=0.12).

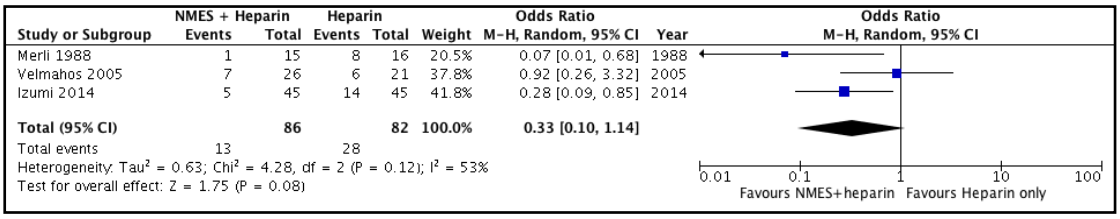


Figure 13 - Forest plot comparing neuromuscular electrical stimulation (NMES) to NMES in combination with heparin in the prevention of deep vein thrombosis

(CI confidence interval; df degrees of freedom; M-H Mantel-Haenszel).

Thromboprophylaxis: NMES compared to IPC

Nicolaides reported a significant reduction in the incidence of DVT with IPC (4% vs 18%; p<0.0025) and UFH (9% vs 18%; p<0.05) compared to NMES (Nicolaides *et al.* 1983). However, the duration of thromboprophylaxis for each group varied; NMES was applied intraoperatively only, IPC for a minimum of 72 hours or until ambulant, and UFH until discharge.

Thromboprophylaxis: Incidence of PE

Five studies reported the incidence of PE in the trials (Doran *et al.* ; Doran *et al.* 1970; Lindstrom *et al.* 1982; Izumi *et al.* 2014; Lobastov *et al.* 2014). Three trials did not use any diagnostic imaging(Lindstrom *et al.* 1982; Izumi *et al.* 2014; Lobastov *et al.* 2014). Two trials by Doran et al, reported a 1%(Doran *et al.*) incidence of fatal PE and 3%(Doran *et al.* 1970) incidence of PE based on clinical symptoms. Izumi et al reported one symptomatic PE

postoperatively (Izumi *et al.* 2014). The remaining trials were not comparable due to differing comparator groups; comparing intraoperative NMES to no thromboprophylaxis (14% absolute risk reduction (ARR) of PE in general surgical patients; $p<0.05$)(Lindstrom *et al.* 1982) and comparing NMES as an adjunct to standard therapy (heparin + IPC when no longer contraindicated) to standard therapy alone (5% ARR; statistical significance not calculated) in high risk surgical patients treated with postoperative NMES (Lobastov *et al.* 2014).

Thromboprophylaxis: Benign/Malignant disease

Rosenberg et al compared the effect of NMES in benign and malignant disease (Rosenberg *et al.* 1975). Rosenberg found that whilst both NMES and UFH reduced the incidence of DVT in benign disease (ARR 18.9% and 26.3%; $p<0.01$ and $p<0.001$, respectively), only UFH reduced the incidence of DVT significantly in malignant disease (ARR 54.6%; $p<0.001$).

4.2.2.3.2 Effect of electrical stimulation on leg oedema/chronic venous disease

Five studies examined the effect of NMES on oedema, four of which were on healthy individuals (Man *et al.* 2003; Goddard *et al.* 2008; Green DA 2008; Broderick *et al.* 2010) and one study involved patients with evening oedema (Bogachev *et al.* 2011). Healthy volunteers showed significant increase in foot and ankle volume when standing still over 30 minutes, which was abolished with NMES use (Man *et al.* 2003). Both leg volume measurements and air plethysmography, in the seated position, demonstrated reversal of fluid pooling in the legs with electrical stimulation of the calf (Goddard *et al.* 2008; Green DA 2008). Broderick tested leg swelling in supine subjects, and unsurprisingly showed no leg swelling over 4 hours bed rest, and no additional effect with NMES (Broderick *et al.* 2010) .

In trials on patients with venous disease, 20 minutes of treatment over a 30 day period resolved evening oedema in 59.4% of cases, reduced it in 34.4%, and remained unchanged in 6.2% of cases (Bogachev *et al.* 2011). This resulted in a significant reduction in group average supramalleolar circumference, reduced pain score and improved quality of life.

4.2.2.3.3 The effect of electrical stimulation in patients with impaired calf muscle pump

Patients with neurological disorders affecting the lower limb will suffer from impaired muscle pump activity. In addition to increasing their risk of developing deep vein thrombosis, this group of patients are at a higher risk of cardiovascular failure because of autonomic dysfunction and have impaired venous return to the heart. Van Beekvelt et al (van Beekvelt *et al.* 2000) using strain gauge plethysmography demonstrated that electrical stimulation can improve muscle pump activity in spinal cord injured patients. Although the spinal cord injury patients could tolerate higher current (60mA), their muscle pump action was significantly lower than that of able bodied subjects (21.5% vs 67.7%). Effects of long term treatment were not investigated.

4.2.3 Discussion

Electrical stimulation has clearly been shown to improve venous haemodynamics, however the reporting of stimulation parameters varies greatly between trials. Waveform shapes have been described as galvanic, biphasic symmetrical, sinusoidal, square wave, biphasic rectangular or trapezoidal symmetric biphasic burst, but have not described the significance of the waveform, or why that choice was made. Pulse width and frequency varied widely, and in some studies were completely unspecified. The intensity of stimulation is the most misleading variable, with different authors using potential difference, current, charge,

arbitrary units or that used to achieve a specific motor response. Electrode placement determines the effectiveness of calf muscle stimulation due to the particular nerve or muscle bulk targeted, with direct comparisons being misleading. The contraction relaxation times are variable which would affect venous filling and therefore the calf muscle pump function. Subject position is heterogenous, and comparing studies with standing, seated, prone and supine subjects may be injudicious. Haemodynamic outcome measures include air plethysmography, photoplethysmography, strain gauge plethysmography, venous occlusion plethysmography and venous duplex. The site of duplex scanning varies between the femoral, popliteal, posterior tibial and peroneal veins, whilst assorted haemodynamic parameters are reported, and the clinical significance of each parameter is poorly understood. Similarly, the techniques for detecting DVT in the early studies lacked the sensitivity and specificity of today's imaging techniques.

NMES is non-inferior to IPC in terms of venous haemodynamic parameters, and does not carry the complications associated with IPC (mainly excessive heat and sweating under the inflatable cuffs), however some find NMES untenable (Lachmann *et al.* 1992). One of the benefits of electrical stimulation over IPC is that the action increases the activity of the users own muscles, as opposed to a passive compression system. A randomised control trial on intensive care patients demonstrated an improvement in muscle strength with electrical stimulation (Karatzanos *et al.* 2012). It has been shown that aerobic exercise of any type, even just the arms, has a positive effect on walking distances in claudicants, and may alter metabolic profile (Zwierska *et al.* 2005). The cardiovascular benefits from NMES are unknown.

Venous stasis is thought to be a major contributor in the pathogenesis of deep vein thrombosis, and is the main mode of action targeted by electrical stimulation in VTE

prevention (Mackman 2012). In this meta-analysis, heparin has been shown to be most efficacious in the prevention of DVT when compared to NMES alone. However where heparin is contraindicated, NMES has a significant effect on risk reduction. In cases of very high risk, the addition of NMES to a heparin thromboprophylaxis regime increases efficacy. Katz observed a fibrinolytic effect with the use of NMES, and when twinned with increased venous velocities in the deep veins may work in synergy with heparin (Katz *et al.* 1987). It must also be kept in mind that there are significant risks associated with heparin, such as major haemorrhage and stroke exacerbation of post-operative bleeding, HIT and osteoporosis, and the need to adjust doses in extremes of body mass, pregnancy and renal impairment (Gouin-Thibault *et al.* 2005). These problems are not encountered with NMES. High risk peri-operative patients are likely to benefit from electrical stimulation in addition to low molecular weight heparin. In particular, patients who are regarded as high risk such as those undergoing bariatric surgery and laparoscopic cancer surgery, or immobile patients with spinal cord injuries. The combination of NMES with graduated compression stockings may further enhance the haemodynamic effects.

The potential clinical application of electrical stimulation in venous disease is largely unexplored, but given the dependence of the venous system on the calf muscle pump, and the large numbers of people with CVD, this is worth exploring. In treating chronic venous insufficiency, the aim is to relieve venous hypertension, and prevent lower limb ulceration. Given that orthostatic oedema can be reversed with NMES, and the ability of the calf muscle pump to be trained over time, NMES may be very successful in this area. Increased impedance (resistance to current flow) of oedematous tissues may limit how tolerable the required parameters are in order to produce underlying muscle contraction. Concentrating on demonstrating the improvement in venous haemodynamics with NMES may be a mistake, or

perhaps a good starting point. Future trials should concentrate on translating these effects into clinical practice, so that data is available to inform clinical decisions. Clinical outcomes (e.g. ulcer healing rates) and quality of life data will be most useful in analysing NMES as a clinical tool, especially given the success of IPC in this cohort (Dillon 1986; Kolari *et al.* 1986; Kolari *et al.* 1988; Smith *et al.* 1990).

The field of electrotherapy is interesting and currently underutilised, despite the emergence of various new devices with improved safety profiles, portability, and tolerable parameters. However, the lack of uniformity of nomenclature impedes comparison between devices. A consensus needs to be achieved on reporting of electrical parameters such as pulse width, frequency of stimulation, intensity and waveform. Research should also investigate the effects of dosing, duration of treatment and long term effects of electrical stimulation in treating venous disease.

4.3 Literature Review: Peripheral arterial disease and NMES

Maximal medical and surgical therapy of peripheral arterial disease (PAD) has been shown in the past to be augmented by the use of medical devices such as intermittent pneumatic compression (Delis *et al.* 2005). In order to identify if this cohort may benefit from treatment with NMES, it is logical to carry out a literature search looking at the efficacy of circulatory augmentation of patients with peripheral arterial disease, whether that be with intermittent compression devices or electrical stimulation.

4.3.1 Methods

A systematic review of EMBASE and Medline was undertaken, with the search string:

("device" OR "electric" OR "stimulation" OR "pneumatic" OR "compression" OR "IPC") AND ("peripheral arterial diseas*" OR "isch*" OR "peripheral vascular diseas*" OR "claudication") AND ("leg" OR "limb" OR "foot") (No Related Terms).

The search was performed on 8th July 2013 and repeated by the Royal College of Surgeons of England library. A PRISMA diagram shows the paper selection process (Figure 14).

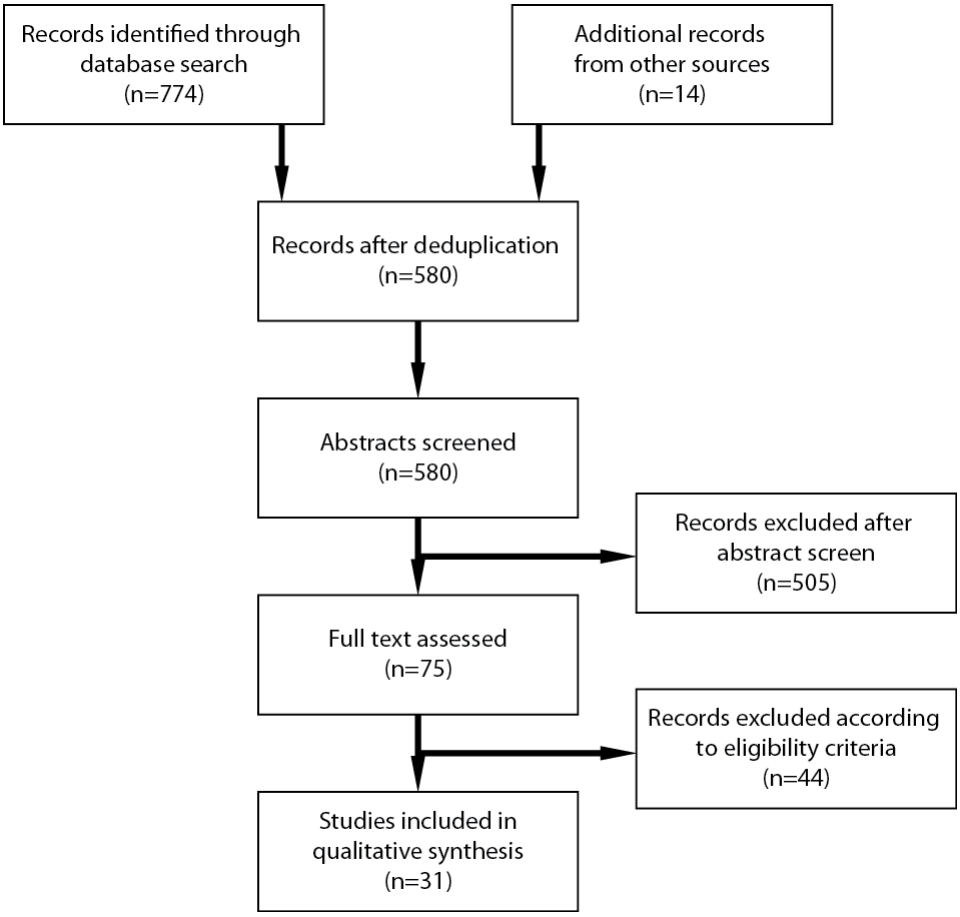


Figure 14 - PRISMA diagram illustrating literature search investigating the use of electrical stimulation in peripheral arterial disease

Abstracts were screened and included or eliminated by title, abstracts and full text for the peripheral use of non-invasive medical devices in humans for the management of lower limb peripheral arterial disease.

Inclusion criteria were clinical or haemodynamic data related to the device use. Exclusion criteria were animal studies, studies pertaining to epidemiology, diagnosis or pure imaging. Non-electrical devices were excluded (e.g. vascular closure devices), as were invasive therapies such as endovascular intervention, spinal cord/epidural stimulation, and

extracorporeal limb perfusion. Functional electric stimulation for spinal cord injury was excluded.

4.3.2 Results

31 papers met criteria for inclusion and were grouped according to indication for use. Despite multiple devices, protocols and trial designs, inferences have been drawn. For tabulated results please see appendix (section 10.2). Heterogeneity of protocols, devices, and endpoints made meta-analysis of little practical use, so was not performed.

4.3.2.1 Devices

1. Intermittent pneumatic/external compression

Six external compression devices were reported: five pneumatic (Circulator Boot, Circulator Boot Company; the AV Impulse, Novamedix Ltd; the DVT-30, Huntleigh Healthcare; the Art Assist 1000, ACI Medical; the ArterialFlow, DJO), and one which uses an external compressive band (FM220TM, FlowMedic, Israel). Pressure settings varied from very low (55mmHg in Circulator Boot) to high (120mmHg in ArtAssist). All applied pressure anatomically to the calf, some additionally to the foot or thigh.

2. Electrical stimulators

A mixture of transcutaneous electrical stimulators were reported. The VeinoplusTM (Adrem Tech, France) delivers electricity to the gastrocnemius muscle (1-250Hz, 50V, pulse width 240ms), whilst the three NMES/TENS machines can operate at variable settings and be placed over muscles or nerves (MedicompexTM, Medicompex SA; NeuroTrac TM TENS; Verity

Medical Ltd; TENS SM1, Schwa-Medico). The Micro-ZTM, Prizm Medical Inc is a galvanic electrical stimulator (50V, pulse width 100µs, sub-threshold for sensation and muscle contraction). Direct current is applied to a specific body area by a conducting garment, contact aided by an electrolyte solution.

4.3.2.2 Evidence by indication

4.3.2.2.1 Intermittent claudication

Haemodynamic

The immediate effect of IPC devices is to increase popliteal artery velocity (49 – 70%) (Delis *et al.* 2000; Delis *et al.* 2000) and flow (49-84%) (Morgan *et al.* 1991; Eze *et al.* 1996; Delis *et al.* 2000; Delis *et al.* 2000; Anthonysamy *et al.* 2012) when compared to baseline levels. When used for a prolonged period of time they have also been shown to improve popliteal artery flow by 3-36% (Delis *et al.* 2000; Delis *et al.* 2005).

Electrical stimulation of the gastrocnemius has been shown to increase superficial femoral artery flow by 140%, although there were a wide range of flow changes in the small cohort study (Abraham *et al.* 2013).

Walking distances

There were a wide variety of protocols, however over the course of 4.5 - 6 months IPC was shown to increase ICD (97 - 150%) and AWD (84 - 112%) by clinically significant distances, with the benefits persisting over 12 months (Delis *et al.* 2000; Delis *et al.* 2000; Delis *et al.* 2005; Ramaswami *et al.* 2005).

External compression of the calf achieved an increase of 85% and 76% in ICD and AWD, which was significant when compared to negative controls, and sustained at 3 months (de Haro *et al.* 2010).

NMES of the calf muscles, using the Medicompex, increased ICD and AWD by 82% and 61-150% over 4 weeks (Hudlicka *et al.* 1994; Anderson *et al.* 2004), and 26% and 34% over 8 weeks (Tsang *et al.* 1994). These trials were small, but results were significant when compared to a sham device group.

Quality of life

Delis et al showed an increase in quality of life at 5 months with IPC therapy, whilst the best medical therapy groups scores were unchanged (Delis *et al.* 2000). These gains were sustained at 12 months post-treatment.

4.3.2.3 Critical limb ischaemia

Pain

IPC reduced ischaemic rest pain in 40-100% of trial subjects reported in the literature (Louridas *et al.* 2002; Sultan *et al.* 2011).

One trial evaluated the effect of high frequency TENS on the relief of laboratory-induced tourniquet ischaemic pain (Seenan *et al.* 2012). A blood pressure cuff inflated to 200 mmHg produced pain on heel-raising against a 40kg backpack. The blinded use of the TENS machine delayed the onset of pain, reduced pain levels, and increased endurance of pain over a period of several minutes. This was significant when compared to sham-placebo. However, the feasibility of clinical use of TENS in peripheral arterial disease does not appear to have been explored.

Wound healing

IPC treatment was shown to have ulcer healing rates of 26% (Louridas *et al.* 2002). Vella et al offer a 79% "favourable outcome" at 40 days, a loose term which covers decreased ulcer size, complete healing, or improved sufficiently to allow revascularisation (Vella *et al.* 2000). Blood flux to the skin of the foot, as evidenced by laser Doppler, was increased significantly over 5 IPC cycles in a cohort of 20 subjects (Labropoulos *et al.* 2005).

Limb salvage

IPC had an early limb salvage rate of 58-83% at 1-3 months, and 58-94% at 1.5 - 3.5 years (Vella *et al.* 2000; Louridas *et al.* 2002; Kavros *et al.* 2008; Sultan *et al.* 2011). Beirne et al quoted an amputation rate of 3% at 18 months, and a mortality rate of 21%, but do not compare to controls (Beirne C 2009).

No studies have reported the use of EMS or NMES in the management of CLI.

Post revascularisation

Two IPC devices have been investigated for their utility in circulatory support after surgical revascularisation. Application to the foot for seven days after femorodistal bypass in 5 subjects led to reduced swelling and increased haemodynamic parameters in the femoral and popliteal vessels over controls not treated with IPC (White *et al.* 1996). No numerical data to support this is given, and conflicts of interest have not been declared. The 2002 paper looks at grafted arteriopaths (femoropopliteal and femorodistal bypass) approximately 18 months post-operation, and shows that a single treatment with IPC increases foot skin blood flux from baseline equally to controls, during device operation (Delis *et al.* 2002).

Galvanic stimulation – a special mention

There were few published clinical studies using galvanic electrical stimulation in peripheral arterial disease. Peter et al investigated subjects who had impaired peripheral perfusion and microvascular insufficiency, as measured by laser Doppler and transcutaneous oximeter (Peters *et al.* 1998). He demonstrated an increase in skin blood flux and oxygen tension in 11 diabetics with impaired microcirculation after five minutes of stimulation, which dropped back to baseline after this. Another study of 40 randomised and controlled subjects looked at the healing of diabetic neuropathic foot ulcers when subjected to a nocturnal program of galvanic skin stimulation over 12 weeks (Peters *et al.* 2001). There was no significant difference in wound healing rate, time to complete healing, or limb salvage between test and control groups.

4.3.3 Discussion

There is evidence to support the use of IPC in the management of intermittent claudication, with clinically important changes in treadmill distances seen with regular use. The greatest volume of published supporting research lies behind the ArtAssist device. The benefit of linking compressions to cardiac cycle, such as with the Circulator Boot, has not been shown to be clinically important at this point in time.

Numerous mechanisms have been postulated for the increase in blood flow after IPC therapy, none of which completely explain the haemodynamic changes in isolation (Delis *et al.* 2005). The interplay of these systems also has a mild systemic effect, which varies according to IPC anatomical site, compression value and duration (Fanelli *et al.* 2008). In healthy subjects, IPC has been shown to cause minor increases in mean arterial pressure, and moderate reduction of cardiac output and heart rate. It remains to be seen if long term IPC use results in angiogenesis in the same way that exercise training is seen to act. Increases in pressure

gradient and therefore flow and velocity around the arterial stenosis may stimulate arterial collateralisation, or fibrinolysis of local clot. Experimental cell culture flow models have shown IPC conditions up-regulation of tissue plasminogen activator and nitric oxide synthase RNA expression (Dai *et al.* 2000). The effect of IPC on plaque biology has not been investigated.

The IMC device has also been shown to have a positive effect on claudicants, used for 2 hours per day, for 3 months; although the sum total volume of supporting evidence is one small trial (de Haro *et al.* 2010).

The use of electrical devices to treat limb pain is not new, and can be traced back to the times of Franklin, Rutherford, Galvani and Hunter (Cambridge 1977). These devices have evolved significantly since their initial introduction as a simple pair of electrodes attached to a generator box, with significant safety concerns – they had been known to cause burns at the interface site, or explode in the operating theatre. They are decreasing in size and cost, some are portable and do not interfere with mobilisation. The manufacturers of NMES state that their device is more tolerable that EMS, and simple bench testing in our laboratory does support this. No published studies in our literature review reported on this.

This review suggests that long term EMS or NMES can positively affect clinical parameters of symptomatic peripheral arterial disease (Curry 1993; Tsang *et al.* 1994; Anderson *et al.* 2004). The similarity to results of exercise training in claudicants can be commented on, but whether this is due to the effect of increased vascular circulation, release of systemic factors that affect general cardiovascular health, or an effect on neuromuscular innervation, remains to be seen. There is plenty of evidence in animal models that changes in muscle stimulation can affect muscle fibre differentiation, although this has not been replicated in adult humans (Buller *et al.* 1960). Nerve cross-union experiments have shown the ability of fast and slow twitch

muscles to change their contraction times and metabolic activity, according to changes in innervation (Buller *et al.* 1960; Pette *et al.* 1999). Forst et al showed a positive effect on diabetic microvascular disease, with NMES able to increase foot temperature, but this was blunted in cases of peripheral neuropathy (Forst *et al.*). It would be interesting if trials using direct muscle stimulators, such as the Veinoplus, could replicate the results of NMES in peripheral arterial disease.

The duration and length of NMES protocol remains contentious, ranging from one 30-min stimulation session per day (Bosi *et al.* 2013), to multiple 20-min session per day (Bogachev *et al.* 2011), to 90-mins a week (Petrofsky *et al.* 2010), to continuous 24-hour usage (Broderick *et al.* 2010). Despite different stimulation methodology, results in these small studies do appear to be consistently beneficial (Curry 1993). There is no general consensus as to an ideal protocol, which may be best explored in a combination of laboratory and clinical trials.

It remains to be seen how effective long term NMES is for treating symptomatic intermittent claudication, and if this is equivalent to supervised exercise training, or a useful adjunct. High level evidence will only be provided by an adequately powered comparative clinical trial.

TENS machines have been used in the management of pain, and are thought to work through a gating mechanism, effectively switching off downstream pain signals by providing an alternative stimulus. This can be used in the treatment of pain symptoms of any cause, and do appear to be effective in the short-term treatment of ischaemic pain. Conclusions beyond this are not supported by the literature.

There is little evidence to support the use of galvanic stimulation in this patient cohort.

There are many devices that are clinically relevant to the vascular specialist managing patients with peripheral arterial disease. A working knowledge of available devices, especially IPC and NMES, expands the range of therapies available for management of symptomatic disease, and may be of special benefit to those with limited exercise capacity. The use of IPC in non-reconstructable critical limb ischaemia is particularly useful for limb salvage.

4.4 Literature review: Diabetes and NMES

Diabetes is a disease of endocrine dysfunction with systemic effects on nerves, blood vessels and the immune system. There has been press recently regarding the efficacy of exercise on the symptoms of diabetic neuropathy, and it might be postulated that NMES may be helpful in this regard(Yoo *et al.* 2015). A systematic review was performed, looking at exercise interventions in diabetes, and how these might pertain to electrical stimulation in this population.

4.4.1 Methods

Medline and Embase databases were interrogated on the 22nd July 2015. The search string used was: ("diabet*" NOT "drug*") AND ("exercis*" OR "electric*") AND "function*" AND "clinical trial", limited to full-text articles in English pertaining to humans.

Eligibility criteria are listed in Table 5.

Table 5 - Eligibility criteria for literature search evaluating neuromuscular stimulation in the management of diabetes

Inclusion	Papers concerning diabetic subjects Diabetes definitions conforming to WHO standard Trials in human adults Controlled trials Intervention administered is either exercise or electrical stimulation Endpoints with functionally relevant endpoints
Exclusion	Case series, case studies, posters, conference proceedings, non-peer reviewed data Unclear methodology (e.g. allocation to groups, control method, blinding) Data pertaining to children Diabetic preventative strategies Drug trials

4.4.2 Results

After deduplication 260 records were returned, of which 19 papers were included in the qualitative analysis. A PRISMA diagram is presented in Figure 15.

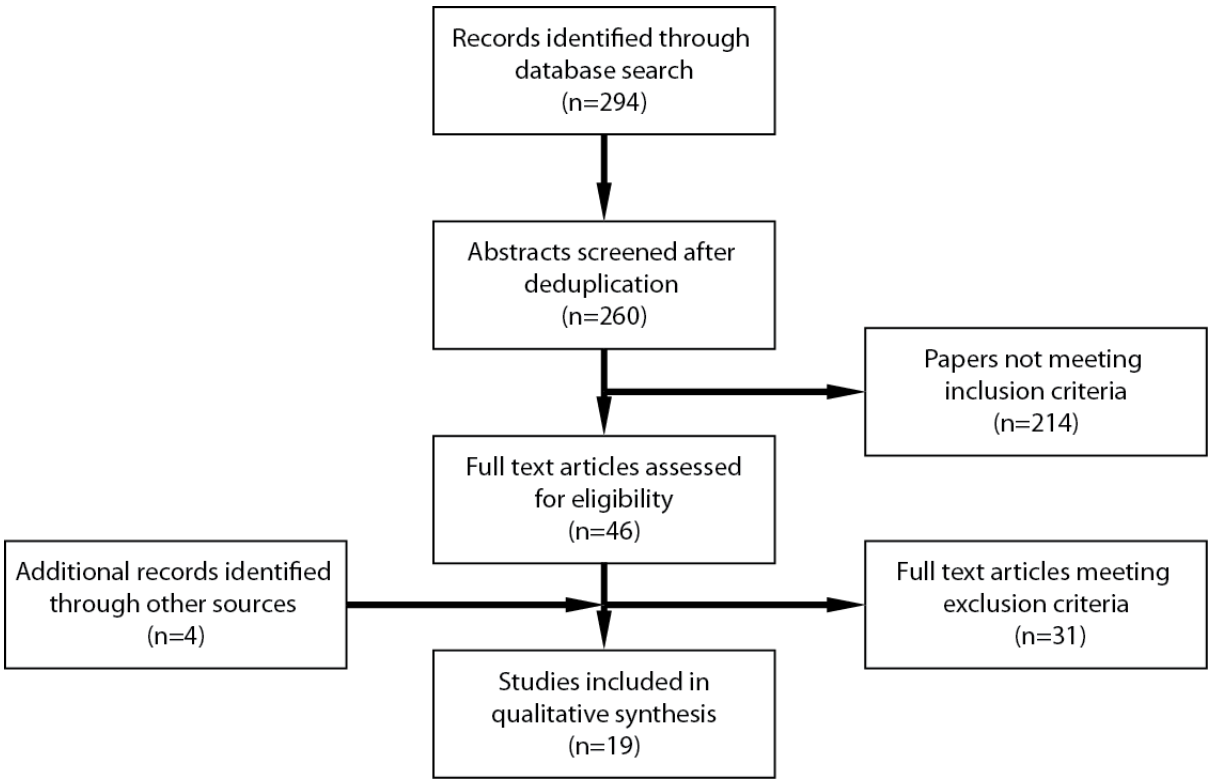


Figure 15 - PRISMA diagram of systematic review for the management of diabetic peripheral neuropathy with exercise or electricity

Results were divided according to intervention, which fell into two natural groups. Those pertaining to the use of electrical devices are presented in the appendices (Section 10.3).

4.4.2.1 Electricity

Population

Three papers investigated the use of electricity in diabetic populations, for the management of diabetic peripheral neuropathy (DPN) (Khalil *et al.* 2007; Moharic *et al.* 2010; Bosi *et al.* 2013). All groups tried to eliminate heterogeneity by isolating diabetics with minimal co-morbidities, resulting in exclusion of large numbers with ischaemic heart disease, foot ulcers, and cardiac pacemakers.

Protocol

TENS was used in two studies, a frequency-modulated TENS in the third (the functional and clinical importance of this technical variation is not clear). All devices used were operated via two electrodes placed on the leg, although Bosi did not specify exact anatomical positioning. Electrical stimulation was sensory only, none of the devices caused muscle contraction. Khalil and Moharic both stimulated unilaterally, and measured outcomes ipsilaterally. Bosi and Khalil used control groups exposed to a "no-stimulation" placebo, whilst Moharic used a case-series. Stimulation protocols ranged from 5 minutes to 3 hours, and were performed once or twice a day for a total of 3 weeks to 3 months.

Outcome measures ranged from electrophysical (nerve conduction) to functional (temperature and pain threshold testing, pain scores) and are clinically important markers of nerve function.

Outcomes

No studies were able to show changes in nerve conduction with the use of TENS, either from baseline or compared to controls. Bosi et al showed a reduction in night pain during the trial, which regressed back to baseline after protocol cessation. Moharic demonstrated

improvements in temperature thresholds over 3 weeks, which persisted for at least one month. The threshold changes reported by Bosi were not statistically significant.

4.4.2.2 Exercise

Sixteen studies examining the changes to diabetic participants with exercise were critically analysed (Richardson *et al.* 2001; Maiorana *et al.* 2002; Malhotra *et al.* 2002; Balducci *et al.* 2006; Middlebrooke *et al.* 2006; Hordern *et al.* 2008; Wycherley *et al.* 2008; Cheung *et al.* 2009; Hordern *et al.* 2009; Allet *et al.* 2010; Hey-Mogensen *et al.* 2010; Madden *et al.* 2010; Morton *et al.* 2010; Kluding *et al.* 2012; Allen *et al.* 2014; Taveggia *et al.* 2014). For the purposes of tabulation they have been separated out into groups where studies concerned chiefly functional reserve (or physical fitness), nerve function, and vessel function (Table 56).

Population

Study populations were generally selected in small numbers, and avoiding high risk subjects (smokers, hypertension, end-organ dysfunction of the eyes, heart or kidneys, those with foot ulcers, and those with significant peripheral vascular disease or stroke). The clinical significance of the data is therefore limited in its generalisability. There was a paucity of powered studies, and the majority of group sizes varied from 10-30. There was only one large study of over 220 participants (Hordern *et al.* 2009).

Protocol

Protocols applied were generally a mixture of aerobic and resistance exercises, often personalised using 50-80% heart rate or $VO2_{max}$ capacity. This seems to be a standard in the literature studied. Some studies used novel therapies such as asanas yoga (Malhotra *et al.*

2002), home-based Theraband^{TM} stretching exercises (Cheung *et al.* 2009), and balance/gait training (Richardson *et al.* 2001; Allet *et al.* 2010). On the whole appropriate controls were used, where no changes were made to subject activities. Exceptions to this were Madden and Teveggia, who stated that controls were given a programme of undefined "less strenuous" exercises (Madden *et al.* 2010).

Protocol length ranged from 3 weeks to 4 years, with significant methodological differences in frequency (1/week to 5/week) and exercise episode length (30mins to 150mins).

Outcome measures included anthropomorphic measures such as height, weight and BMI, skin fold thickness, and waist circumference. Blood tests for HbA1c, lipid profile and fasting glucose were common. Specialist tests included nerve conduction studies, exercise ABPI, treadmill tests, ECG changes, and exercise capacity (VO2max). One study looked at vascular function by taking blood samples for plasma nitrate and other markers of endothelial function (Allen *et al.* 2014).

Outcomes

Seven studies reported observed changes to physical fitness, exercise capacity, or stamina (Maiorana *et al.* 2002; Hordern *et al.* 2008; Cheung *et al.* 2009; Allet *et al.* 2010; Hey-Mogensen *et al.* 2010; Morton *et al.* 2010; Taveggia *et al.* 2014). In four weeks (5 hours per week, treadmill and strength exercises), diabetic neuropaths did not show any improvement in objective physical tests compared to controls, although exercise stamina did improve (Taveggia *et al.* 2014). Given longer periods of exercise (8-12 weeks, treadmill or circuits) resting heart rates was shown to fall, VO2max increase by approximately 5-7% ($p<0.05$ compared to controls) (Maiorana *et al.* 2002; Morton *et al.* 2010), and muscle oxidative function increase by an average of 63% (Maiorana *et al.* 2002; Hey-Mogensen *et al.* 2010).

Time to exhaustion on treadmill testing was increased by 50% compared to non-exercise controls (Morton *et al.* 2010). No differences in pancreatic function or insulin resistance between groups was observed at the end of the trial period (Hordern *et al.* 2008). Some interventions were shown to be equivalent to controls. Unsupervised Theraband™ prescribed exercise had no additive effect on functional movement, quality of life or HbA1c (Cheung *et al.* 2009).

Five studies used endpoints specific to peripheral neuropathy (Richardson *et al.* 2001; Malhotra *et al.* 2002; Balducci *et al.* 2006; Madden *et al.* 2010; Kluding *et al.* 2012). The longest study was conducted over 4 years, in diabetics with no symptoms of neuropathy at baseline. Using a protocol of supervised treadmill walking prescribed according to heart rate (50-80% reserve), they report excellent compliance, with greater than 90% attendance to classes. It is reported that there were significant differences between exercise intervention group and no-exercise controls in both objective nerve conduction studies (peroneal and sural nerves), and the development of clinical symptoms of neuropathy (30% versus 7%). 10-12 weeks appeared to have significant influences on cardiovascular autonomic function, neuropathic pain, and quality of life. Against this, objective nerve conduction studies over this shorter period failed to reach statistical significance, however Kluding et al report significant signs of nerve growth evident in skin biopsy compared to baseline (there were no controls used for this study). One to three weeks of light exercises such as yoga and balance exercises failed to show any improvements in conduction velocities, and are vague in their reporting of functional scores (Richardson *et al.* 2001; Malhotra *et al.* 2002).

Four studies looked specifically at vascular and endothelial function in diabetic subjects having undergone a programme of exercise (Middlebrooke *et al.* 2006; Wycherley *et al.* 2008;

Hordern *et al.* 2009; Allen *et al.* 2014). None managed to show any appreciable improvement in myocardial contractility, endothelial function or microcirculatory response to stimuli, even with 6-months exercise training. Allen et al comment that the beneficial effects of exercise on the vascular system of claudicants are attenuated in diabetics compared to non-diabetics (they used non-diabetic exercised controls) (Allen *et al.* 2014).

4.4.3 Discussion

From the literature review it appears that no reported study has ever tried utilising neuromuscular stimulation for the benefit of the diabetic population, and there are areas where it may prove useful. Limited conclusions may be drawn from experiments using TENS, as a therapy that makes muscles contract is likely to have different physiological effects to that which just activates sensory nerves. It is questionable whether a true negative control could ever be applied in such circumstances, and derived benefit is subject to a large placebo effect. The sensation of electrical stimulation may have beneficial effects on experience of pain, whether by a gating mechanism (now out of favour with neurophysiologists) or by a more psychological moderating influence (Syrjala *et al.* 1995). Kluding et al's results indicate that it may be useful particularly in those experiencing extreme pain, and pain specifically at night. The positive effect on thermal thresholds surely bears some further investigation, especially considering the frequency with which diabetics are subject to thermal injury (erythema ab igne, scalds from bath water).

The results show that it takes as little as 4 weeks of exercise to improve muscle control, gait, and stamina, but what is interesting is that this includes not only those protocols that used heavy aerobic activity. Protocols of balance and stretching exercise, even yoga, caused reported functional improvements. Is this purely a muscle strength or coordination effect, or

are there more factors involved here, such as muscle activity, or increased neuromuscular junction activity? A trial comparing sedentary neuromuscular stimulation to exercise would be very interesting, especially if end-points looking at neurochemical activity could be measured practically. Currently non-invasive technology to assess this is not available.

The clinical importance of the lack of endothelial response to exercise in diabetics is unknown, and whilst it may offer insights into underlying biochemical and physiological mechanisms, it offers no guidance into extrapolating the effect of electrical stimulation on macro- and micro-vascular function. Ultimately, functional and quality of life differences will matter more to both patients and physicians when evaluating potential therapies.

It has been shown in the management of claudicants that supervised exercise is more effective than unsupervised and home-based exercise (Cheetham *et al.* 2002; Cheetham *et al.* 2004; Fokkenrood *et al.* 2013) in terms of increasing claudication and absolute walking distance, as well as quality of life changes. However the cost of facilitating supervised exercise classes has precluded their uptake by physicians and surgeons (Shalhoub *et al.* 2009). In the case of symptomatic diabetes, it is clear that a sustained period of exercise is beneficial in terms of exercise capacity and functional movement. Would neuromuscular stimulation possibly offer similar benefits to exercise, and would attaching a device to someone for a few unsupervised hours a week be more cost-effective than running classes? Practically you would need one visit for device familiarisation and supply, a telephone contact for any queries, and outpatient appointments at appropriate intervals to make a scheme work. Compare this to a weekly meeting with facilities costs, the time of qualified staff such as specialist nurses and physios, and the cost of equipment hire and maintenance, and one may start to see the cost-benefit implications.

In the literature studied a large group of diabetic subjects were excluded, namely those with diabetic foot ulcers. It has been made clear in the introduction what a large problem this is for patients and the health care system, taking massive resources to manage. They are also at massive risk of life threatening infection, necrosis, and loss of limb or life. If an intervention were found to improve the healing rates of ulcers, this is a population with which it would make a huge difference. If neuromuscular stimulation is found to improve circulation in diabetics, a trial looking at foot ulcer healing would be of large clinical importance.

5 End-point Measurement

The evaluation of specific endpoints in a scientific trial need to be specific and relevant to the cohort investigated. Measures need to incorporate both objective and subjective measures of health, comfort and well-being, as well as measure relevant physiological changes. Changes to disease-models can be recognised using a mixture of objective physiological measurements, functional changes, and quality of life assessments. Here I will define the measures used in the clinical testing, with validation studies where appropriate.

Section		Page
5.1	History and examination	125
5.2	Quality of life questionnaires	125
5.3	Functional assessment	131
5.4	Tissue oxygenation	142
5.5	Wound healing	151
5.6	Blood-flow assessment	160
5.7	Lower limb oedema	170

5.1 History and examination

Where appropriate to establish or confirm a clinical diagnosis, a structured and targeted medical history and examination of appropriate organ systems was performed by an appropriately trained medical staff member.

5.2 Quality of life questionnaires

Biological and physiological testing can convey information about biological disease processes, however there may be poor correlation between these measures and the functional impairment of the patient in a community setting. Questionnaires that encompass both disease-specific and global aspects of well-being are essential measures of disease impact on an individual.

5.2.1 Generic

Measurements of the effects of disease on physical, social, and role functioning provide a comprehensive insight into the degree of disability experienced by the patient as a result of the disease.

5.2.1.1 Short Form 12

The Medical Outcomes Study (MOS SF-36) questionnaire consists of 36 questions, and pertains to evaluate physical function and general health perceptions as well as limitations due to mental health, social function, and vitality (Ware *et al.* 1992). Several areas are interrogated – physical and mental limitations due to either health or mental state, activities

of daily living, pain, general mental health, vitality, and general health perception. It has been designed for use in the general populations of both the USA and UK, as well as others. Validated in most European countries, it correlates with other measures of health in various different disease states: outcomes pertaining to neurological disease, stroke, arthritis, HIV, diabetes, cardiac rehabilitation, and post-operative recovery following colorectal or orthopaedic intervention (Anderson *et al.* 1996; Ahroni *et al.* 2000; Arpinelli *et al.* 2000; Brown 2003; Anderson *et al.* 2009; Antonescu *et al.* 2014; Awdeh *et al.* 2015). Internal consistency is reportedly good, with Cronbach's α >0.8 in all domains (Jenkinson *et al.* 1994). A truncated form of the questionnaire, the Short Form-12 has been validated for use in health care evaluation (Ware *et al.* 1996). It can reliably predict component SF-36 scores with Spearman correlation coefficients >0.9. Health summary measures can be split into physical and mental components (PCS-12, and MCS-12 respectively). They have a test-retest (2 weeks) ICC of 0.76 and 0.89.

5.2.1.2 EuroQol-5D

Taken with the Short Form questionnaires mentioned above, the EQ-5D is the other most widely used generic measurement instrument of health used internationally. One of this questionnaires great strengths is the ability to use a country-appropriate dataset, applicable to the studied population. For each health domain evaluated, a score between 0 (dead) and 1 (full health) is given, which can be used to estimate quality-adjusted life years (QALYs)(Devlin *et al.* 2013).

5.2.2 Depression

Depression has been well-documented to co-exist, and perhaps exacerbate, physical symptoms and disease prognosis (Musselman *et al.* 1998; Lustman *et al.* 2000; Musselman *et al.* 2003; Shimbo *et al.* 2005). It is therefore an important outcome-measure when looking at disease modification in the context of clinical trials.

5.2.2.1 Centre for Epidemiologic Studies Depression Scale (CES-D)

The CES-D was created by Radloff et al in 1977, and revised in 2004 (Radloff 1977; Eaton WW 2004). The CES-D has been the standard of depression epidemiology reporting in the western world since the 1970's, and has been validated in both general and specific populations (including the elderly, those on a low-income, cases of child abuse, and cancer patients) (Eaton *et al.* 1981; Orme *et al.* 1986; Hann *et al.* 1999; Thomas *et al.* 2001).

5.2.3 Venous disease

5.2.3.1 Aberdeen Varicose Vein Score

The clinical and anatomical status of venous disease is often disconnected from symptomatology (Guyatt *et al.* 1993; Bradbury *et al.* 1999; Bradbury *et al.* 2000). The Aberdeen Varicose Vein Score (AVVQ) is a patient-reported survey assessing multiple elements of varicose vein disease (Garratt *et al.* 1993; Garratt *et al.* 1996). It asks the patient to rate symptoms experienced over the last two-weeks, and is scored from 0 (asymptomatic) to 100 (severe symptoms).

The questionnaire rates physical symptoms (pain, pedal oedema, and ulcer presence), cosmesis-related problems, use of compression hosiery, and impact on patient social life.

5.2.3.2 Venous Clinical Severity Score

The Venous Clinical Severity Score (VCSS) is an assessment tool completed by the treating physician, ratified in 2010 by an international working group of the American Venous Forum (Vasquez *et al.* 2010). It consists of sections covering specific symptom areas common to venous disease. This includes pain, varicose veins presence, pedal oedema, skin pigmentation, inflammation, induration, use of compression hosiery, and presence or severity of foot ulcers. Each section is marked on a severity scale of 0 to 3.

The clinical and research value of the VCSS is its ease of use, along with a weighting towards severe manifestations of venous disease. Vasquez et al report that this weighting renders it most sensitive to clinical changes over time, allowing accurate tracking and quantification of improvement (Vasquez *et al.* 2012).

5.2.3.3 Venous Disability Score

This is a three point score introduced in 1996 by venous consensus committee (Rutherford *et al.* 2000). An additional clinician scoring system, it bluntly evaluates disease-related effects on employment and functional activity, and classifies patients into one of four categories. It is intended as an adjunct to CEAP and VCSS to provide an over-all picture of the patient.

Using the scale, asymptomatic patients score 0. Patients who are symptomatic but able to complete normal activities without compression score 1. Those dependant on compression to complete daily activities score 2, and incapacitated patients score 3.

As stated by Sritharan et al, this instrument is easy to use, but relatively insensate to change in clinical status (Sritharan *et al.* 2012).

5.2.4 Arterial disease

5.2.4.1 Intermittent Claudication Questionnaire

The Intermittent Claudication Questionnaire (ICQ) is a validated 16-item self-administered instrument that measures patient-reported health outcomes related to typical PAD symptomatology. It is highly consistent, with Cronbach's α value of 0.94, and an ICC at weeks 0 and 2 of 0.95 ($p<0.01$) (Chong *et al.* 2002). Test scores correlate well with absolute walking distance.

5.2.5 Diabetes

5.2.5.1 Problem Areas in Diabetes

The Problem Areas in Diabetes (PAID) questionnaire is a self-administered tool that measures the effect size of common causes of diabetes-related emotional distress (Welch *et al.* 1997). 20 areas are covered, and are scored from 0 (not a problem) to 4 (serious problem). The sum of the total score is then multiplied to give a final score out of 100.

Internal reliability has been shown to be high (Cronbach's α 0.95). Responses are relatively sensitive to clinical severity changes, and scores in diabetics correlate with both glycaemic control and other diabetic quality of life scores (Welch *et al.* 1997; Welch *et al.* 2003; Reddy *et al.* 2013) .

5.2.5.2 Diabetes Quality of Life

The Diabetes Quality of Life (DQOL) tool was specifically developed as an outpatient tool (Group 1988; Burroughs *et al.* 2004). Specific routine and clinically important items are addressed that directly influence treatment options, measurement of adherence, and patient

satisfaction. It has been validated in patients with both diabetes type I and II. The overall score can be broken down into three subsets: treatment satisfaction, treatment impact on patient life, and patient anxiety. Each question is marked out of 5 (1-no effect to 5-large effect), giving a total score between 46 and 230. Internal consistency is good, (Cronbach's α 0.92), and a good test-retest reliability (Pearson correlation coefficient r=0.92) (Group 1988).

5.2.5.3 Michigan Neuropathy Screening Instrument (MNSI)

The MNSI is a tool designed to screen for diabetic peripheral neuropathy (332). It is divided into two parts: the first is filled out by the patient and relates to specific symptoms of neuropathic pain, numbness and temperature thresholds. They are answered in a binary fashion (yes or no) and carry a maximum score of 13. The higher the score, the more severe the symptoms. The second part is physician-scored, and consists of physical examination with testing of ankle reflexes, microfilament sensation, and vibration sense. Scoring more than 2 points on the clinical scale (maximum 10) merits full neurological evaluation.

MNSI scores are considered by some to be reliable and accurate enough to diagnose DPN with no further diagnostic tests (Moghtaderi *et al.* 2006). A 2-year study of 176 diabetic patients, using an intern and a neurophysiologist to carry out the questionnaires and examination in a university teaching hospital. When MNSI scores cut-offs of 1.5, 2, 2.5, and 3.0 were used, it displayed 79%, 65%, 50% and 35% sensitivity for NCS confirmed DPN, and 65%, 83%, 91% and 94% specificity respectively.

5.3 Functional assessment

In the vascular cohort useful evaluation procedures must have adequate precision, clinical applicability or correlation, and reproducibility. Heterogeneity of functional status between patients can be a problem, as vascular disease can impair activities of daily living, mobility, social interaction, and mental well-being to different extents. This is dependant not only on the disease-process itself, but also complex psycho-social interplays in the life of the affected individual.

For that reason, a combination of functional assessments will be carried out in each pilot trial.

5.3.1 Treadmill distances

An important part of the clinical evaluation of peripheral arterial disease, particularly with reference to intermittent claudication, are walking distances. Treadmill testing is designed to obtain objective data on the functional exercise capacity of patients with claudication.

Constant-load exercise testing

Popularised by Carter, low-speed treadmill tests were introduced as part of clinical assessment of the PAD cohort in 1972 (Carter 1972; Laing *et al.* 1986). Typically these are of a fixed grade (0-12%) with a constant belt "walking-speed" of 1.5-2mph. The distance at which the patient first reports onset of claudication pain is termed the intermittent claudication distance (ICD), and the point at which the patient terminates the test due to untenable pain is termed the absolute walking distance (AWD). Coefficients of variation of 30-45% for both

measures have been reported, necessitating repeated testing to establish a true baseline (Laing *et al.* 1986).

There exists great heterogeneity of walking distances in the PAD population, which makes the grade of treadmill testing difficult to standardise. If the grade is too steep, early pain may make it impossible for the patient to demonstrate meaningful results. If the grade is set too low, chances are high that patients may terminate the test due to other co-morbidities, such as chronic respiratory problems. Upper and lower ranges for walking distance are necessarily enforced for the constant load exercise test, and walking distance that fall outside these limits are considered invalid. This is a particular problem in therapeutic trials, which tend to underestimate clinical improvement with effective therapies. International trials employing this method of assessment have excluded up to 20% of potential recruits as the treadmill protocols range of accuracy was not appropriate (Balsano *et al.* 1989; Lindgarde *et al.* 1989).

Graded exercise testing

Graded exercise testing was originally developed by Bruce et al, for the clinical assessment of cardiac patients (Bruce *et al.* 1973). Protocols were developed that allowed performance evaluations of both low- and high-performance patients. Workload is initiated at an easy level, and then incrementally increased until each patient reached a discernible, reproducible peak workload during a test of moderate duration. The full spectrum of disease severity is able to be assessed, and has been utilised in many clinical trials evaluating therapies such as exercise training, drug therapy, coronary angioplasty, and coronary bypass surgery(Lee *et al.* 1979; Hill *et al.* 1984; Parisi *et al.* 1992). Reported coefficients of variation for the ICD in 15-25%, and AWD 12-13% (Hiatt *et al.* 1988; Gardner *et al.* 1991). Gardner et al describe a study of claudicants where intraclass correlation coefficients for constant-load treadmill tests were

0.53 and 0.55 for ICD and AWD(Gardner *et al.* 1991). When a graded protocol was used, the ICCs increased to 0.89 and 0.93. The graded treadmill test has shown good acceptability by patients and physicians, and there is no need to exclude patients on the basis of age or ABPI (Hiatt *et al.* 1995; Gardner *et al.* 2007).

GARDNER PROTOCOL	HIATT PROTOCOL
2mph, +2%/2mins	2mph, +3.5%/3mins

Changes in exercise performance have been shown to correlate with improved ambulatory function in patients with PAD (Hiatt *et al.* 1990; Regensteiner *et al.* 1993). This association is important as it allows even small percentage changes to be interpreted as clinically relevant. As the workload of the test increases, the importance of a time improvement in walking distance is of greater significance, and is non-linear. Therefore percentage changes in fixed and graded protocols should not be directly compared.

5.3.2 Nerve function

Electrodiagnositic testing of nerves and muscles are frequently performed for diagnosis and monitoring of neuromuscular dysfunction. These include nerve conduction studies (NCS) which measure the properties of the nerves, and needle electromyography (EMG) for the properties of the muscle. In the appropriate clinical context, NCS can evaluate the exact conduction properties of peripheral nerves, and characterise disease of the different axon types, myelin sheath, or both.

5.3.2.1 Nerve conduction studies

Nerve conduction studies use electrical stimulation to evoke activity in nerves and muscles. From the response to the stimulus, evidence regarding the health of the nerves and muscles can be derived. A depolarising square wave is applied to a peripheral nerve producing a propagated nerve potential at a distant point over that nerve, and a compound muscle action potential produced from muscle fibre activation (Mallik *et al.* 2005). Electrodes used can be surface or needle, depending on the indication. Choice of stimulation and recording points depend on the suspected pathology.

Waveform

Typical studies present both motor and sensory conduction velocities, latencies, and amplitudes (Figure 16). Onset latency is measured from the time of stimulus to the first initiation of potential, and represents the fastest fibre conduction speed. Peak latency is measured at the peak amplitude, and represents the majority of conduction speeds. Both are affected by disorders or damage to the myelin sheath, interfering with salutatory conduction. Reduction of signal amplitude generally signifies axonal damage.

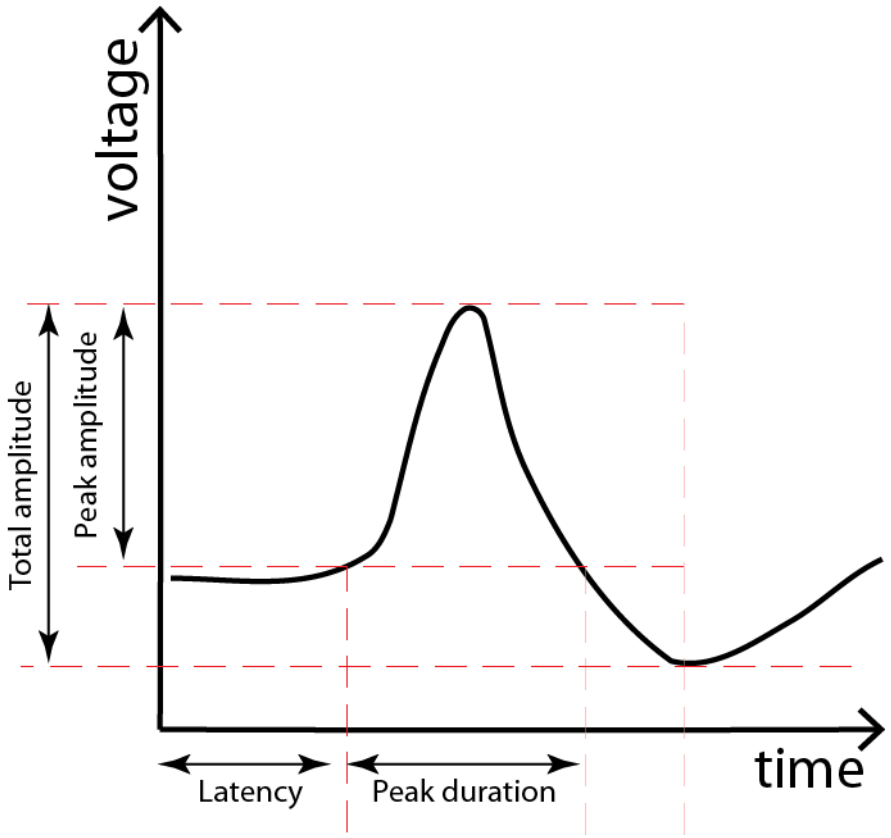


Figure 16 - Normal motor (median) nerve conduction waveform

Original diagram adapted from experimental work. The recording electrode was placed over adductor pollicis brevis, and the median nerve was stimulated proximally.

Sources of error

The two sources of error in nerve conduction studies are measurement of the nerve length between stimulation and recording, and waveform misidentification of waveform onset. Mallik et al estimate 92% error is due to time measurement, and 8% measurement of distance (Mallik *et al.* 2005). Accuracy is reported to be correlated to experience, and at our centre there is an experienced consultant neurophysiologist.

Clinical correlates

Distal symmetrical polyneuropathy is the most common form of diabetic neuropathy (Kakrani *et al.* 2014). Involvement of the tibial and sural nerves is more common in diabetic neuropathy compared to neuropathic subjects without diabetes. Polyneuropathy is associated with decreased conduction velocities, amplitudes, and increased latency (Kohara *et al.* 2000). Changes in these parameters correlate strongly with neuropathic pain onset and severity, functional deficits, and glycosylated haemoglobin levels (de Souza *et al.* 2015).

5.3.2.2 Sympathetic skin response

The conductance of the skin changes in response to the application of electricity, and has been used historically by neurophysiologists to explore human autonomic activity (Gutrecht 1994). The difference in potential between two points local to each other, in response to an unpleasant stimulus, is termed the sympathetic skin response (SSR). In health this has a typical waveform, but is heavily influenced by environment, temperature, hydration, and psychological priming (Toyokura 2012). Spinal transection will abolish SSR distal to the lesion (sympathetic chain is located at T1-T6 for upper limb, T8-12 for lower limb), as will local administration of atropine (Kumru *et al.* 2009).

Epidermal and peri-follicular sweat glands in the skin are innervated by sympathetic cholinergic neurons. Efferent sweat fibres originate in the hypothalamus, and descend ipsilaterally through the medulla and intermediolateral cell column neurons. The preganglionic fibres emerge from anterior spinal nerve roots to reach the sympathetic chain via the white rami communicantes. Unmyelinated postganglionic fibres emerge from the

sympathetic chain and travel peripherally with somatic nerves to innervate autonomic functionality in target organs. Release of stress hormones (catacholamines, adenosine triphosphate etc.) and activation of the sympathetic nervous system causes changes in the potential of the skin, as well as the production of sweat. These combine to give fast changes in the electrical properties of the skin, which can be measured electrophysically (Wilcott 1962; Knezevic *et al.* 1985). The outcome is used as a surrogate for sympathetic sudomotor function.

Recording

Recording of the SSR can be obtained from any part of the body with a skin surface. Common sites are the dorsum and palm of the hand, forehead, or genitals (Ertekin *et al.* 1987). It can be elicited via many different methods (touch, injection, painful pressure over a bony prominence, loud noise, bladder tap, magnetic stimulation are but a few). Generally, the more intense the stimulus, the greater the SSR recordable (Elie *et al.* 1990). Reliably repeatable and controlled stimuli can be applied electrically, and stimulation of the median nerve is used frequently. This is mainly due to the ease of application and delivery, but can be applied to the peroneal and pudendal nerves for successful effect (Park *et al.* 1988). When applying electrical shocks, it is recommended that this be applied contra laterally to the recording electrodes to avoid artefact. A square pulse of 10-30mA for 0.1-0.2ms is usually sufficient to evoke a response, but thresholds vary from person to person (Vetrugno *et al.* 2003). Delivery of stimuli at irregular intervals can help to combat habituation, and care must be taken to separate impulses by >40s to avoid immediate habituation effects. The subjects should be instructed to relax with their eyes shut, and loud noises should be minimised. Electrodes

should be placed on clean skin using appropriate gel-interface electrodes. Recorded waveforms can be separated for analysis, or summated.

Waveform of the SSR usually consists of positive and negative deflection phases, and can be bi- or tri-phasic (Figure 17). Amplitude is age-dependant, and responses in the feet tend to disappear with time before that in the hands (Drory *et al.* 1993).

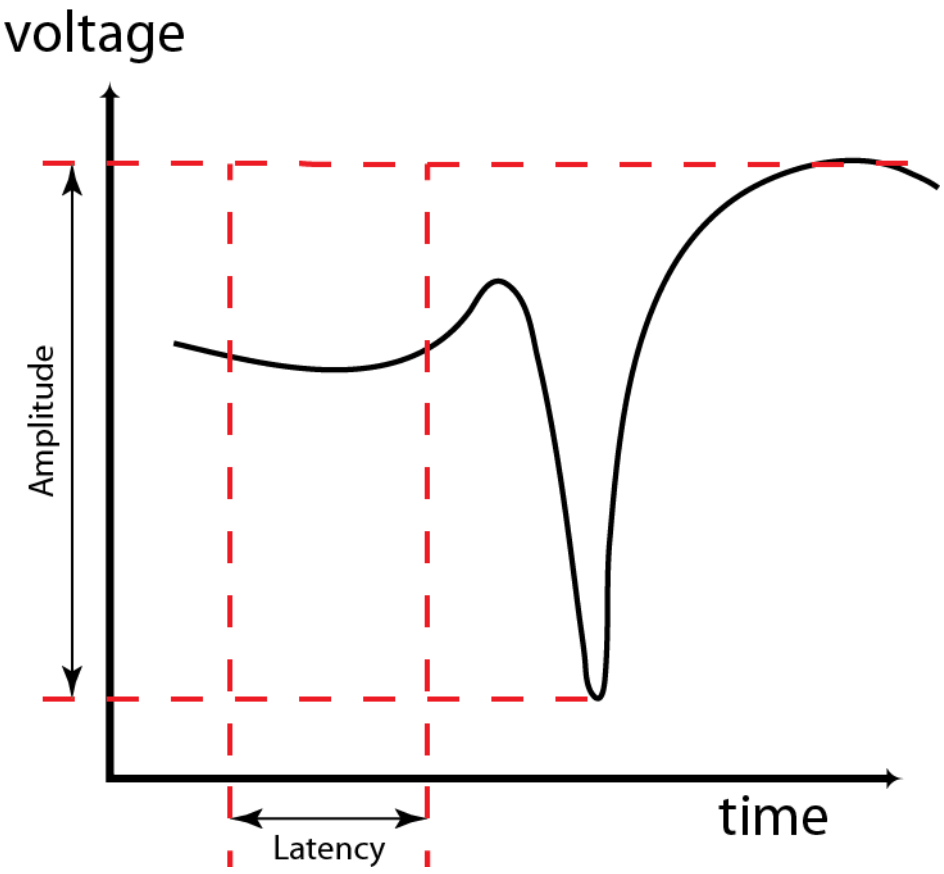


Figure 17 - Representative recording of the human sympathetic skin response

(latency and amplitude markers are shown)

The use of SSR in patient groups with neuronal dysfunction may be response-limited (Charcot-Marie-Tooth, alcoholics, chronic renal failure, lumbar sympathectomy patients, spinal injury, and scleroderma).

Clinical use

Soliven et al noted that absent SSRs are more common in subjects with autonomic neuropathies, and that this is more sensitive for the diagnosis of autonomic neuropathy than a Valsalva test or the presence of orthostatic hypotension (Soliven *et al.* 1987). SSRs are absent in two thirds of patients with diabetic peripheral neuropathy (DPN), which correlates with severity of axonal damage (Niakan *et al.* 1988; Watahiki *et al.* 1989; Dellantonio *et al.* 1992). SSR has high concordance with the quantitative sudomotor axon reflex test (Q-SART) in patients with DPN (Maselli *et al.* 1989).

Repeatability

Recorded waveform morphologies tend to vary between subjects, and can vary in the same subject from trial to trial. Toyokura et al have evaluated the consistency of SSR over time, and also looked at responses to differing stimuli in the same subjects (Toyokura 2012). When stimuli were applied at intervals of 40-60s, amplitudes decreased significantly over 4 recorded SSRs. This is termed habituation. Responses to auditory stimuli had significantly smaller amplitudes than those evoked electrically or magnetically, however statistically significant correlations of amplitude between modalities were found (Spearman correlation coefficient 0.4-0.5, $P<0.05$). The coefficient of variation for electrically evoked SSR was 68%.

5.3.3 Pain

Assessment of pain forms an important part of clinical care, but is very difficult to measure. On the surface, a negative reaction to pain and subsequent evasive behaviours might seem a common human and animal trait, and therefore it might follow that this reaction is well-preserved and directly translatable. However experimentally, it has been found that a painful stimulus to one subject may be non-noxious to another, and a change in context may change the reported pain experience completely (Leknes *et al.* 2013). Both laboratory and clinical models have been used to characterise the complex neurobiology of pain modulation. These approaches include placebo analgesia, nocebo hyperalgesia, hidden administration of analgesics, and the manipulation of the pain–reward relationship (Carlino *et al.* 2014). Pain is now popularly thought of as both a physical and emotional experience that is modulated by many factors. The biopsychosocial model describes how the subjective experience of pain is made up from a complex interaction between biological neurochemical and endocrine signals, and the psychological state of the subject (Engel 1981; Syrjala *et al.* 1995; Truchon 2001). This has been applied to many different situations (post-operative, trauma, lower back pain, cancer-related), but seems to be more apparent in experiments conducted with subjects suffering from chronic pain (Gatchel 2004). Person specific qualities such as gender, ethnicity, family factors, and even neonatal care experience can affect reported pain levels (Lipton *et al.* 1984; Grunau *et al.* 1994; Unruh 1996). There are many studies that have reported that incidence of chronic pain is linked to depression, and the presence of depression can perpetuate and exacerbate the pain felt by that subject (Jones *et al.* 2002). The Cochrane Collaboration have reported prevention of chronicity in the treatment of both short- and long-term pain using multidisciplinary biopsychosocial rehabilitation programs (Karjalainen *et al.* 2003).

Despite these difficulties, there are validated scoring systems for pain. These tend to focus on the immediate point in time, and can be compared in the same subject between points in time. The advantages and limitations of the tools used in this project will be discussed.

5.3.3.1 The Visual Analogue Score

The visual analogue score (VAS) for pain consists of a line drawn on an A4 page, divided into equal sections of one unit. It represents a continuum of pain from 0 (no pain) to 100 (worst imaginable pain). The subject is asked to mark on the line where they feel their pain today falls. Their score is then calculated as a mark out of 100.

The VAS pain score has been validated in both experimental and clinical pain scenarios to grade the intensity and unpleasantness of pain sensation, but cannot discriminate between the various dimensions of pain (Price *et al.* 1983). It is simple to administer, versatile, and relatively insensitive to bias effects (Huskisson 1974; Joyce *et al.* 1975). It is relatively sensitive to changes in pain intensity (Ohnhaus *et al.* 1975).

5.3.3.2 The Verbal Rating Score

The verbal rating score (VRS) is a 5-point discrete scale, consisting of a list of phrases describing increasing levels of pain intensity: no pain, mild pain, moderate pain, intense pain, maximum pain. This has been validated for assessment of unidimensional pain in emergency, peri-operative, and palliative settings (Hjermstad *et al.* 2011).

5.4 Tissue oxygenation

5.4.1 Laser doppler fluximetry

The measurement of skin microperfusion via laser Doppler fluximetry has been widely utilised in both research and clinical spaces for over 30 years.

The development of coherent light emitters, or lasers, in 1960 allowed the emission and manipulation of narrow beam and uniformly polarised optical radiation. The first functional laser was developed by Maiman(Maiman 1960), and it was demonstrated that the Doppler frequency-shift of backscattered light could be used to measure red cell velocity and concentration in vitro (Cummins *et al.* 1964; Riva *et al.* 1972). The first in vivo study was reported by Stern et al (Stern 1975), where He-Ne red laser light was used; the spectral shift of reflected light was manipulated to generate an electrical signal. The He-Ne has now been largely replaced by laser diodes. Real-time monitoring is made possible by displaying the root-mean-squared (RMS) of the photocurrent, and signal to noise ratio is improved by the usage of dual channel detectors in each optical lead (Nilsson *et al.* 1980; Bonner *et al.* 1981).

Wavelengths commonly used in commercial machine are around 780nm, which can penetrate ~2mm into the skin, and signal is largely unaffected by skin colour. The adsorption of light by both oxygenated and deoxygenated haemoglobin at this wavelength are almost equal, therefore any dependence on oxygen saturation is eliminated.

Laser Doppler Fluximetry is sensitive to changes in temperature, posture, respiratory pattern and emotional stimulation (Bircher *et al.* 1994). Movement artefact may be introduced by movement of the fibre-optic cables, and manifests as fluctuations of signal intensity. This noise has been demonstrated over a range of frequencies, and can be several orders of

magnitude greater than the perfusion signal (Newson *et al.* 1987). Ideally, it is generally accepted that laser doppler measurements should be conducted in a temperature controlled room, stabilized to ±1'C, with a 15 minute subject acclimatization period to the testing environment (Leahy *et al.* 1999). Subject limb movements and speech should be limited, and stimulants should be avoided in the pre-testing period. Standard measurement protocols should be implemented for experimental conditions.

Laser Doppler fluximetry units cannot be used in absolute terms, which makes widespread interpretation of results difficult. Single readings from any anatomical site are poorly reproducible when separated in time. However the variation in response to physiological stimuli are reproducible, and ratiometric or percentage changes are usually employed (Leahy *et al.* 1999). Calibration of the laser Doppler flux signal has been achieved in certain tissue-specific circumstances with techniques such as isotope wash-out, radioactive labelled microspheres, photo-plethysmography, hydrogen clearance and capillaroscopy. Preparations of feline small intestine and human bowel have shown good correlation between fluximetry signal and venous outflow (Ahn *et al.* 1984; Lindhagen *et al.* 1984). In clinical practice, a suspension of latex microspheres as a motility standard is used, based on two-point calibration.

The limitations of laser Doppler are that there are many factors which affect either the actual or measured perfusion of the microcirculation, and determining their relative importance is not easy. Limiting environmental and physiological factors aids accuracy, but the lack of widespread validation in different tissues hampers widespread interpretation and acceptability of results.

5.4.1.1 Laser Doppler generic protocol

Laser Doppler fluximetry and skin temperature measurements will be recorded from the dorsum of the hand and/or foot using the dual channel moorVMS-LDF (Moor Instruments, UK). Fibre-optic leads will be attached to the surface of the skin via transparent double-adhesive pads. Baseline measurements will be taken at rest, and then repeated after the cessation of 20 minutes device activation. Measurements during NMES device operation will be discounted as movement artefact is deemed to be large. Experimental conditions will be kept as constant as possible (temperature, humidity) by keeping the door shut, avoiding disturbances in the treatment room, and asking the subject to avoid stimulants (caffeine, sports drink etc.) in the hour preceding testing. Access to an environmentally controlled room is not financially viable at this point.

5.4.1.2 Experimental reliability

A Moor laser doppler was attached to the dorsum of foot of a healthy subject. They were allowed to rest for 10 minutes, then continuous measurements of fluximetry taken for 20minutes. Coefficient of variation was 8.7% (n=10) for repeat measurements of fluximetry performed on one trace. When comparing resting values for two periods separated by 24 hours, correlation was extremely poor (Spearman correlation coefficient 0.04, p=0.8). Bland-Altman analysis showed a mean error of 23% (confidence intervals -157 to 110). This is presented graphically in Figure 18.

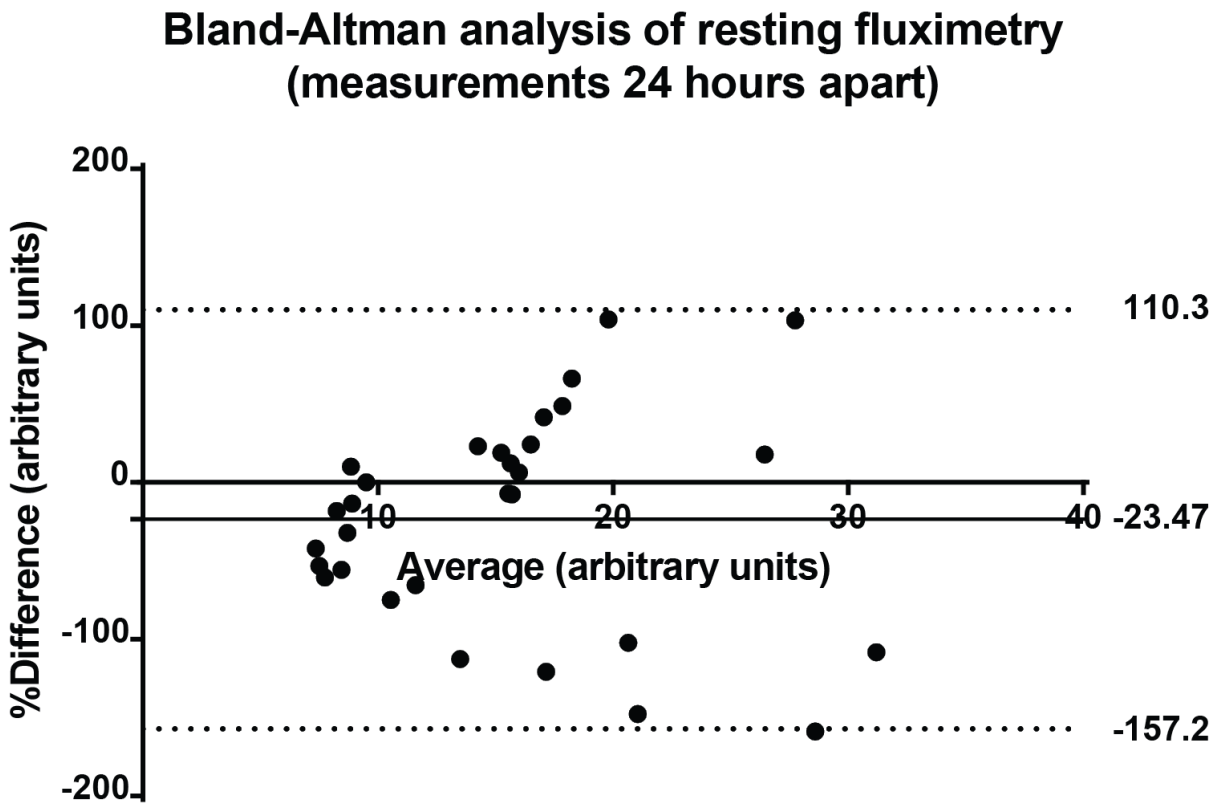


Figure 18 - Graph showing Bland-Altman analysis between repeat laser Doppler measurements taken from the dorsum of a foot of a healthy subject.

"Rest 1" and "rest 2" recording periods were separated by 24 hours. Line of mean error between readings, and 95% confidence intervals shown.

In order to compare changes from baseline values for a repeatable intervention, three subjects were asked to perform the above protocol, then 10 heel-toe manoeuvres. Laser doppler fluximetry recording was continued for 10 minutes, and measurements whilst subject was motionless were compared, in order to minimise movement artefact. One example trace is shown in Figure 19. Ten measurements for each subject were performed. This protocol was repeated again on all three subjects, 24 hours later.

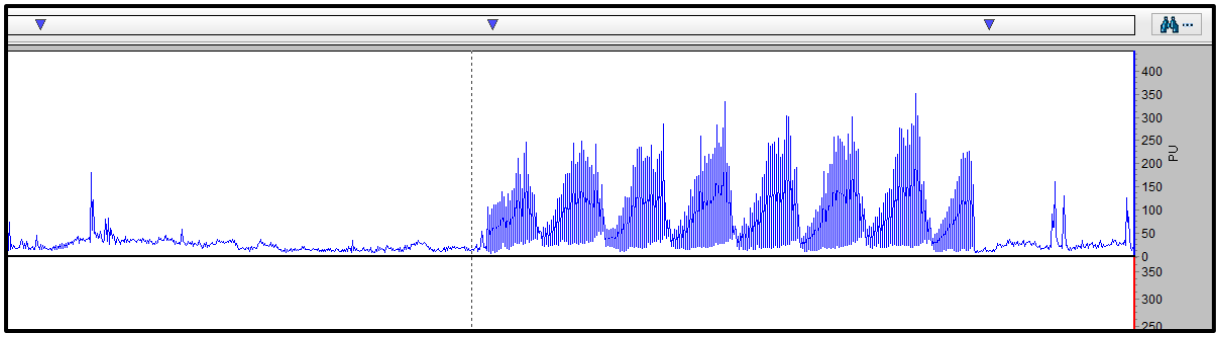


Figure 19 - Fluximetry trace over time, first phase at rest, and second phase during heel-toe exercises

Post-exercise measurements were taken immediately after cessation of exercise (marked by technician on the trace).

Changes in absolute values between rest and post-exercise are presented in Figure 20. Changes between phases were found to be statistically significant ($p<0.05$, Wilcoxon matched-pairs).

When fluximetry percentage changes (rest versus post-exercise) on day 1 and day 2 are plotted against each other, correlation was present but not strong (Spearman correlation coefficient 0.5, $p=0.005$). Bland-Altman analysis showed a mean bias of 121% (confidence intervals 60 to 303). This is presented graphically in Figure 21.

Measurements taken using laser doppler can give information about the red blood cell activity under the surface of the skin, and can discriminate between rest and exercise states. Error measurements can be large. Results separated in time are non-comparable and units can be treated as arbitrary. Percentage changes to baseline with intervention may be more reliable for comparison in the same subject.

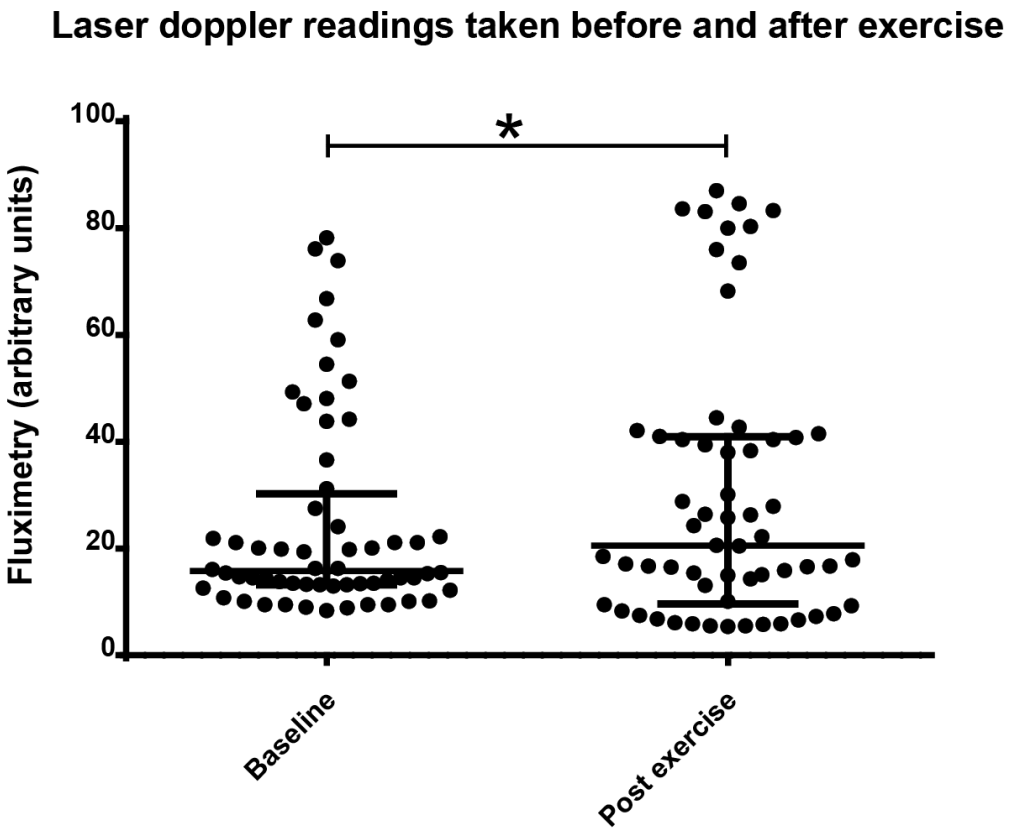


Figure 20 - Fluximetry readings taken at rest, and after exercise

(* – p<0.05, Wilcoxon matched-pairs)

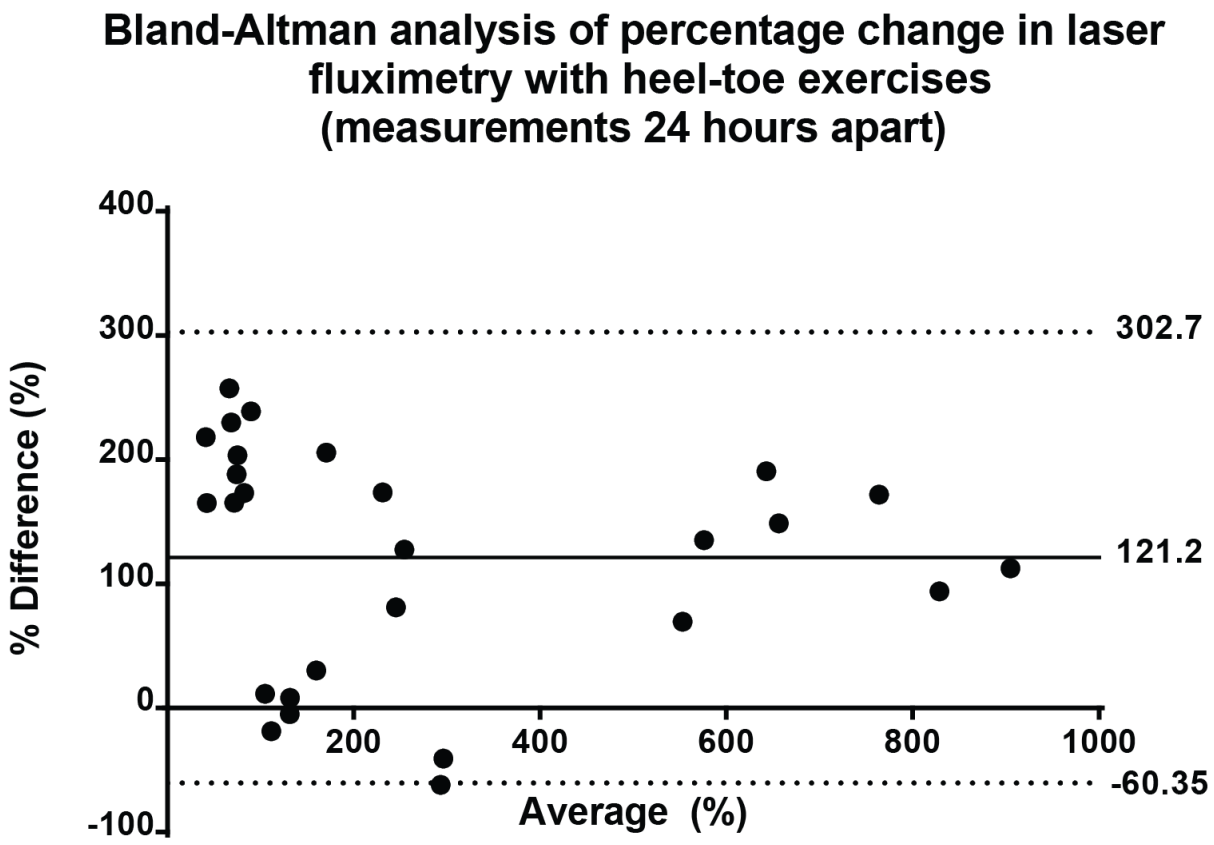


Figure 21 - Graph showing Bland-Altman analysis between laser doppler measurement percentage difference measurements taken 24 hours apart.

Mean error shown with solid line, 95% confidence intervals shown with dashed lines. The exercises performed were 10 heel-toe manoeuvres

5.4.3 Near-infrared spectroscopy

Near-infrared spectroscopy (NIRS) is a non-invasive imaging technique that is commonly used in research looking at tissue oxygenation (brain and muscle). Light in the spectrum of 650-950nm has the peculiar property of being strongly absorbed by haemoglobin, and has a penetration depth of up to 4cm in the human body (Arridge 1999). The adsorption spectra of haemoglobin changes according to oxygenation status, and according to wavelength of light applied (Figure 22). By shining transcutaneous light at multiple wavelengths into human tissue and measuring the reflected light, the relative levels of oxy- and deoxy-haemoglobin can be quantified (hence tissue oxygenation) (Gibson *et al.* 2005).

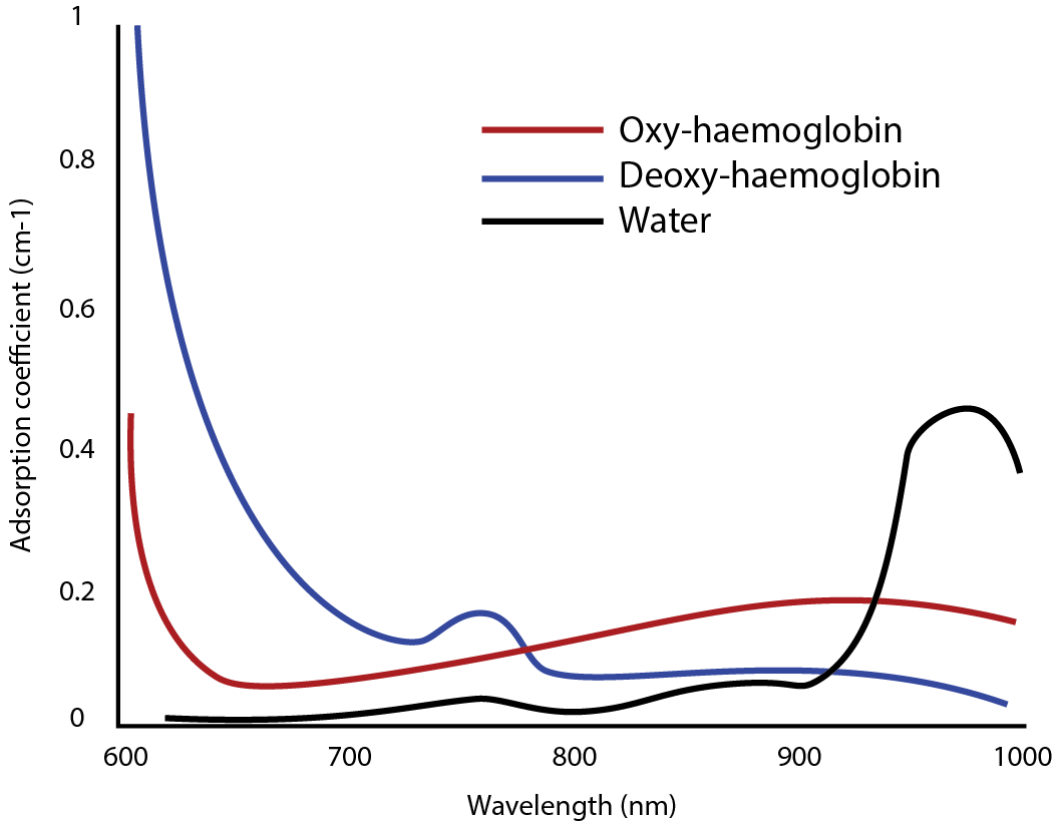


Figure 22 - The adsorption spectra of haemoglobin and water

Adapted from Phan et al - (Phan *et al.* 2010)

NIRS devices consist of a small emitting/receiving device and a recording device. Modern machines are able to communicate wirelessly, allowing measurements on any part of the body and during movement. This would be ideal to measure muscle oxygenation over time during exercise (Scholkmann *et al.* 2014). Komiyama et al have shown that a protocol of NIRS spectrographic measurement during exercise is possible, and in claudicants correlates with the onset and severity of claudication (Komiyama *et al.* 1996; Komiyama *et al.* 2002). Work performed by McCulley et al have shown that NIRS adsorbance values from the calf after exercise correlate with time-linked magnetic resonance spectroscopy of oxyhaemoglobin (McCully *et al.* 1994). The depth limitation of 4cm should not be a problem in measurement of muscle oxygenation in the leg. The main problem lies in the fact that a machine is expensive, costing around £10,000 each.

5.5 Wound healing

Skin ulceration is a common problem, and one that represents significant challenges for both patients and clinicians. The success of management techniques to promote wound healing is commonly evaluated using the parameter of 'time to complete healing' (Armstrong *et al.* 2009). A trend towards healing or non-healing can guide management plan decisions towards those most likely to succeed (Lavery *et al.* 1996). However, this process takes, on average, between 3 to 5 months in diabetic foot ulcers, with some ulcers failing to completely heal at all (D'Hemecort P A 1998; Jensen *et al.* 1998). Wound healing is a multi-factorial process consisting of angiogenesis, deposition of extracellular matrix, contraction and epithelialisation (Falanga). Management strategies, especially those which are expected to provide small, non-linear incremental benefits, require an objective and easily reproducible method of quantification. Measurements of wound healing are therefore an important and valid endpoint in clinical trials on vascular patients.

Clinical practice often requires that measurements are taken at appropriate time intervals. Direct measurements of maximum height and width have been shown to overestimate ulcer surface area by approximately 40% (Rogers *et al.* 2010). Digital planimetry, where the wound is traced onto acetate, scanned into electronic form, and then measured using calibrated software, is common in the context of clinical trials (Mayrovitz *et al.* 2009).

Much research has focused on tracking wound surface area, but it is becoming increasingly apparent that wound volume is also important, as the latter picks up both rates of granulation and circumferential epithelialisation. An initial wound volume decrease is the first and most sensitive marker of wound healing, followed by a slower decrease in circumference and area

(Jezernik *et al.* 2004; Kecelj-Leskovec *et al.* 2007). It seems sensible therefore that when using changes in wound size to inform management, that volume changes be measured as a matter of importance. Previously volume measures have been attempted clinically by using mouldable capacitance wound fillers, such as putty or water (Covington *et al.* 1989; Berg *et al.* 1990). This was abandoned due to concerns regarding patient discomfort, risks of infection and cross-contamination, and lack of reproducibility.

5.5.1 Planimetry and 3D cameras

3D cameras are now widely available for purchase, and two are licensed for clinical use in wound management. The accuracy and reliability of these cameras were tested using a simple wound model, comparing them to experimental values measured using planimetry and a water displacement method.

Method

Ulcer models were created using circular imprints into modelling clay (Fimo™, Germany). Four different colours were used to imitate differing skin and ulcer colours – white, pink yellow, and brown. Three different types of lesion were simulated in each colour of modelling clay, one broad-based and flat, one punched out lesion, and one very small lesion. The models were then baked in an oven until cured (Figure 23). An intraclass correlation power analysis calculated that a sample size of 9 lesions with 5 observations each gave 90% power (alpha 0.05, beta 0.09). Therefore our sample of 12 should be sufficient to show significant differences between methods.

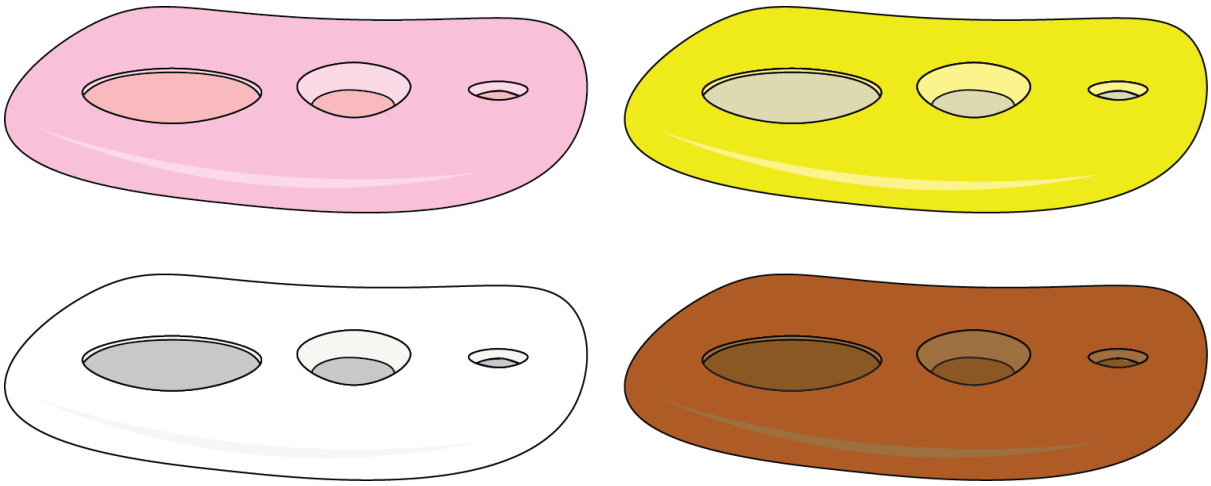


Figure 23 - Cured modelling clay ulcer models

Three methods were used to measure wound surface area and wound volume: digital planimetry/water displacement, Eykona camera (Fuel 3D, UK), and Silhouette camera (ARANZ, New Zealand). The cameras are shown in Figure 24. Measurements were performed by 5 naïve observers, who had a brief 10 minute training session to familiarise themselves with all the equipment. It was universally agreed that measurements would be taken from the inner lip of the ulcer as defined by each observer.

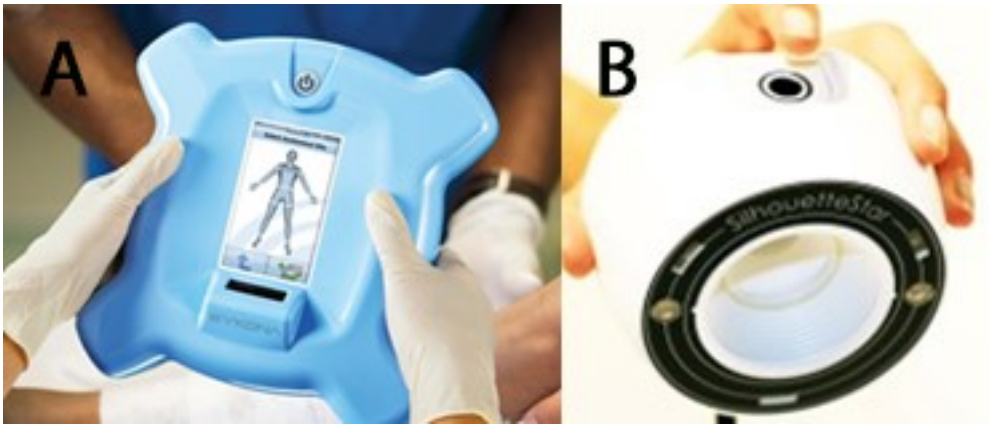


Figure 24 - Three-dimensional cameras licensed for clinical use

A) Eykona (Fuel 3D) B) Silhouette (ARANZ)

Digital planimetry was performed by tracing ulcer outlines onto a transparent 10x10cm planimetry grid (ConvaTec Inc., UK), scanned into an electronic image file, and analysed using open source software (ImageJ, NIH, USA), using the grid for calibration.

For the water displacement measurements, it was found that the formation of a meniscus was problematic for accurate measurement. Trials of surfactant found that the addition of a very small amount of washing-up liquid removed surface tension enough to prevent a meniscus forming. Water was added drop-wise until the relief was filled, and just before overflow occurred. Water was removed using an insulin syringe for accurate measurement.

The Eykona 3D camera is a large portable unit that uses calibrated stickers within the visual field to allow wound measurement. The camera uses a summation of four photographs, coordinating these with 4 in-built light sources, which flash in quick succession to eliminate shadow artefact. Images are then analysed offline using software provided by the manufacturer.

The Silhouette is a small handheld device that uses a camera with laser system for measurement purposes. The camera is held at a specific distance from the wound that allows the lasers to converge. Image data is then analysed offline using manufacturer's software.

Both 3D cameras were used according to manufacturer instructions.

Data was analysed using both Prism (Graphpad , version 6) and SPSS Statistics (IBM, version 21), significance taken at 0.05.

Results

The 5 observers were all healthy middle-aged health professionals, two of whom wore glasses for reading. None had any experience of measuring ulcer size or volume.

Wound surface area

Eykona and Silhouette tended to underestimate surface wound area by mean 1.7% (standard deviation 12, confidence interval -22 to 25) and 3.7% (15, -25 to 32) respectively. Bland-Altman plots are shown in Figure 25. Intra-class correlations for planimetry and the two cameras were 0.999, 1, and 0.999 ($p<0.001$). Spearman correlation coefficients for Eykona and Silhouette compared to planimetry were 0.94 ($p<0.001$) and 0.92 ($p<0.001$). This is shown in Figure 26.

Wound volume

Comparison of water displacement to Eykona and Silhouette resulted in a bias (95% limits of agreement) of 0.6515cm3 (-0.2 to 1.5) and 0.3413cm3 (-1.073 (-1.1 to 1.8). Bland-Altman plots are shown in Figure 25. Intra-class correlations for water displacement and the two cameras were 0.996, 1, and 0.961. Spearman correlation coefficients for Eykona and Silhouette compared to planimetry were 0.92 ($p<0.001$) and 0.72 ($p=0.01$). This is shown in Figure 26.

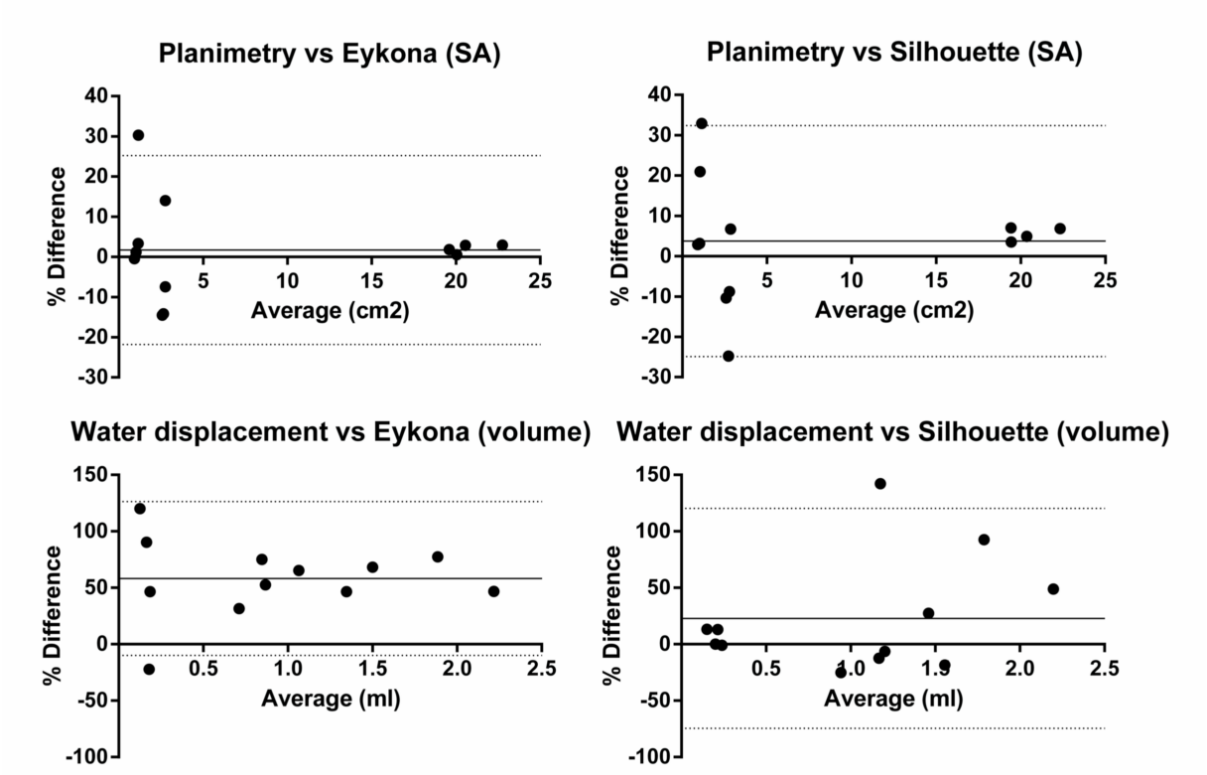
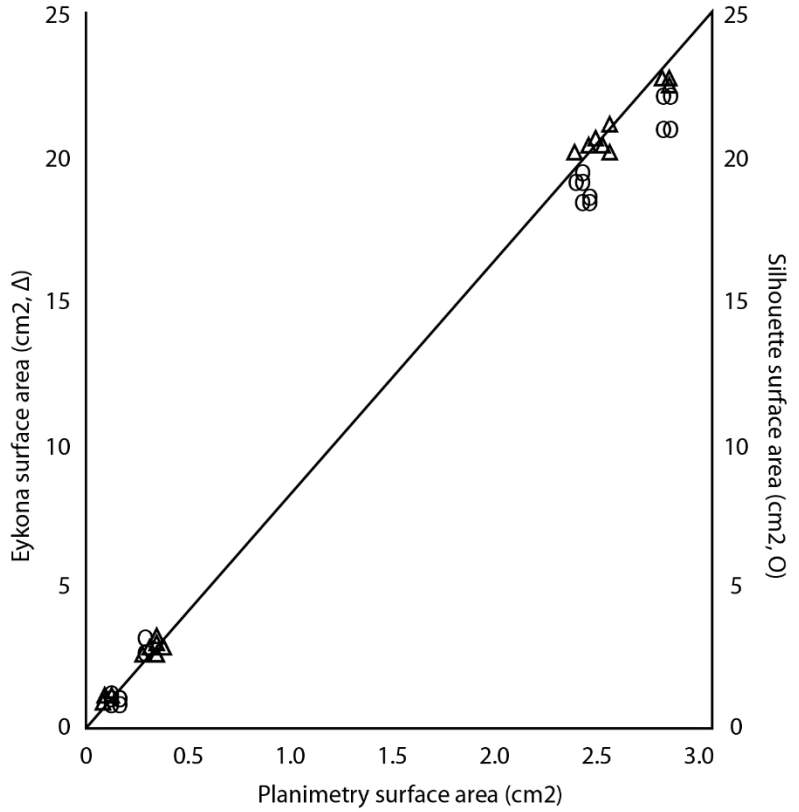


Figure 25 - Bland-Altman plots of measurement system comparisons for both surface area and wound volume.

(Bias shown as bold line, 95% limits of agreement with broken lines)


Eykona surface area (cm2, Δ)
Silhouette surface area (cm2, O)
Planimetry surface area (cm2)
0
5
10
15
20
25
0.5
1.0
1.5
2.0
2.5
3.0

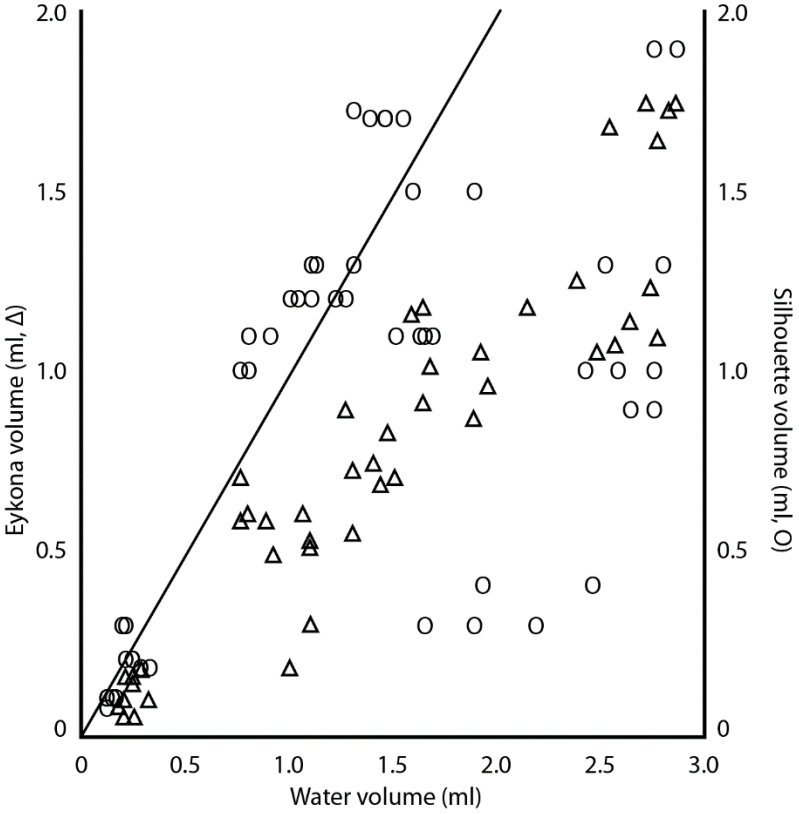

Eykona volume (ml, Δ)
Silhouette volume (ml, O)
Water volume (ml)
0
0.5
1.0
1.5
2.0
2.5
3.0

Figure 26 - Graphs showing correlations between digital planimetry and 3D cameras, for both surface area (A) and wound volume (B) measurements.

Lines of equivalence are plotted. Eykona values shown with triangles, Silhouette with circles.

Colour

One-way ANOVA analysis of modelling clay colour is shown in Table 6. There was no effect of clay colour on measurement of dimensions.

Table 6 - Significance of colour of ulcer model analysed using one-way ANOVA, compared to planimetry.

(p values shown)

	Surface area	Volume
Eykona	0.12	0.65
Silhouette	0.95	0.29

Discussion

Intraclass correlations for planimetry and camera methods are good, and concordant with other reported trials (Majeske 1992; Griffin *et al.* 1993; Lucas 2002). Previous trials evaluating digital planimetry found great difficulty standardising calibration, given the varying distances of the camera from the wound(Lagan *et al.* ; Shaw *et al.* 2007; Shetty *et al.* 2012; Sprigle *et al.* 2012), which seem to have been solved using laser guided protocols and/or the use of calibration stickers(Thawer *et al.* 2002). The lack of difference in accuracy between the Eykona and Silhouette suggests that shadowing is not a significant factor in clinical measurements. Colour of material measured did not affect measurement accuracy in this study.

Serial, accurate and objective area measurements are feasible as part of on-going clinical assessment of wounds and ulcers. However, replacing manual planimetry with three-

dimensional measurements is unlikely to result in improvements in wound measurement accuracy. The added dimension that these cameras bring is sensitivity, through the ability to take volume measurements. This may better reflect the physiological process of granulation upwards from the ulcer base. This alone may justify the expense of equipment purchase over simple grid planimetry.

Real ulcers, in comparison to simulated wounds, are more complex to measure, and therefore results must be interpreted in a clinical context with caution. Plassmann and Jones described the main difficulties faced when trying to obtain objective wound measurements (Plassmann *et al.* 1998). The wound boundary is difficult to define and depends on clinician judgement. Changes in the patient's position affects the accuracy of the measurement. Curvature of the body, extension across a joint, and wound undermining represent significant challenges (Charles 1998; Humbert *et al.* 2004).

Wound measurement forms an important part of clinical care for patients with ulcers, as it can inform care and support or negate new therapies. It is therefore an important part of any trial observing wound healing over time. The results reported here suggest that use of digital planimetry or 3D cameras would both be acceptable in terms of accuracy and reliability.

5.6 Blood-flow assessment

5.6.1 Haemodynamic Ultrasound

Assessment of blood flow through an anatomical structure can be performed using medical ultrasound equipment. The placement of an external transducer on the skin allows the transmission and reflection of sound waves through tissue, with information deduced from the properties of the echoes.

The term ultrasound applies to any sound above the range of human hearing, which is up to about 20 kHz. Commonly used diagnostic ultrasound frequencies are 2-20MHz for externally applied transducers.

A real-time two-dimensional image of the signal intensity provides information about tissue structure, termed "brightness mode" (B-mode) image.

Blood is a heterogeneous fluid, consisting of cells and small particulate suspended in plasma. These components form a mixture which scatter ultrasound, due to the components different densities and compressibility properties. The large number and size of red blood cells means that they dominate this scattering effect, and haematocrit can affect the strength of the ultrasound signal. The strength of the signal received by the transducer will depend on the backscattering of ultrasound waves by blood and attenuation by intervening tissue. Compared with adjacent tissue such as muscle, the echogenicity of blood is very low (by approximately 43dB(Oates 2001)), therefore the lumen of a vessel appears dark on B-mode images.

Colour Doppler mode allows speeds of fluids to be assessed in a non-invasive fashion. Utilisation of the physical properties of sound waves reflected from particles allows relative speeds to be calculated. For a stationary target, the frequency of echo returning to the transducer will equal the transmitted frequency. For a target moving towards the transducer, the reflected frequency will be increased, and for a target moving away the frequency will be reduced. The change in reflected frequency is termed the "Doppler phenomenon" and is described by the equation:

$$f_D = 2\frac{v}{c} f_T \cos(\theta_D)$$

where f_D is mean doppler frequency shift, v is the velocity of red blood cells, c is the speed of sound in tissue (assumed to be 1540m/s), f_T is frequency emitted by the transducer, and θd is the angle of the transducer relative to the vessel (Brody *et al.* 1974; Nakayama *et al.* 1974). In any vessel there will be large numbers of moving "targets", resulting in a spectrum of Doppler frequencies. In a colour flow image a single quantity, namely the mean frequency shift or signal power, is calculated at each point of the 2D image and super-imposed as a colour code on the B-mode image. Alternatively this information can be displayed in spectral form, calculated in real-time and plotted as a frequency-shift-against-time plot.

Measurements of velocity can be used to deduce clinical information about the vessel under visualisation, such as degree of stenosis. In an artery, the normal spectral waveform is smooth, with intensities bunched around a maximum with little broadening of the spectrum to lower frequencies. This correlates with laminar flow, where within the sample volume the blood velocity vectors are of similar magnitude and direction at any instant in time. Blood moving through a stenotic lesion speeds up as it passes through, then enters a turbulent post-

stenotic region. This appears as spectral broadening and high frequency spikes. The peak systolic velocity can be used to interpolate percentage stenosis using standardised tables

Volume flow

In 1986, the concept of measuring flow via doppler ultrasound was realised, and validated by Lewis et al in a flow rig (Lewis *et al.* 1986). An algorithm used a product of the measured flow rates and vessel cross-sectional area, but was critically dependant on insonation angle and selected sample volume.

When recording haemodynamic data from a vessel using ultrasound, the transducer is placed longitudinally and moved about an axis perpendicular to the skin until a clear B-mode image of both parallel vessel walls are seen, with a maximal inter-intimal distance (Lewis *et al.* 1986). Making the assumption that the vessel is perfectly circular, cross-sectional area is calculated from this diameter using callipers. It is not physically possible to measure the angle between the transducer and the longitudinal diameter of the vessel, but when both vessel walls are parallel on the B-mode image, approximate alignment to practical levels is universally accepted.

After switching to doppler mode, the direction of the ultrasound beam is then adjusted using the "steer" function to produce a corrected angle of 60-65°. This is a compromise between obtaining an optimal B-mode image at 0' to axis, and obtaining an optimum doppler signal at 90°. By convention, 60° has been chosen and is taken as standard. As flow is a product of speed and cross-section, insonation of 60 gives a cosθ value of 0.5. Sampling volume is positioned in the centre of the vessel (Figure 27). Maximal axial diameter is set to insonate the entire depth of the vessel in longitudinal section, as speeds across the vessel will vary with laminar flow affects.

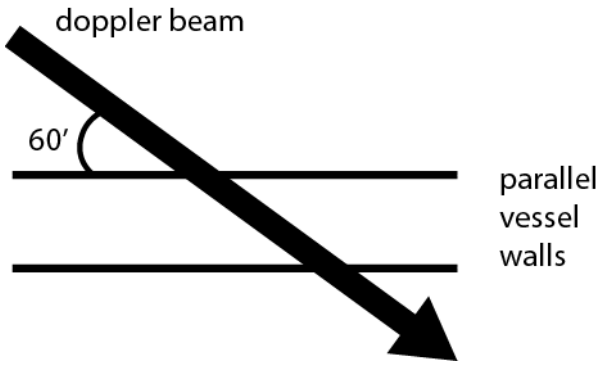


Figure 27 - Method for measuring volume flow in a vessel using doppler ultrasound

(showing transducer parallel to vessel longitudinal axis, and both vessel walls in parallel section. Insonation angle is corrected to 60° and sampling volume set to maximal vessel diameter)

Lewis et al warn of the importance of transducer placement relative to the vessel, as if the section sampled is not taken at the maximal diameter, the doppler shift waveform will be significantly blunted (Lewis *et al.* 1986). Accuracy of flow measured using haemodynamic ultrasound is reported as good, with a mean error of 5ml/min when compared to a flow rig. In healthy subjects, coefficient of variability for arterial flow in the superficial femoral artery was 12-20.2% (Lewis *et al.* 1986; Hussain *et al.* 1996). One quarter of this can be abolished by holding the transducer in a clamp, with the remaining three quarters presumably due to physiological changes (Lewis *et al.* 1986). It is well known that inter-rater variability is an issue regarding measurements taken with ultrasound, and inter-observer variations of up to 20% are typical in experienced hands (Hussain *et al.* 1996). Hussain et al recommend recording 5 measurements and calculating an average, in an effort to minimise the effects of respiration, vessel wall movement, and thermoregulatory patterns. Image resolution limits this technique to the measurement of parameters in those vessels where diameter (and therefore cross-sectional area) can be accurately quantified.

Significant differences in venous and arterial flow rates are observed in healthy subjects when standing, sitting, supine, and elevated (Morgan *et al.* 1991). Elevation of the limb increases common femoral blood flow by 17% transiently, which then falls back to baseline levels. Dependency decreases CFA flow by 17%, and standing erect decreased flow by 47%. This has been corroborated by radioactive clearance technique (Amery *et al.* 1969). Flow rate changes to positioning are altered in disease states: for example in critical limb ischaemia flow rates increase by 31% on dependency, and increases by 42% on standing (Morgan *et al.* 1991). Elevation of the limb decreases flow by 53%. Care must be taken to reproduce scanning protocols and subject positioning if comparing values over time. The importance of an equilibration period at rest is also recommended, around 10 minutes is conventionally used unless an experimental condition has been imposed (e.g. exercise treadmill).

Changes to blood composition (and therefore its acoustic properties) are unlikely to contribute significant error to ultrasonic haemodynamic measurements. Multiple studies have shown that ultrasound propagation through blood is independent of frequency in the range 1-15MHz, and that it varies weakly with temperature, haematocrit, and protein content of blood (Bradley *et al.* 1972). Narayana et al have shown that an assumed variance in body temperature of ±1'C, ±10% haematocrit, and ±0.75g/100ml in protein content, will result in a total range of variation of ±9m/s (±0.6%) in the propagation speed of ultrasound through blood (Narayana *et al.* 1984). Problems with turbulence of blood have been shown to increase the sonoreflectivity of blood (Shung 1983), but this is unlikely to be of any practical importance as turbulent flow is often associated with a relatively flat velocity profile, where non-uniform signal scattering causes very small error in flow measurement (Gill 1985).

Table 7 summarises the potential sources of error to be minimised in any ultrasound protocol.

Table 7 - Sources of error in measuring limb volume flow using haemodynamic ultrasound

Estimation of vessel cross-sectional area (spatial resolution of ultrasound, misalignment along vessel axis, non-circular vessels) Important considerations - careful placement of measurement callipers on B-mode image, intimal-to-intimal, avoidance of applying too much pressure to cause vessel deformation.
Estimation of insonation angle Important considerations - keep transducer and vessel parallel, and use corrected insonation angle of 60'.
Ultrasound scattering assumptions (uneven distribution of signal intensity within sample volume, non-representative sample volume within vessel, non-uniformity of signal scatterers). Important considerations - sampling entire width of vessel, intimal surface to intimal surface.
Physiological changes within scanning protocol (temperature, autonomic activation with vessel diameter changes, cardiac cycle pressure changes). Important considerations - taking an average of five measurements.

5.6.1.1 Generic ultrasound protocol

Where indicated by screening demands, initial duplex ultrasound of the venous system (for thrombosis and reflux) was performed with subject standing, using iU22 xMATRIX ultrasound system with L9-3 transducer and vascular protocol (Philips, Seattle, WA, USA). Presence or absence of reflux was assessed using a variety of established techniques (e.g. calf squeeze, Wunstorf manoeuvre (Mendoza *et al.* 2001), Parana manoeuvre (Franceschi 1997)). Abnormal reflux was deemed present if measured reflux time was equal to, or greater, than 0.5 seconds.

For haemodynamic assessment, subjects were placed supine and semi-recumbent, and allowed to quietly rest for 10 minutes. A pillow was placed behind both knees, and the lower limb to be imaged is flexed at the knee and externally rotated at the hip. This produces a "lazy frog leg" position which enables clear views of the proximal superficial femoral artery, and femoral vein. The appropriate vessels were identified with B-mode ultrasound, and arteriovenous nature double-checked using colour doppler and transducer pressure compression. Transducer positioning sites were marked on the skin for repeat measurements as per Williams el al (Williams *et al.* 2015). Where the vessel view was obscured (e.g. body habitus, calcified plaque) an alternative site immediately proximal or distal was chosen, and noted in the patient case report form.

Using B-mode imaging, the proper orientation of the transducer relative to the vessel of interest was checked to be parallel, with optimal views of both vessel walls. Insonation angle of the sampling gate was corrected to 60', and adjusted to incorporate the whole vessel from intima to intima. Time scale was adjusted to 15 seconds total screen width, and 15 seconds of doppler trace recorded. Measurements of TAMV and volume flow were performed and a

screen shot captured. Images were exported to disk, and stored electronically. Peak velocity was calculated offline using calibrated ImageJ 1.47v software (NIH, USA).

5.6.1.2 Experimental reliability

One healthy subject (age 26, BMI 24) was subjected to the ultrasound protocol specified above, semi-recumbent and relaxed. The right groin vessels were positioned and visualised. Ten repeat measurements were taken by two technicians (KW and RR).

Intra-rater reliability

Coefficients of variation (COV) for measurements made by KW were: arterial diameter 5.0%, TAMV 23.1%, and volume flow 24.9%.

Coefficients of variation for measurements made by RR were: venous diameter 4.6%, TAMV 18.5%, and volume flow 16.2%.

Inter-rater reliability

Bland-Altman charts comparing measurements taken by KW (n=20) and RR (n=20) are shown in Figure 3. Average bias (over- or under-estimation) of arterial diameter, TAMV and volume flow were 11%, 33%, and 52% respectively. Average bias for venous diameter, TAMV and volume flow were -13%, 10%, -9%.

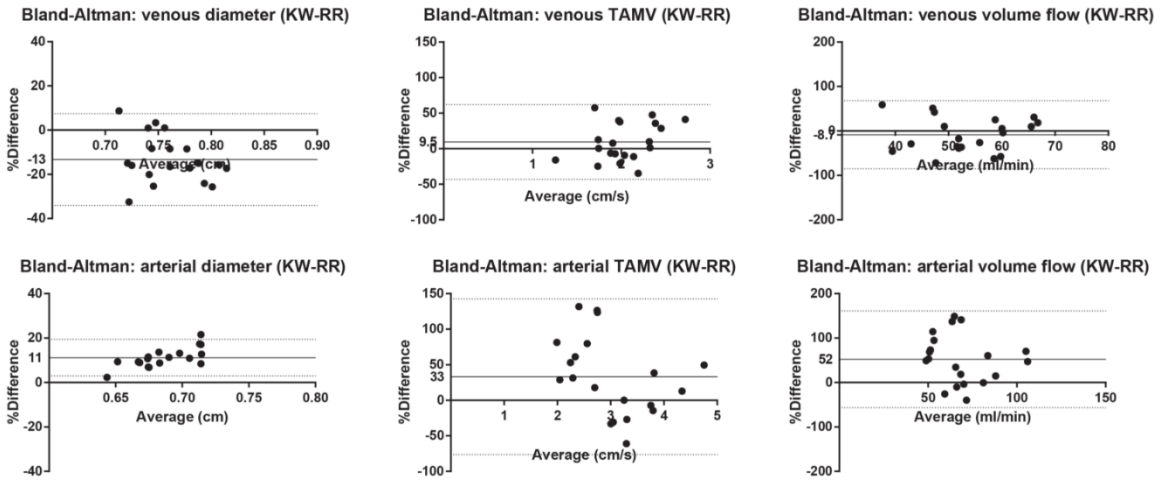


Figure 28 - Bland-Altman charts of measurements taken with ultrasound from the right femoral vein and superficial femoral artery of a healthy subject.

Bold lines show bias, and 95% confidence intervals shown with dashed lines. TAMV - time averaged maximum velocity.

Intra-rater reliability for speed and volume measurements of blood flow in the femoral vessels is acceptable, and concordant with the existing literature (coefficient of variation <25%). Inter-rater reliability is poor, and worst in arterial haemodynamic studies. Whenever possible the same sonographer will be used for all scans. Where subject volume concerns mean that more than one scanning technician is used, accuracy of reporting will suffer, and results are much less likely to reflect physiological changes.

5.7 Lower limb oedema

Swelling of the limbs is a common symptom associated with vascular disorders, particularly those pertaining to venous and lymphatic dysfunction. Measurements of limb circumference and volume can be used to measure objective changes in swelling over time, and are important objective endpoints in trials where reduction of swelling is an important clinical concern.

5.7.1 Water displacement

Water displacement is considered the gold standard for limb volume measurement. A water filled receptacle is used to measure the displacement that anything placed in it exerts, utilising the Archimedes principle. It is low cost, with chemically safe components, however there are serious health and safety concerns regarding its clinical use. The first is the potential for spread of infections - both bacteria and fungi colonise the feet and lower legs of normal subjects, and subjects with clinical infections may have suppurating wounds, and may be resistant to antibiotics. The potential for subject to subject transmission must be minimised. The use of solutions in a clinical environment also present problems. Water on the floor can become a fall hazard, and can damage electrical equipment. In the context of electrical stimulation, there is also the potential of delivering electricity in an uncontrolled fashion, endangering both subject and technician.

For the purposes of these pilot clinical trials, this method has been deemed unsuitable.

5.7.2 Tape measure

A tape measure can be used to measure limb circumference, commonly at the ankle and at the maximal calf circumference

To evaluate the reliability of circumference measurements, consecutive measurements were taken from the right leg of a healthy subject by KW. The subject was sat upright, with knee joint flexed at 90' and foot flat on the floor. The subject was asked to relax their leg muscles. Twenty measurements were taken, each from two points on the leg, as illustrated in Figure 29. At location A, mean circumference was 23.5cm±0.08, coefficient of variation 0.35%. At location B, mean circumference was 39.7cm ±0.3, coefficient of variation 0.75%.

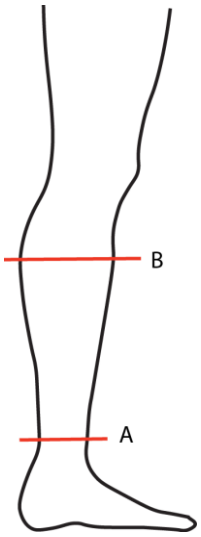


Figure 29 - Diagram of location of repeat measurements taken from the leg of a healthy subject

A limited leg volume (V) can be calculated by using ankle and maximal calf diameters, measuring the vertical distance between the two points (h), deriving ankle and calf radii (R1 and R2), and applying an inverted truncated cone model:

$$V = \frac{\pi h}{3}(R1^2 + R1R2 + R2^2)$$

This will only estimate the volume between points A and B in Figure 29, and makes geometric assumptions about the shape of the leg. The increased number of anatomical measurements may make this unreliable when comparing two points in time. The information from the reliability experiment above was used to calculate lower leg volume, with h= 30cm. Mean and standard deviation of lower leg volume between the specified points was 2439.7cl ±23.0, coefficient of variation 0.94%.

All measurements were repeated by another observer (Y). Bland-Altman graphs are shown in Figure 30. The bias for each measurement between observers was negligible, with 95% confidence intervals less than 3%.

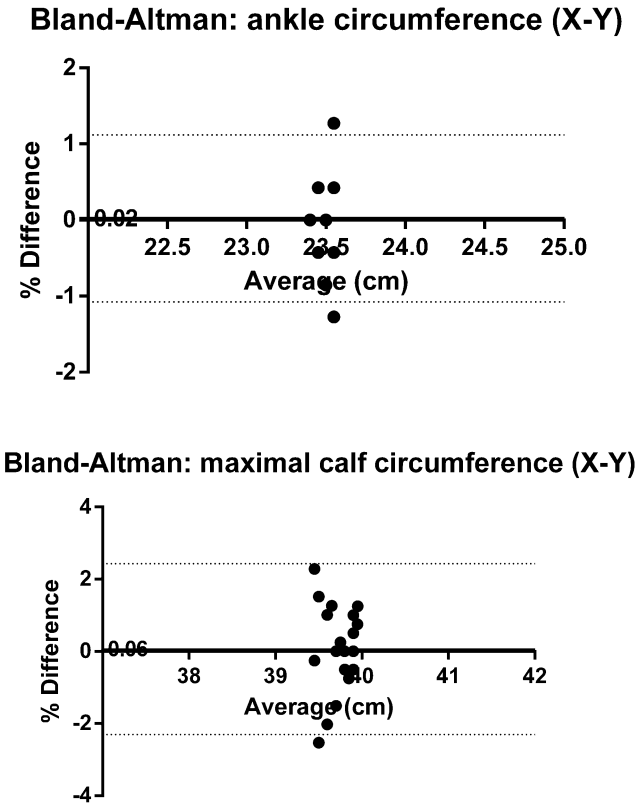


Figure 30 - Bland-Altman graphs of measurements taken by observers X and Y

Bias is shown in bold line, 95% confidence intervals are shown with dashed lines.

5.7.3 Perometer

The perometer is an optoelectric limb volume measurement system (Type 35 S,Pero-system Messgeräte GmbH, Germany, using PeroPlus 2000 software). The limb is placed inside the frame, and then the frame is moved proximally in a slow continuous motion. A circular arrangement of inwardly facing infrared light transmitters and photosensors on a rigid square frame interact to create an electronic image of the presented limb (Tan *et al.* 2013). Multiple diameter measurements are captured at 4.7mm intervals as the frame is moved. From this, measurements of circumference and volume can be calculated, relative to bony landmarks. A perometer is shown in Figure 31. The perometer has been previously validated for limb circumference measurements (ICC 0.997) when compared to a tape measure method (Stanton *et al.* 1997; Deltombe *et al.* 2007).

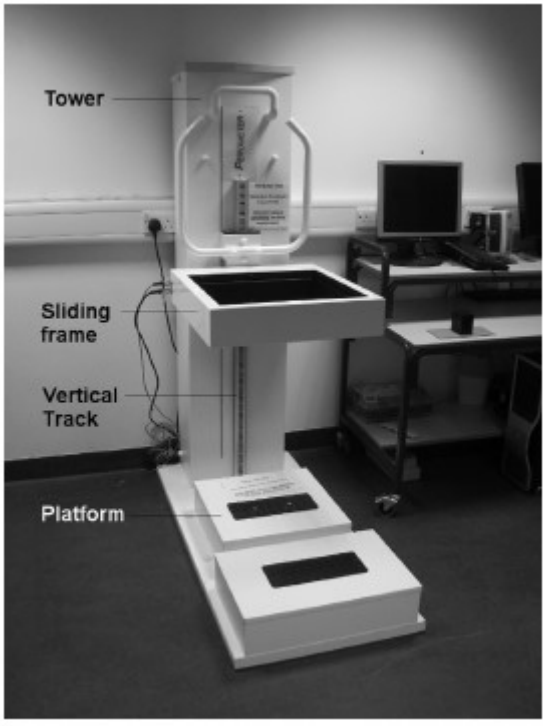
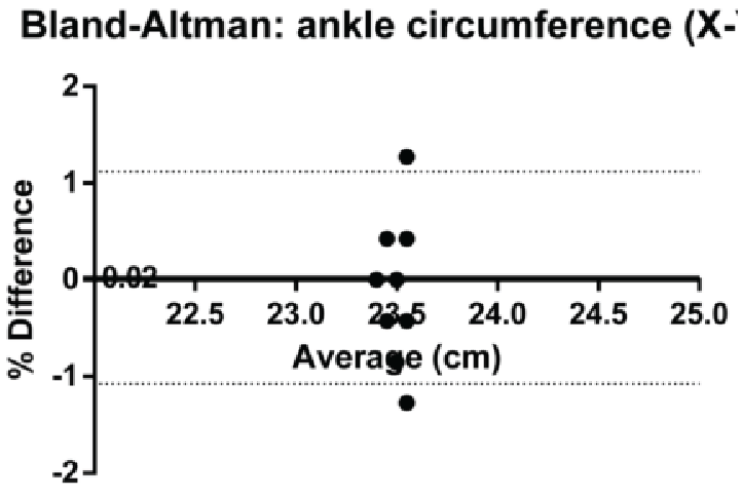


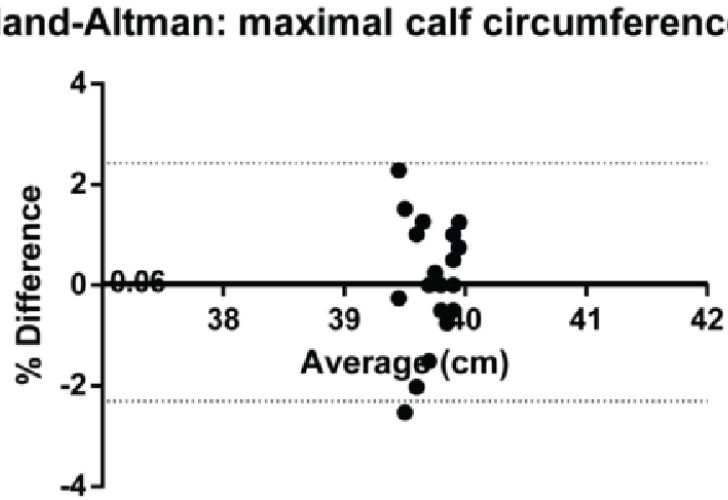
Figure 31 - A perometer, used for assessing leg volume

A small study was conducted to assess the variability of readings in our department. One subject was seated on a standard office chair, positioned with their leg extended and foot placed flat on the footplate. Care was taken to minimise space between sole and footplate, and relax the leg as much as possible to minimise leg muscle contraction. A baseline scan was taken and the length of footplate to middle of patella noted. All successive measurements of volume used this length value as a standardised cut-off, particular to that trial subject. Two observers each took 15 consecutive perometer scans, 30 in total. Reliability testing co-efficient of variation were 0.2% and 0.19%. The mean difference (±SD) between observers was 2% (±4.2). Bland-Altman plots are shown in Figure 32.

A)


Bland-Altman: ankle circumference (X-Y)
% Difference
Average (cm)
0.02
2
1
0
-1
-2
22.5
23.0
23.5
24.0
24.5
25.0

B)


Bland-Altman: maximal calf circumference (X-Y)
% Difference
Average (cm)
0.06
4
2
0
-2
-4
38
39
40
41
42

C)

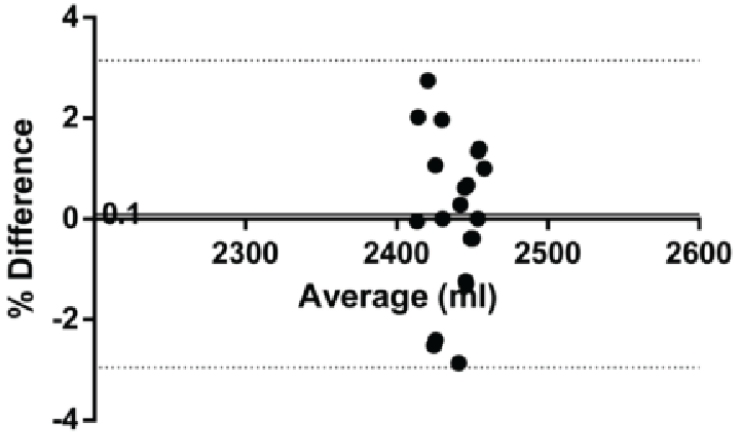

Bland-Altman: leg volume (X-Y)
% Difference
Average (ml)
0.1
4
2
0
-2
-4
2300
2400
2500
2600

Figure 32 - Bland-Altman chart of repeated measures taken from a volunteers leg

6 Bench testing and small preliminary trials using NMES in healthy subjects

In order to plan practically sensible and clinically relevant trials looking at manipulation of the circulation in both health and disease, preliminary studies need to be carried out.

To this end several small preliminary projects were planned and executed, evaluating the action of NMES in healthy subjects. These explore the effects of applying stimulation to different muscle groups, the use of different commercially available products, and comparison with other medical devices known to enhance the circulation of the leg.

Section	Title	Page
6.1	Femoral blood flow and laser doppler fluximetry changes with electrical stimulation of selected muscles in the leg	178
6.2	The effect of common peroneal nerve stimulation	196
6.3	The effect of electrical footplate stimulation	201
6.4	Concomitant use of common peroneal nerve stimulation and graduated compression stockings	208
6.5	Comparison of NMES with intermittent pneumatic compression	217
6.6	NMES compared to graduated compression stockings	232
6.7	Changes in autonomic activation with NMES of the legs	242
6.8	**Summary of findings from bench testing and small trials**	257

6.1 Femoral blood flow and laser doppler fluximetry changes with electrical stimulation of selected muscles in the leg

The three lower limb muscle pumps show varying degrees of efficacy in improving blood flow under different electrical stimulation conditions, and there is no consensus as to the most effective therapeutic target. The following study aims to address which muscle, or group of muscles, is most effective at improving femoral vessel haemodynamics in healthy subjects, by selecting and stimulating seven key anatomical sites within the thigh and lower leg muscle pumps. Secondary aims include evaluating the microvascular changes in the skin, and recording tolerability of electrical stimulation at each muscle site.

6.1.1 Methods

Ethical approval was granted by Imperial College Research Ethics Committee (study ref. 15IC2528). Healthy subjects were recruited from the vascular department at Charing Cross and Imperial College Medical School, and written informed consent was obtained. Inclusion and exclusion criteria are presented in Table 8. All subjects were screened with a medical history, physical examination, ankle-brachial pressure index (ABPI), and venous ultrasound of both legs.

Table 8 - Inclusion and exclusion criteria for trial subjects in muscle pump trial

Inclusion Criteria	18 years or older Current good health
Exclusion Criteria	History of cardiovascular disease ABPI<0.9 Venous reflux>0.5s Pregnancy

Protocol

Participants were positioned on an examination couch, semi-recumbent with attention to subject comfort. The right leg was exposed from the groin downwards, and the rest of the subject kept warm. All measurements were taken from the participant's right leg (Figure 33). Electrical stimulation sites were cleaned with an alcohol wipe and electrodes placed. A blanket was then placed over the subjects legs so they were not in view for the protocol.

The selection of muscles to include in the protocol was based on anatomical relationship to veins and venous plexi, the amenability to transcutaneous stimulation, and comfort of the subject during stimulation. The seven sites stimulated in this study were: *vastus medialis, rectus femoris, vastus lateralis, tibialis anterior, peroneus longus,* and the *medial* and *lateral heads of gastrocnemius* (Figure 34). These muscles are well-placed anatomically to compress deep veins when contracted, and superficial enough that tenable current values can be used (Delitto *et al.* 1992; Maffiuletti 2010; Gobbo *et al.* 2011). Stimulation of muscles in the foot and the hamstrings was found to be very uncomfortable, and at the request of study participants were not included. Optimal muscle motor stimulation points (usually the point at which the motor neurone enters the muscle) were identified using a motor point atlas, and correspond to the locations on the skin above a muscle in which an electrical pulse evokes a muscle twitch to visual inspection and manual palpation (Botter *et al.* 2011). The negative

electrode was placed over the motor point, and the dispersive electrode 1-2cm from this, over the muscle in question. Increasing the output current in a stepwise fashion allowed motor threshold identification for each muscle. Motor threshold values were defined as observed contractions of the entire muscle. This value was doubled for the stimulation phase of the study to ensure adequate venous compression, whilst avoiding untenable discomfort. This also complies with instructions for some modern stimulation devices (Varatharajan *et al.* 2014). The order in which the muscles were stimulated in each participant was altered on a consecutive basis in an attempt to reduce application bias (Table 9). Stimulation lasted for a total of 5-minutes per muscle, followed by a 5 minute relaxation phase (Figure 35).

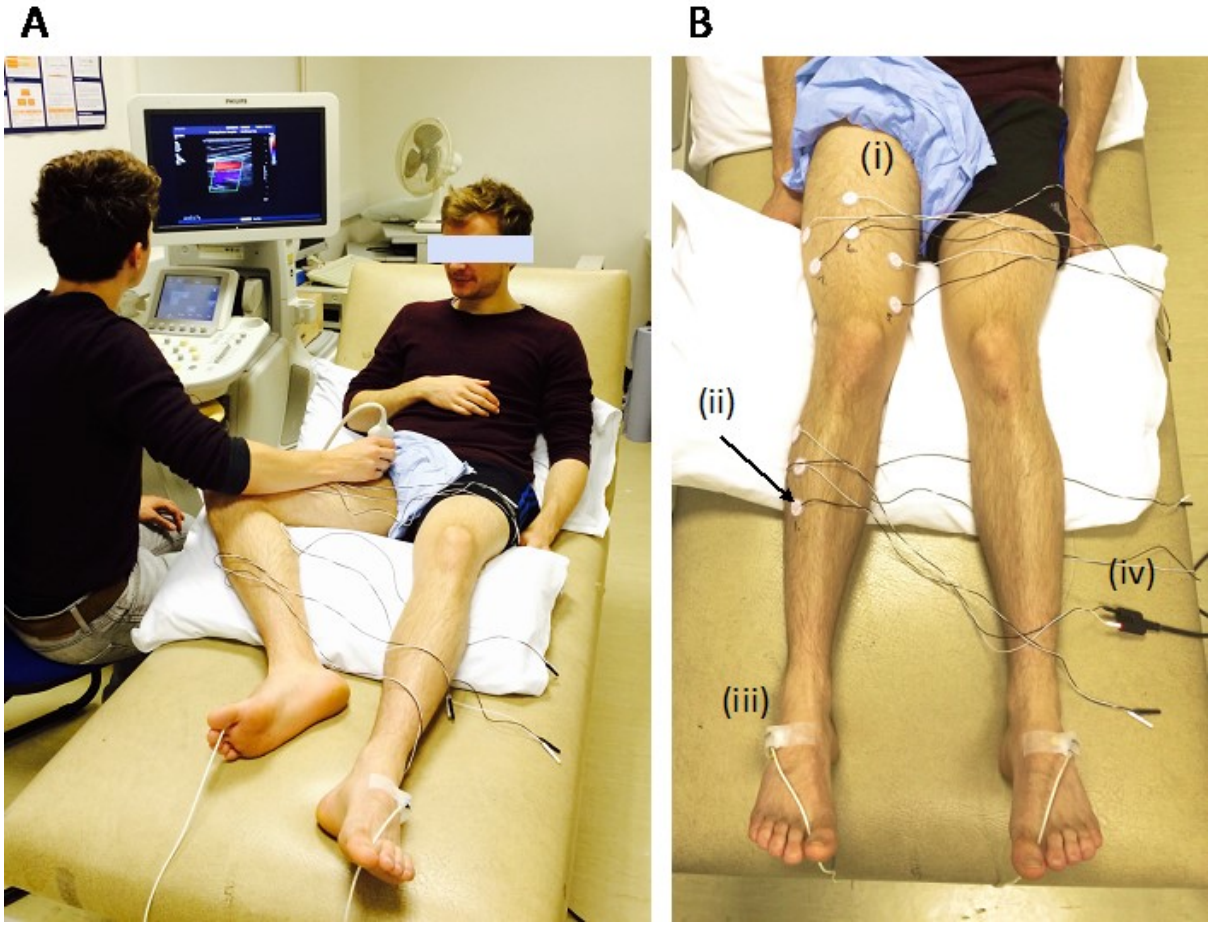


Figure 33 – Subject positioning for MUSCLE PUMP protocol

(A) Patient supine on reclined bed during ultrasound scanning. (B) Arrangement of leg, (i) femoral triangle where scanning took place, (ii) electrode pairs on muscle, (iii) laser Doppler probes recording microvascular changes, (iv) cable connecting Digitimer™ to electrode pairs to deliver stimulation.

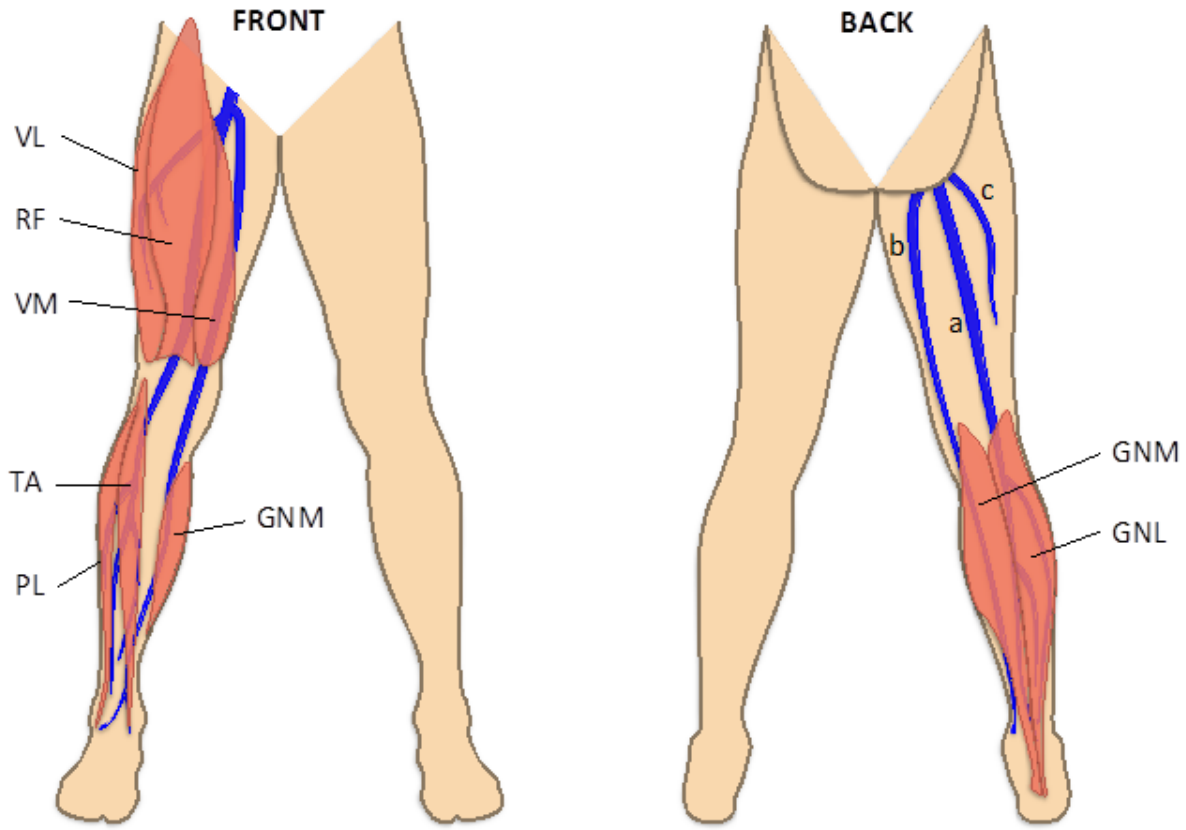


Figure 34 - Venous vasculature draining lower limb with stimulated muscles.

(a) femoral vein, (b) great saphenous vein, (c) deep femoral vein.
TA = tibialis anterior, PL = peroneus longus, GNM = gastrocnemius (medial head), GNL = gastrocnemius (lateral head), VL = vastus lateralis, RF = rectus femoris, VM = vastus medialis.

Table 9 - Allocation order of muscle stimulation for trial protocol

Subject		1	2	3	4	5	6	7	8	9	10	11	12
Muscle stimulated	TA	1	2	3	4	5	6	7	1	2	3	4	5
	PL	2	3	4	5	6	7	1	2	3	4	5	6
	GNM	3	4	5	6	7	1	2	3	4	5	6	7
	GNL	4	5	6	7	1	2	3	4	5	6	7	1
	VL	5	6	7	1	2	3	4	5	6	7	1	2
	RF	6	7	1	2	3	4	5	6	7	1	2	3
	VM	7	1	2	3	4	5	6	7	1	2	3	4

TA = tibialis anterior, PL = peroneus longus, GNM = gastrocnemius (medial head), GNL = gastrocnemius (lateral head), VL = vastus lateralis, RF = rectus femoris, VM = vastus medialis.

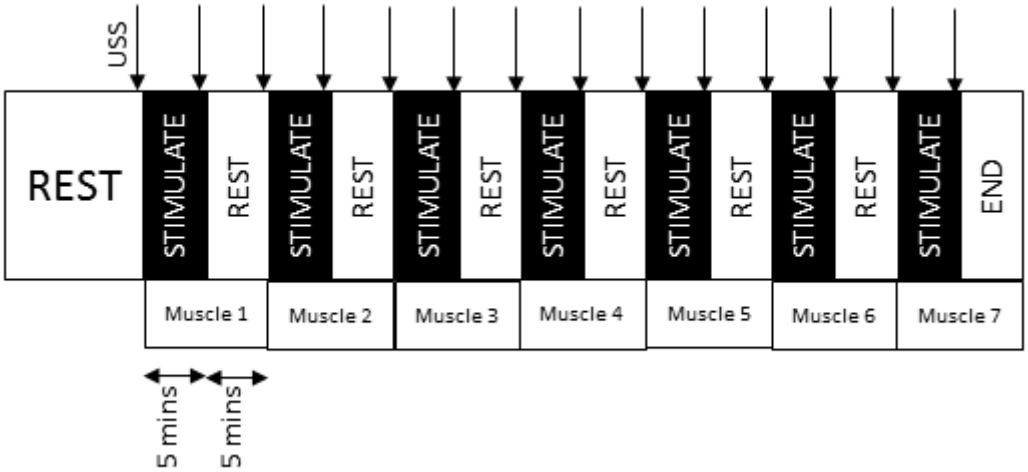


Figure 35 - Outline of the MUSCLE PUMP study protocol.

Protocol continued for 7 muscles in total. Arrows indicate when ultrasound measurements were taken.

Laser Doppler

Laser Doppler (VMS-LDF2, Moor instruments, UK) adhesive skin electrodes were attached to each foot in order to measure changes in dermal microcirculation and temperature throughout the protocol. A marker was placed on the trace to identify the exact time at which each stimulation started and stopped. Average skin temperature and blood flow was measured offline taking the largest proportion of the reading as possible (between 30s and 60s), while minimising movement artefact. Laser Doppler readings were calculated just before stimulation and immediately following cessation of stimulation.

Ultrasound

Blood velocity and flow rate were measured using haemodynamic ultrasound (Philips iU22 vascular system, linear transducer) at the level of the groin vessels as per Williams et al (Williams *et al.* 2015). The right leg was externally rotated at the hip and flexed at the knee

to ensure the vessels were optimally aligned for identification and taking measurements. The right femoral vein was identified and a mark was made on the skin for consistency in repeat measurements. Baseline ultrasound readings were taken in the required position on the examination bed after a 10-minute quiet relaxation period of physiological equilibration. New baseline readings were taken immediately before each stimulation phase, 5-minutes after cessation of previous electrical stimulation. Three ultrasound readings, of 15s each, were taken during each measurement and stored as screen shots for offline calculation of mean values. Both arterial and venous readings were recorded. Traces were saved to disk as a screenshot and analysed offline. Flow volume (VF) and time averaged maximum velocity (TAMV) were automatically calculated by the Philips software, whilst venous peak velocity (PV) was calculated offline using ImageJ 1.48 software.

Electrical Stimulation

Electrical stimulation was delivered to each of the seven sites through neonatal electrodes (1041PTS, Kendall/Covidien, USA). Signal v.3.13 (CED Ltd., UK) software was used to programme a stimulation frequency of 1Hz. An adjustable electrical stimulating unit (DS7A HV, Digitimer Ltd, UK) delivered the electrical stimulation at a constant pulse duration of 1ms and maximum compliance voltage of 200V for each participant. Output current causing contraction of the muscle was doubled in value for the purposes of the protocol.

Environmental considerations

A room temperature of 25-28^{o}C was applied to the testing area in an effort to control environmental factors.

Sample size calculation

The website www.statstodo.com was used to calculate the sample size needed to detect a 50% difference in peak velocity for: seven muscle stimulation groups, with a type one error rate of 0.05, a power of 0.8, and a within-sample standard deviation of 60% (unpublished data on healthy subjects, under peer-review). This showed that a sample size of 12 was needed.

Statistical Analysis

Statistical analysis was carried out using IBM SPSS Statistics, v.22. Data were analysed for normal distribution and appropriate parametric or non-parametric tests were applied. Significance was taken at $p<0.05$.

6.1.2 Results

Twelve healthy participants completed the study in full, seven females and five males. The mean age (± standard deviation) was 23.1±3 years and body mass index 23.1±3 kg/m^2.

Haemodynamic parameters

Screenshots of venous and arterial waveform Doppler traces were captured at baseline and during stimulation. The effects of muscle stimulation and contraction can be identified in venous traces as "spikes" in the trace with a frequency of 1Hz, illustrated in Figure 36.

A large variation in haemodynamic response to stimulation was observed between participants. An example of the variability between subjects is displayed in Figure 37.

PV in the femoral vein was significantly increased by electrically-induced contraction of the leg muscles, and this was independent of which muscle was stimulated ($p<0.05$, Wilcoxon signed-rank). The range of percentage change from baseline with stimulation were 5% (*tibialis*

anterior) to 22% (*vastus medialis*). No muscle displayed a significantly greater effect than the others (ANOVA).

TAMV and VF were significantly increased by the action of medial gastrocnemius (29% and 52% respectively, both $p<0.05$, Wilcoxon signed-rank), but not by any other in the protocol. Results are tabulated in **Error! Reference source not found.** (Appendices, page 397) , and presented graphically in Figure 38.

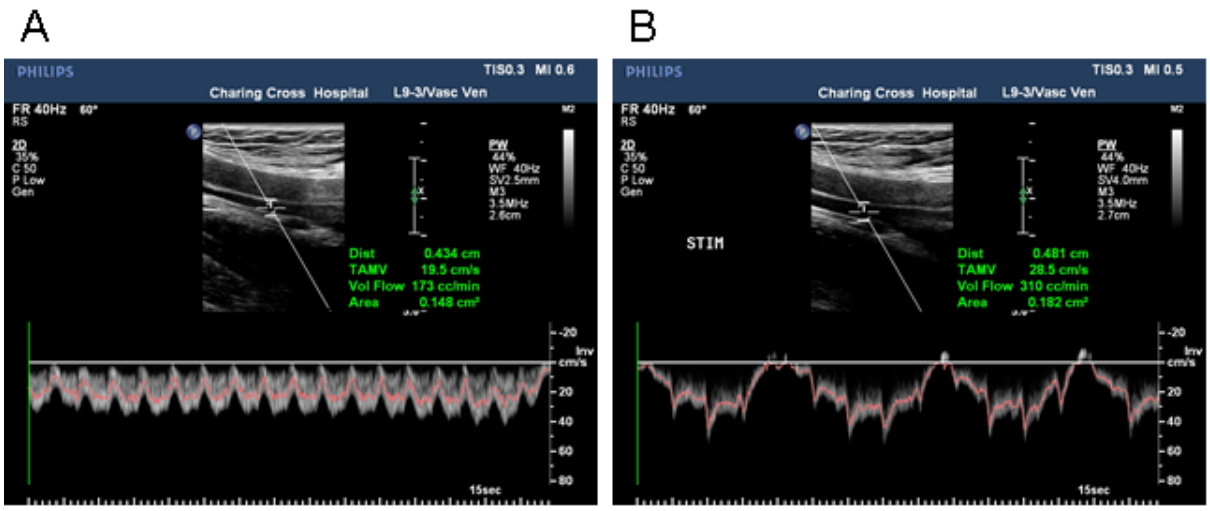


Figure 36 - Duplex ultrasound recordings taken from the femoral vein during MUSCLE PUMP protocol

(A) Baseline (B) Recording during electrical stimulation of peroneus longus.
The venous flow pattern is altered, showing 'spikes' in the waveform which correlate with muscle stimulation (1Hz)

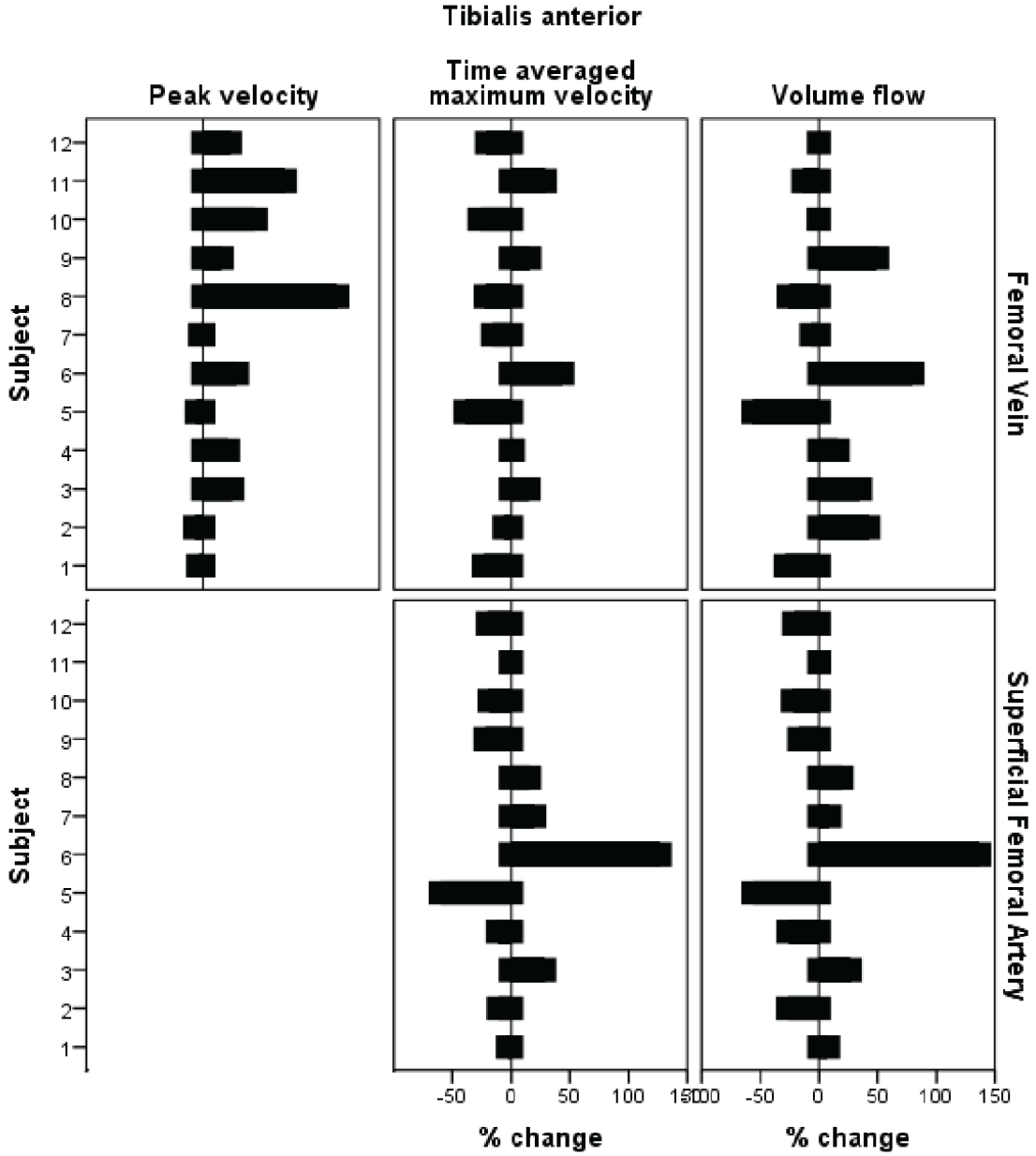


Figure 37 - The percentage change to haemodynamic parameters with electrical stimulation of tibialis anterior, to illustrate between-subject variability

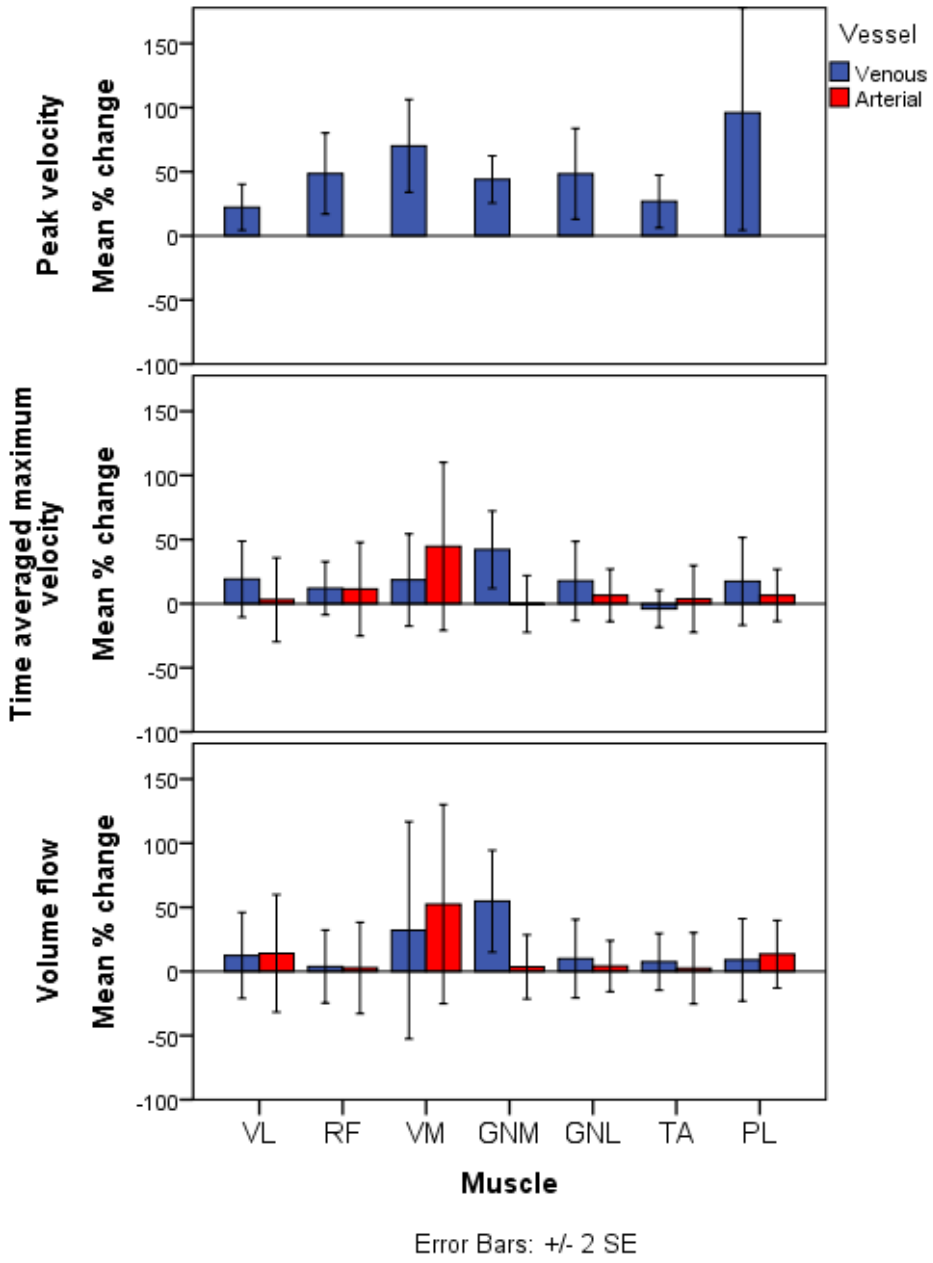


Figure 38 - Box-plots depicting percentage change in femoral vein haemodynamic parameters from baseline with stimulation.

VL = vastus lateralis, RF = rectus femoris, VM = vastus medialis, GNM = gastrocnemius (medial head), GNL = gastrocnemius (lateral head), TA = tibialis anterior, PL = peroneus longus,
Significance from baseline tested using Wilcoxon signed-rank, *p<0.05

Laser Doppler

Percentage change laser doppler fluximetry signal in the stimulated foot increased significantly with stimulation of each muscle ($p<0.05$, Wilcoxon signed-rank). This ranged from 43% (*rectus femoris*) to 91% (*vastus lateralis*), and is illustrated in Figure 7. Freidman analysis of percentage change by muscle was not significant. The Doppler signal in the contralateral foot significantly increased with stimulation of the right leg thigh muscles, and with either head of *gastrocnemius*. *Tibialis anterior* and *peroneus longus* did not cause significant change in flux signal in the contralateral leg.

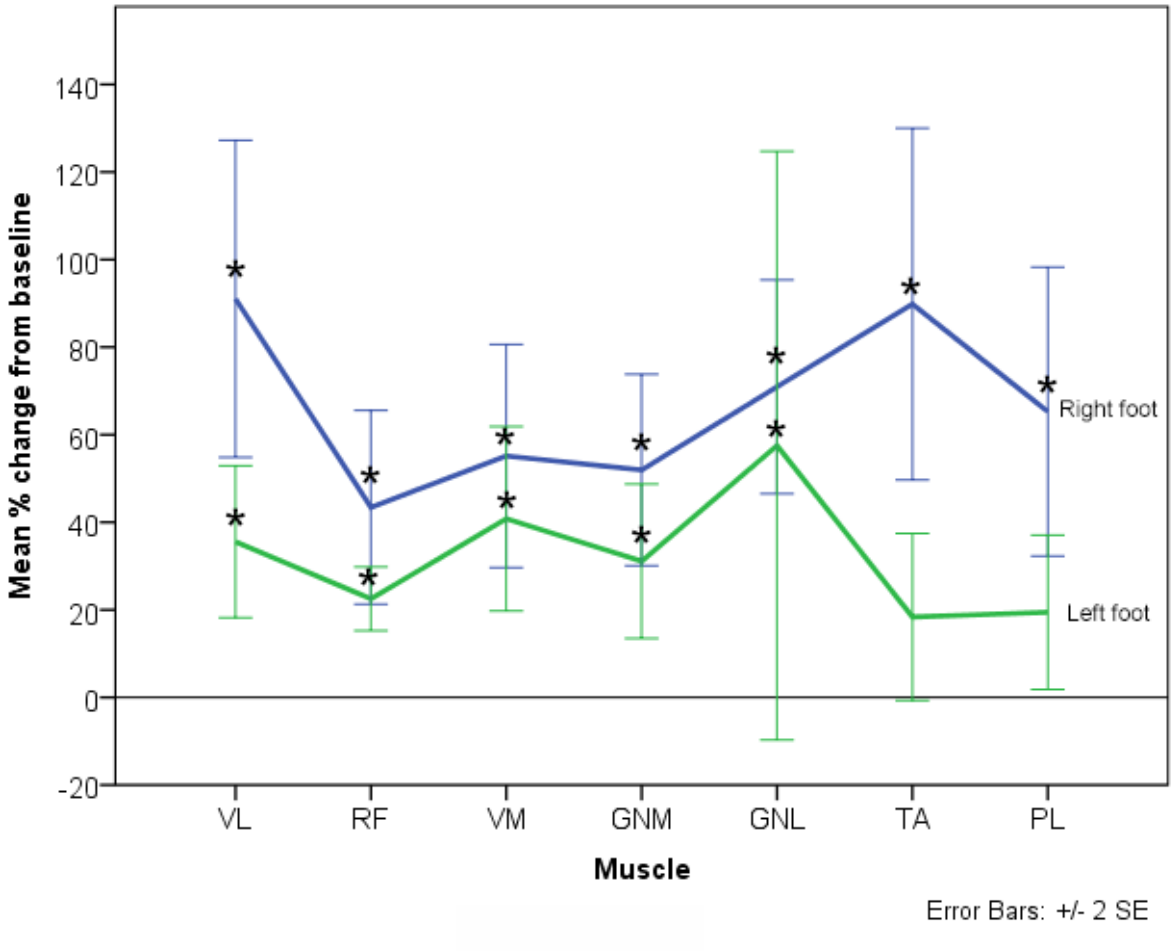


Figure 39 - Laser Doppler changes in blood flow during rest and stimulation in the right and left foot.

(*-p<0.05, Wilcoxon signed-rank; VL-vastus lateralis, RF-rectus femoris, VM-vastus medialis, GNM-gastrocnemius medial head, GNL-gastrocnemius lateral head, TA-tibialis anterior, PL-peroneus longus)

Tolerability

Participants were asked to express how uncomfortable they found the muscle stimulation, assigning a number between 0 and 100 (0 = no discomfort, 100 = maximum discomfort). Values are presented in Table 10, alongside the current applied to each muscle. The muscle with the lowest discomfort value was *vastus lateralis* (15.0) and the highest scoring muscle was *rectus femoris* (66.3). Stimulation of *rectus femoris* was found to be significantly more painful than all other muscles (significance tested using one-way ANOVA, $p<0.05$ in all comparisons).

When plotted against current applied, discomfort score was weakly correlated (Spearman's correlation coefficient 0.2, p=0.3). A line of best fit is illustrated in Figure 40, with R^2 of 0.1. When individual data points are labelled with the protocol muscle stimulated, it can be observed that whilst large muscles such as *rectus femoris* needed high currents to achieve the desired contraction, and caused large amounts of discomfort, smaller muscles such as *peroneus longus, vastus medialis* and *lateralis* tended to caused less discomfort than might have been expected (below the line of best fit). Interestingly, stimulation of *gastrocnemius* was found to be very variable in terms of discomfort experience.

Table 10 - Current intensity applied for protocol with subject tolerability scores

Muscle	Mean discomfort score/100	Mean stimulation current, mA
Vastus lateralis	15.0	19.9
Rectus femoris	66.3	26.2
Vastus medialis	21.3	12.9
Gastrocnemius (medial head)	28.8	18.3
Gastrocnemius (lateral head)	31.3	21.8
Tibialis anterior	21.3	12.1
Peroneus Longus	30.0	17.4

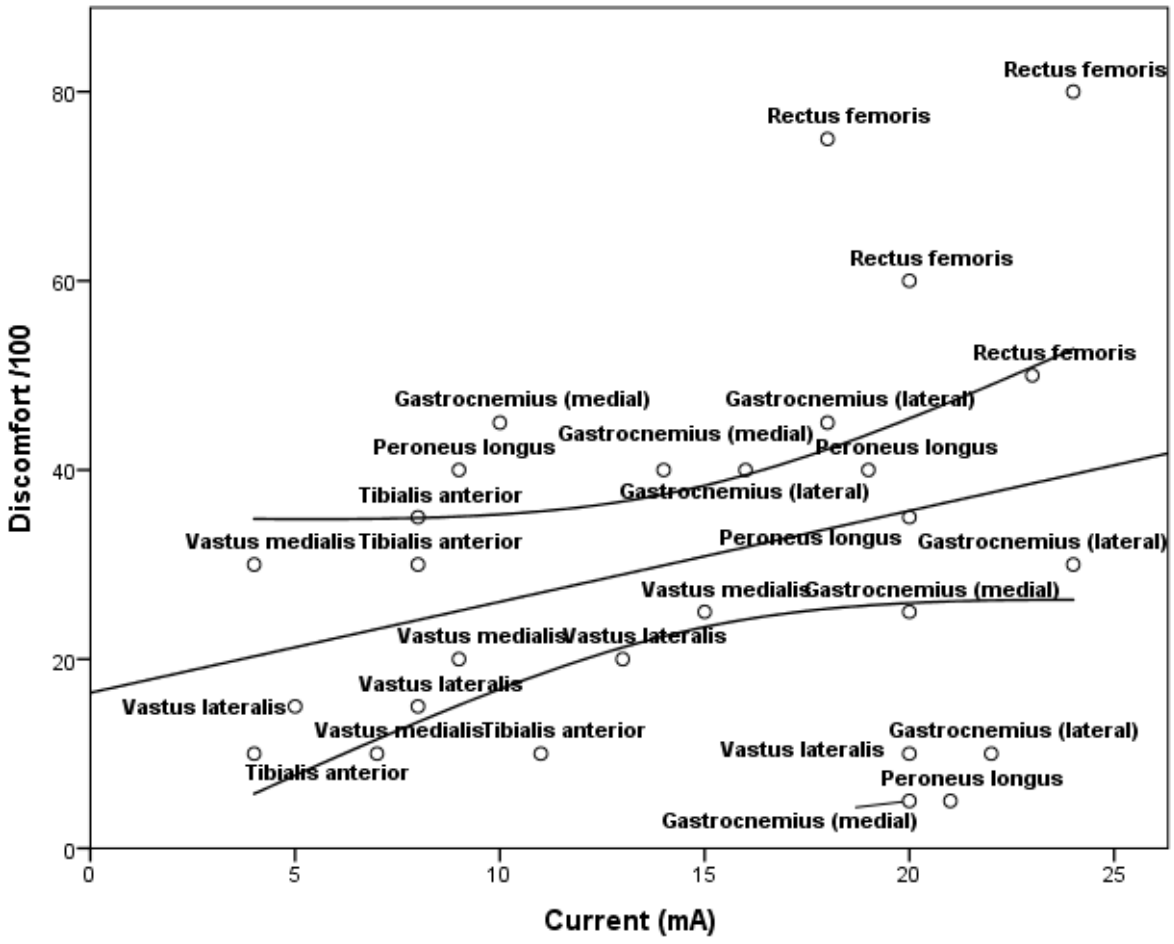


Figure 40 - Graphs showing correlation between discomfort score and current applied with electrical myostimulation during MUSCLE PUMP

Shown with line of best fit and 95% confidence intervals, R2=0.1. Data points labelled with stimulated muscle

6.1.3 Discussion

The current study compared the efficacy of seven lower limb muscles in enhancing venous and microvascular blood flow in the lower extremity. Over 5 minutes, electrical stimulation and contraction of the muscles of the leg caused increased venous velocities in the ipsilateral femoral vein, and increased fluximetry signal in the skin of both feet. This is concordant with the existing literature, and confirms that there are many different muscles that can be utilised through electromyostimulation for the purposes of circulatory support (Izumi *et al.* 2010; Broderick *et al.* 2013; Warwick *et al.* 2013; Lobastov *et al.* 2014; Varatharajan *et al.* 2014; Williams *et al.* 2015; Williams *et al.* 2015). It is not possible to definitively identify the most effective muscle target, as there was such wide variation between individuals. This variation shows how subject-specific muscle stimulation can be. Efforts were made to standardise the protocol by defining threshold values for each subject, yet the haemodynamic response to stimulation markedly varied. This could be linked to individual muscle mass, muscle composition, or differing electrical impedance of body tissues (fat/water distribution, skin moisture).

All individuals progressed through all stages of the protocol, so this should to some extent control for general physiological differences in age, BMI, sex, and hormonal profile. The relatively small sample size limits the statistical representation of findings within the study, which is prone to type one error.

It is noted by the authors that gastrocnemius contraction had a large effect on all venous parameters. If the power calculation had been performed for the parameters of TAMV or VF, it may have been seen to have a statistically significant effect over and above the other muscles. Gastrocnemius is intimately related to a rich plexus of veins, both lying directly

underneath it, and perforating through it (Uhl *et al.* 2015). As many as twelve gastrocnemius vascular pedicles have been described (Aragao *et al.* 2006). Contraction of gastrocnemius causes high speed ejection of blood into the popliteal vein, making it an extremely powerful and efficient pumping unit. Vastus medialis and peroneus longus also appear to have a large enhancing effect on PV, with their respective actions on the thigh and foot pumps. The reality is that physiologically the pumps of the legs work together in synergy, and both ambulation and maintenance of an upright posture will activate many muscles together. It is upon this principle that active clinical manoeuvres to potentiate reflux during ultra-sonographic examination are based. The Wunstorf manoeuvre is executed by asking the subject to flex the toes; the oscillation and Parana manoeuvres depend on moving the subjects centre of gravity anteriorly, with maintenance of upright posture achieved through contraction of multiple antagonistic muscle groups(Mendoza *et al.* 2001; Escribano *et al.* 2003). In the future this may be aided by electromyostimulation of certain muscles over the vein or plexus of interest, and may add to the arsenal of diagnostic gambits.

For the purposes of enhancing venous haemodynamic parameters, causing contraction of any of the muscles in this protocol may be useful in preventing venous stasis. Previous studies have shown that the muscle-pumps of the leg play a crucial role in optimising circulation, and dysfunction may be associated with the onset of clinical symptoms of venous disease (Haenen *et al.* 2000; Simka 2004; Simka 2007). Repeated or regular electromyostimulation of the leg muscle-pumps might have a training and bulking effect on the stimulated muscles, in a similar action to physiotherapy or exercise training (Yang *et al.* 1999; Kan *et al.* 2001). The latter has been shown to alleviate some symptoms of chronic venous disease, and may in the future be an important therapeutic target. The authors anticipate benefits of electrical stimulation

devices in selected vascular cohorts, for relief of pedal oedema, prevention of venous thromboembolic events, or the management of chronic vascular disorders.

It is striking that even brief stimulation periods of under 5 minutes have caused large increases in fluximetry, a surrogate measurement of red blood cell velocity in the skin. This may be due to several factors, including an elevated arteriovenous gradient, possible pressure effects on capillary sphincters, and perhaps even the systemic release of endogenous vasoactive substances. The systemic and distant effects on the microcirculation by electromyostimulation in this study may indicate potential benefits to general cardiovascular health, tissue regeneration (skin ulceration), and recovery after injury or surgery. Previous metanalysis have shown that use of electrical stimulation in the leg can increase transcutaneous oxygen tension, with the potential to expedite healing (Lundeberg *et al.* 1992). There have been no definitive and adequately powered trials, but the hypothesis would not be hard to test.

There was a large variation in tolerability between stimulation of different muscles, and for meaningful conclusions to be drawn efficacy must be interpreted in the context of patient experience. Subjects stated that the majority of reported discomfort came from the electrode-skin interface as opposed to the muscle contraction itself. An effective but painful therapy is of limited use if compliance is low. It is generally understood that increasing the current applied increases the pain experienced by the subject (RM Fish 2009). The higher the current amplitude, the greater the depolarising effect on the structures underneath the electrodes, the greater the number of motor units recruited, and the stronger the contraction effected. Transcutaneous devices tend to use amplitudes of 15-30mA, whilst in our protocol the range of mean current values used were 12.1-26.2mA, which seems appropriate. Intensity

of contraction can also be manipulated by varying other parameters such as frequency, pulse width, pulse interval, and voltage. Some devices such as the geko-T1™ (Firstkind Ltd, UK) use variations in pulse width for increasing contraction strength, whilst others like the Revitive™ (Actegy Ltd, UK) use a combination of cycling parameters. Stimulation of a motor nerve (such as the peroneal nerve) can allow usage of lower settings when compared to direct muscle stimulation (Tucker *et al.* 2010).

The medium and long-term effects of muscle stimulation were not observed in this study. It would be clinically useful to understand the effects of electrical stimulation over hours, days and weeks, especially if stimulation induces a systemic response. The authors would also recommend further evaluation of stimulation in cohorts of patients with vascular disease, as both stimulation variables (tissue impedance, oedema) and vascular factors (reflux, obstruction) will influence subject experience and haemodynamic efficacy. It is not yet fully understood how local pressure effects interact with electrically influenced neurochemical changes, and progress in this area would facilitate further development of tenable electric devices.

Electrical muscle stimulation of skeletal muscle can be used to increase haemodynamic parameters both locally and systemically, and represents a therapeutic target with very few risks or side-effects. The most effective muscle at improving circulation was not identified, but marked variation in efficacy and discomfort between subjects was observed. It may be that subject comfort and adherence to treatment protocols may be more important if used in the management of clinical symptoms. Ideally there should be a balance between differing subject requirements for energy delivery, and the need to design a marketable and economically viable device.

6.2 The effect of common peroneal nerve stimulation on blood flow measurements

Intermittent stimulation of the common peroneal nerve casues muscle contraction of the calf and movement at the ankle. Compression of the deep veins should aid passage of venous blood towards the body. The haemodynamic effect of a transcutaneous device causing peroneal nerve stimulation was evaluated in a small series of healthy subjects.

6.2.1 Method

Ten healthy volunteers were recruited from the Academic Section of Vascular Surgery. They were screened for the presence of peripheral vascular disease via a medical history, full vascular examination, and measurement of ankle brachial pressure index (ABPI). Inclusion and exclusion criteria are displayed in Table 11.

Table 11 - Inclusion and exclusion criteria for small trial assessing haemodynamic effect of geko device

Inclusion criteria	Good physical health
Exclusion criteria	History of cardiovascular disease ABPI <0.9

Vascular ultrasound (Phillips IU22, vascular transducer L12-5, vascular protocol) was used to evaluate arterial and venous flow velocities and volumes in the femoral vessels (femoral vein, superficial femoral artery). A quiet period of 20 minutes was allowed for venous equilibration prior to starting the protocol. The vessels locations were marked on the skin for repeat measurement. During the procedure the subject was positioned semi-recumbent, with knees slightly flexed and externally rotated, supported by a pillow. The geko device was applied to

the right leg, and operated according to manufacturer instructions. Measurements were taken from the right leg: at baseline, and then during stimulation.

6.2.2 Results

Ten healthy recruits completed the protocol in full. Mean ages were 32±4years, BMI 28.9±9kg/m^2. No adverse events were encountered or reported.

The mean increases over time in arterial diameter, peak velocity, TAMV, and volume flow were 0.02cm ($p=0.03$, wilcoxon matched-pairs signed rank; 3%), 24.6 cm/s ($p<0.01$, 57%), 3.9cm/s ($p<0.01$, 159%), and 93.4ml/min ($p<0.01$, 190%). Percentage changes from baseline of all parameters were significant, illustrated in Table 57 (appendices, page 398). Mean absolute changes from baseline were all significant.

Mean increases in venous diameter, peak velocity, TAMV, and volume flow were -0.2cm ($p<0.001$, -16%), 17.2cm/s ($p<0.001$, 173%), 2.4cm/s ($p<0.001$, 185%), and 51.1ml/min ($p<0.01$, 105%). This is shown in the appendices, Table 58 (page 397). Results are presented graphically in Figure 41, showing the changes from baseline over time.

For both arterial and venous components, the last set of measurements (time =720mins) show statistically different values to baseline ($p<0.05$, Wilcoxon matched-pairs signed rank).

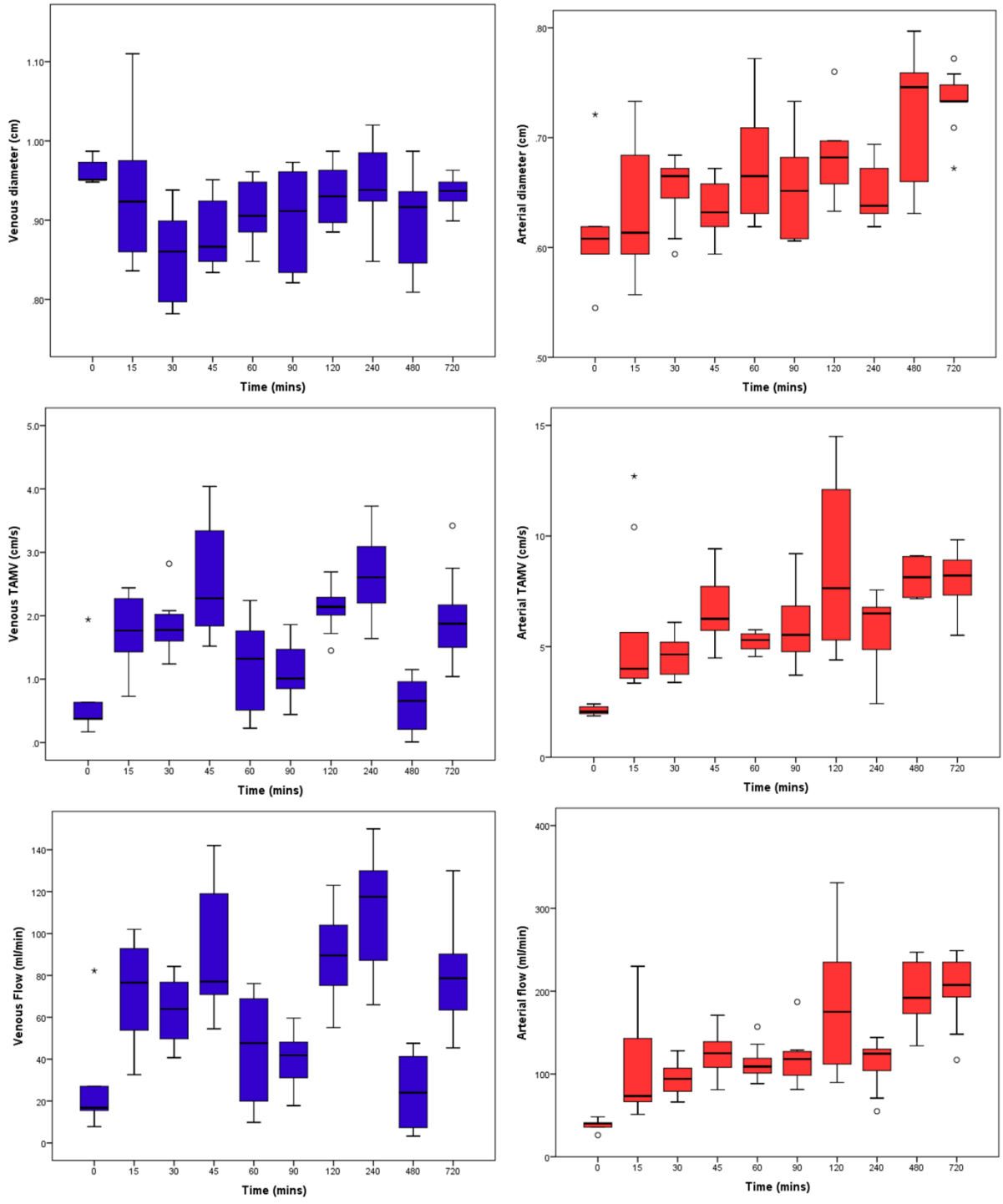


Figure 41 – Haemodynamic parameter percentage changes from baseline over time with common peroneal nerve stimulation

6.2.3 Discussion

Use of the geko NMES device on the common peroneal nerve increases ipsilateral venous and arterial blood velocities and flow rates. This effect is statistically significant in the small group studied here, and represents a physiological doubling to trebling of resting values.

The venous and arterial systems responded to NMES stimulation by differing magnitudes. Venous volume flow rate increased by 93ml/min, compared to 51ml/min in the artery measured. With an expected mean error of ±5ml/min (section 5.6.1.2) this discrepancy needs explanation. It could be misrepresentative of total blood flow changes in the leg, as measurements were taken from the femoral vessels only. The main inflow to the leg is via the common femoral artery, of which the superficial is only one large branch. Likewise, taking measurements from the femoral vein neglects changes in distribution of flow between the deep femoral vein and the great saphenous vein. Collateral flow through complimentary vessels would be unaccounted for. If this were the case to a significant extent, changes to measured inflow and outflow should not necessarily equal each other.

The discrepancy might also be due to changes in venous capacity (and therefore ejection volume) of the leg, or could be due to differential changes in peripheral resistance of the arteries, veins, and microcirculation. Arterial diameter at the level of the femoral vessels was significantly increased with stimulation of the lower leg vessels. This could be a local neuroendocrine reaction to reduced peripheral resistance or muscle metabolism, or as a result of local release of vasoactive substances to vessel wall shear stress (such as nitric oxide). Collection of local and systemic blood samples for vasoactive substances may be advantageous, but might be technically challenging, especially with substances such as nitric oxide which have a very short half-life.

The cumulative effect over time of stimulation on arterial TAMV and volume flow may be advantageous in the management of limb ischaemia. Even over the time period of 12hours, the effect had still not reached a plateau in healthy subjects. If these results can be replicated in a cohort of patients with peripheral arterial disease, then it may open a whole new area of therapeutic intervention for this section of society.

6.3 The effect of electrical footplate stimulation on blood flow measurements

Foot plate NMES devices, such as the Revitive^{TM}, have their effect via stimulation of the foot and calf muscles and nerves. It has been postulated that this might have a differing haemodynamic effect on muscle contraction and blood flow in the lower limb to that effected by stimulation of the common peroneal nerve.

The haemodynamic effect of the device was evaluated in a small series of healthy subjects.

6.3.1 Method

Ethical approval was granted by Imperial College Research Ethics Committee. Healthy volunteers were recruited and informed consent was obtained.

Inclusion criteria were good general health, age 18 years or above, and ABPI 0.9-1.0. We excluded those with a pacemaker or implantable cardiac defibrillator (AICD), pregnant women, an existing or previous DVT, or clinical evidence of vascular disease. The effects of neuromuscular stimulation on lower limb venous and arterial blood flow and velocity were investigated in ten and twenty healthy volunteers, respectively. The same inclusion criteria was applied for both groups. Volunteers were not given any specific instructions regarding diet or clothing for the study. All examinations were carried out in the same room which was temperature controlled.

The Revitive^{TM} device (Actegy Ltd, UK) was applied bilaterally for 30 minutes, whilst the user remained in the seated position. The level of stimulus used in each individual was determined

by doubling the minimum stimulus intensity required to evoke visible calf muscle twitching, the motor threshold. This was thought to elicit a reasonable yet comfortable compressive force on the vasculature.

Using the Phillips IU22 ultrasound system, with L12-5 vascular probe and standard flow volume measurement protocols, the blood flow (cc/min) and time averaged mean velocity (TAMV; cm/s) of the superficial femoral artery and vein were measured using ultrasound after 10 minutes at rest (baseline). The skin was marked for ease of repeat measurements. This was then compared to those after 15 minutes stimulation, and immediately after stimulation cessation at 30 minutes. The protocol is illustrated in Figure 42. Three readings were taken at each time point and an average taken. Data was analysed on GraphPad Prism 6 using the Wilcoxon matched-pairs signed rank test (significance fixed at 95%).

Volunteers gave verbal feedback with regards to what level of stimulation intensity was tolerable and they were informed that if they found the device intolerable at any point then their assessment would be terminated.

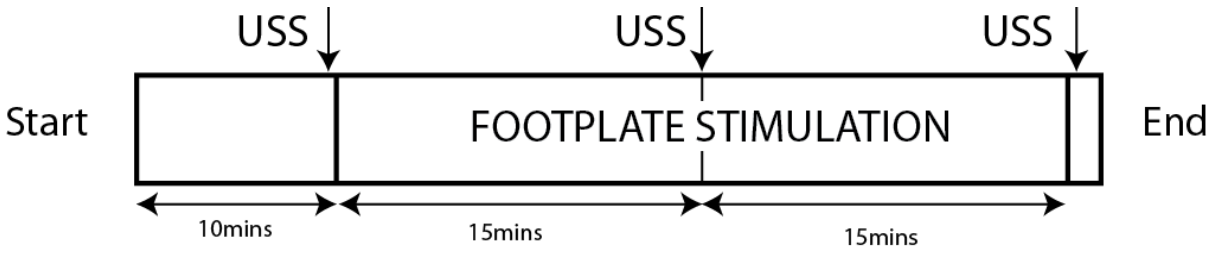


Figure 42 - Protocol for measuring the effect of footplate stimulation on blood flow changes

(USS – ultrasound scan measurement)

6.3.2 Results

Venous data

Ten healthy volunteers (six males, four females) were studied, mean age 34 years (range 21-63).

Median blood flow and TAMV increased from baseline at 15 minutes stimulation by 88ml/min (31%, IQR 7.2 - 186; p=0.014) and 1.1cm/s (38%, IQR -2.5 - 127, p=0.065) respectively (Figure 43). Immediately after cessation of stimulation they decreased by 27ml/min (22%, IQR -53 - 68; p=0.38) and 0.92cm/s (31%, IQR -64 - 69; p=0.38) compared to stimulation values.

Arterial data

Twenty healthy volunteers (10 males, 10 females) were studied, mean age 41 years (range 23-64).

At 15 minutes of stimulation, there was a significant increase in median blood flow and TAMV compared to baseline by 39ml/min (53% increase, IQR 13 - 86; p<0.0001) and 2.2 cm/s (62% increase, IQR 30 - 71; p=0.0003), respectively (Figure 43). After stimulation flow and TAMV decreased from baseline by 4.0ml/min (3.8%, IQR -18 - 42; p=0.77) and 0.5cm/s (6.7%, IQR -27 – 63; p=0.71).

Changes to baseline measurements with the protocol are illustrated in Figure 43, whilst percentage changes to baseline are illustrated in Figure 44. Results are tabulated in the appendices, Table 59 (page 398).

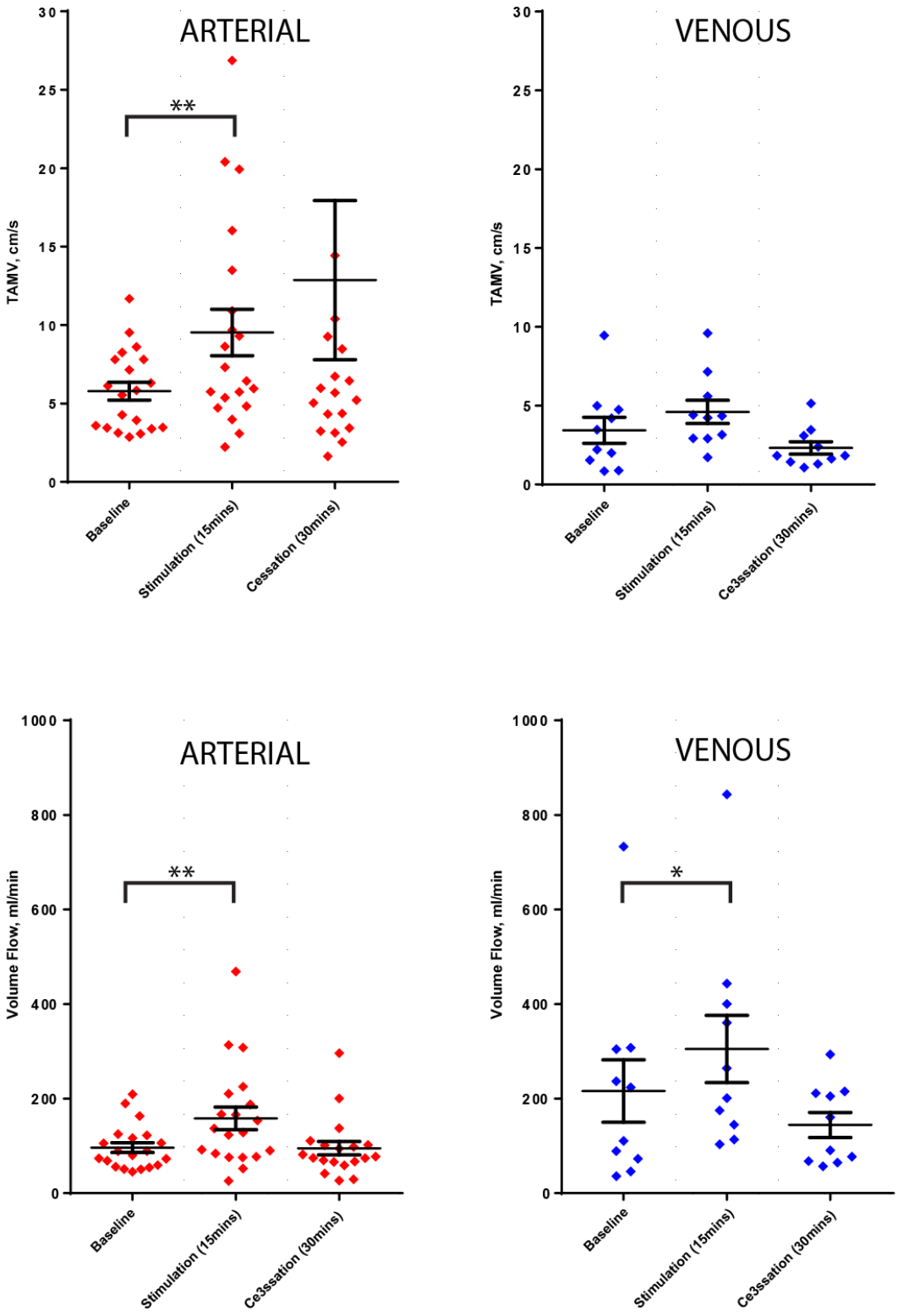


Figure 43 - Haemodynamic ultrasound from the femoral vessels with use of a footplate NMES device

(TAMV - time average maximum velocity, *-p<0.05, **-p<0.01, Wilcoxon matched-pairs signed rank)

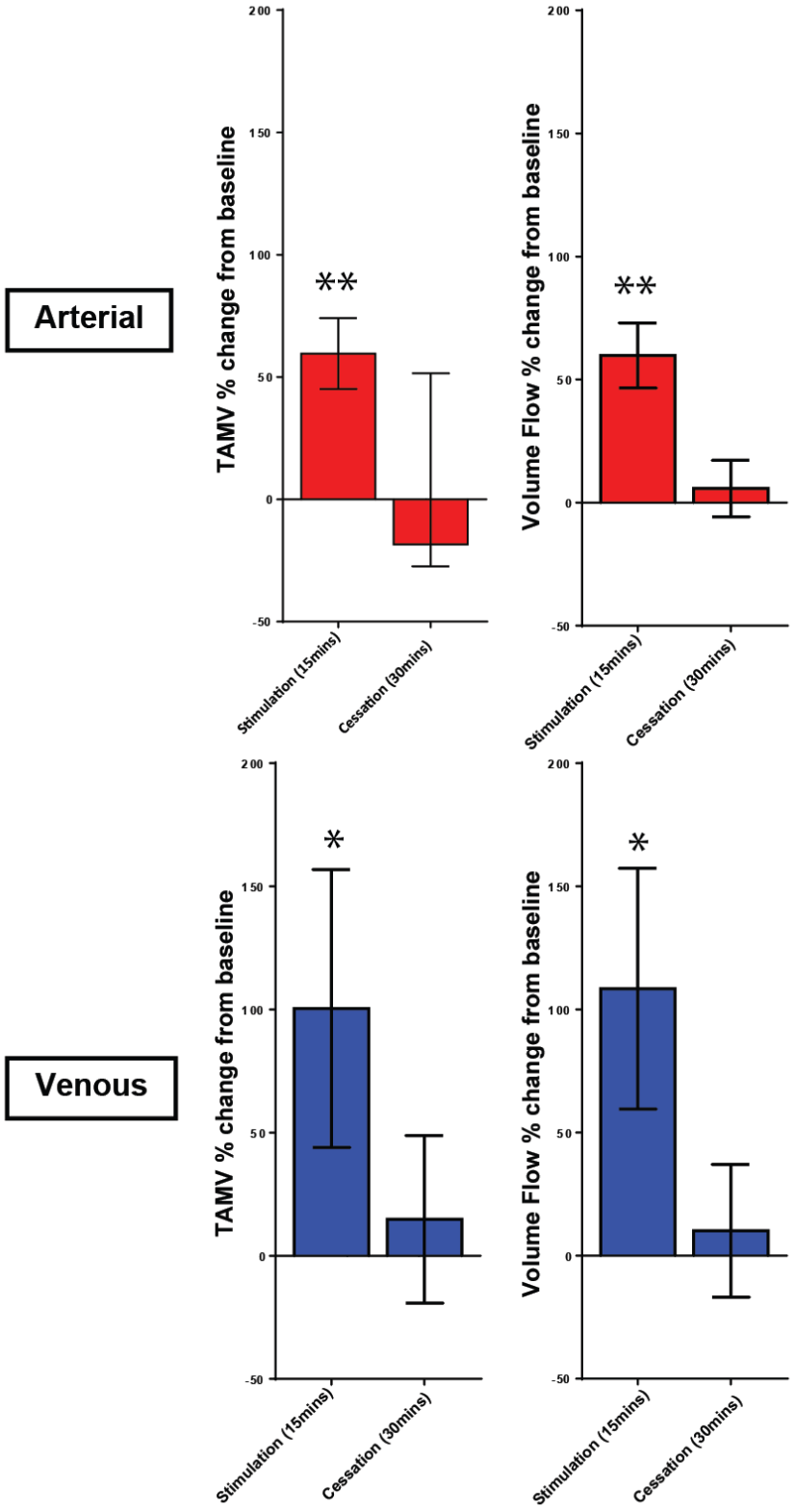


Figure 44 - Percentage changes from baseline in ultrasound parameters with use of NMES footplate device.

(TAMV - time average maximum velocity, *-p<0.05, **-p<0.01, Wilcoxon signed-rank)

All subjects completed the protocol in full, and no adverse events were reported. One subject disliked using the device, and turned the intensity setting down for some programmes. Vital signs remained in normal range throughout.

A comparison of device methods and their effects on the blood flow in the femoral veins (the results from sections 6.2 and 6.3) have been combined in Table 12, comparing median values recorded at 0 minutes and 15 minutes. The geko^{TM} results used are tabulated in the appendices (Table 60, page 399). Please note that these readings were not recorded during a unified protocol, and testing conditions were different.

Table 12 - A comparison of changes to blood flow in the femoral vessels with two different types of NMES stimulation

	Arterial		Venous	
Stimulation type	Common peroneal nerve	Footplate	Common peroneal nerve	Footplate
TAMV change from baseline	1.94cm/s (94%)	2.2cm/s (62%)	1.5cm/s (556%)	1.1cm/s (38%)
Volume flow change from baseline	34ml/min (84%)	39ml/min (53%)	63ml/min (447%)	88ml/min (31%)

(median values, time at 15 minutes stimulation)

6.3.3 Discussion

These results provides evidence that footplate NMES significantly increases both venous and arterial blood flow in the lower limbs. The device was tolerated well by most subjects, and no adverse events were experienced. The significant advantage of the footplate device application during the protocol was that it was extremely intuitive, with subjects able to operate the device with minimal input from the trial technician. This compares to the

common peroneal nerve adhesive device, which for the purposes of bench testing was applied by the technician after examination of the limb, boney landmarks and a bit of trial and error testing to optimise position and stimulation level.

After cessation of stimulation, parameters were not statistically significant to baseline levels. The effect of the footplate device on blood flow appears to be limited to the usage time only.

A comparison of blood flow measurements between two NMES devices was attempted in Table 12, where medians from two different protocols were compared side-by-side. The protocol for the common peroneal nerve device was carried out in the semi-recumbent position, whilst the protocol for the footplate device was performed in the seated position. This may have had a large effect on the venous filling of the legs, and the gravitational resistance to venous return. Results should be interpreted with this in mind. Changes in arterial parameters for both devices are comparable, causing a 50-100% increase in TAMV and volume flow. Venous measurements for TAMV and volume flow were 556/38% and 447/31%. This appears to suggest that one device is superior to the other in this respect. However, when the absolute change values are interrogated (1.5/1.1 cm/s and 63/88 ml/min) this massive discrepancy in percentage change may be an artefact caused by differing baseline values in the two populations. The only way to properly compare these two devices in a more accurate fashion would be in a unified protocol under the same testing conditions.

6.4 Concomitant use of common peroneal nerve stimulation and graduated compression stockings

Neuromuscular stimulation has part of its haemodynamic effect through activation of the muscle pumps of the leg. Bench testing was performed to see if the application of compression hosiery might enhance the effect of NMES on blood flow in the lower limb.

6.4.1 Method

Five healthy subjects were recruited from the Department of Vascular Surgery at Imperial College London. The eligibility criteria are shown in Table 13, and subjects were screened via structured clinical history and examination of the peripheral vascular system. An ABPI was performed on each leg.

Table 13 - Eligibility criteria for adjuvant effect of graduated compression stockings with use of geko

Inclusion criteria	Age greater than 18 years old
Exclusion criteria	History of peripheral vascular disease Evidence of venous reflux in the leg >0.5s ABPI<0.9

Subjects were seated on a chair, with feet resting on the floor. Laser doppler fluximetry probes were attached to the dorsum of the left and right feet. A below-knee grade 2 graduated compression stocking (Medi, UK) was placed on the left leg, non-compressive hosiery for warmth placed on the right.

NMES devices (geko-T1, FirstKind Ltd, UK) were placed bilaterally over the common peroneal nerve according to manufacturer's instructions. Stimulation levels used were titrated to give a definite dorsiflexion movement of the foot, whilst still ensuring comfort.

Haemodynamic ultrasonography was performed using Philips iU22, linear transducer, and vascular protocol. Measurements were taken from common femoral artery and femoral vein, and marked on the skin for repeat measurement. Baseline values were recorded after 15 minutes relaxation. Stimulation values were recorded 10 minutes after initiation of stimulation, with devices on.

After 10 minutes stimulation with the NMES devices, the compression and non-compression hosiery were swapped, with devices left on. Stimulation values were compared to baseline. The protocol is illustrated in Figure 45.

Statistical analysis was performed using Prism v6.01 (GraphPad Software, CA, USA).

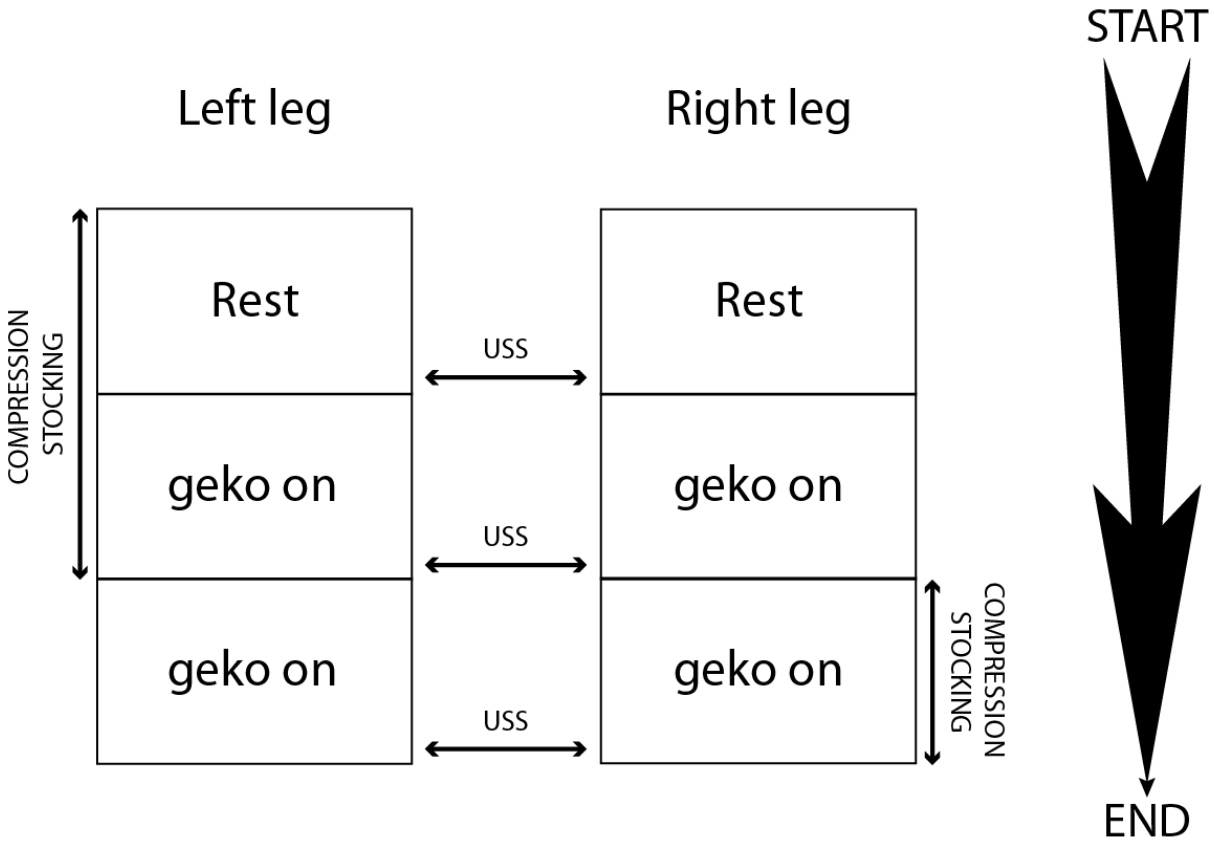


Figure 45 - Protocol comparing haemodynamic effect of the geko, with and without concomitant compression

6.4.2 Results

5 subjects completed the protocol, mean age 26.4±1years and BMI 26.1±1kg/m^2. No adverse events were encountered.

Mean arterial changes to peak velocity, TAMV and volume flow with geko™ usage (without/with compression) were 6.9±6/6.5±2 cm/s, 2.4±1/2.2±3 cm/s, and 28.7±18/57.3 ±39 ml/min. Mean venous changes were -0.4±6/-35.4±28cm/s, 0.2±4/0.0±4cm/s, and 45.0±132/0.9±48 ml/min. Percentage changes are shown in Table 14 and Figure 46.

Mean changes in laser doppler fluximetry were 58.8% with no compression, and 297.4% with compression.

Table 14 - Percentage change to arterial and venous haemodynamic parameters with NMES stimulation, both with and without compression hosiery

(mean ±standard deviation; *-p<0.05, Wilcoxon signed-rank; $ - p<0.05, Wilcoxon matched-pairs signed rank)

		No compression	Compression hosiery	*P value (Wilcoxon matched-pairs signed rank)*
Arterial	**Peak velocity**	8.1 ±7*	7.0 ±2*	*0.5*
	TAMV	40.1 ±16*	52.0 ±64	*0.6*
	Volume flow	20.3 ±13*	49.3 ±38*	*0.1*
Venous	**Peak velocity**	5.5 ±41	-57.1 ±18*	***0.002***
	TAMV	14.3 ±56	17.9 ±66	*0.8*
	Volume flow	27.1 ±59.2	4.9 ±31	*0.2*

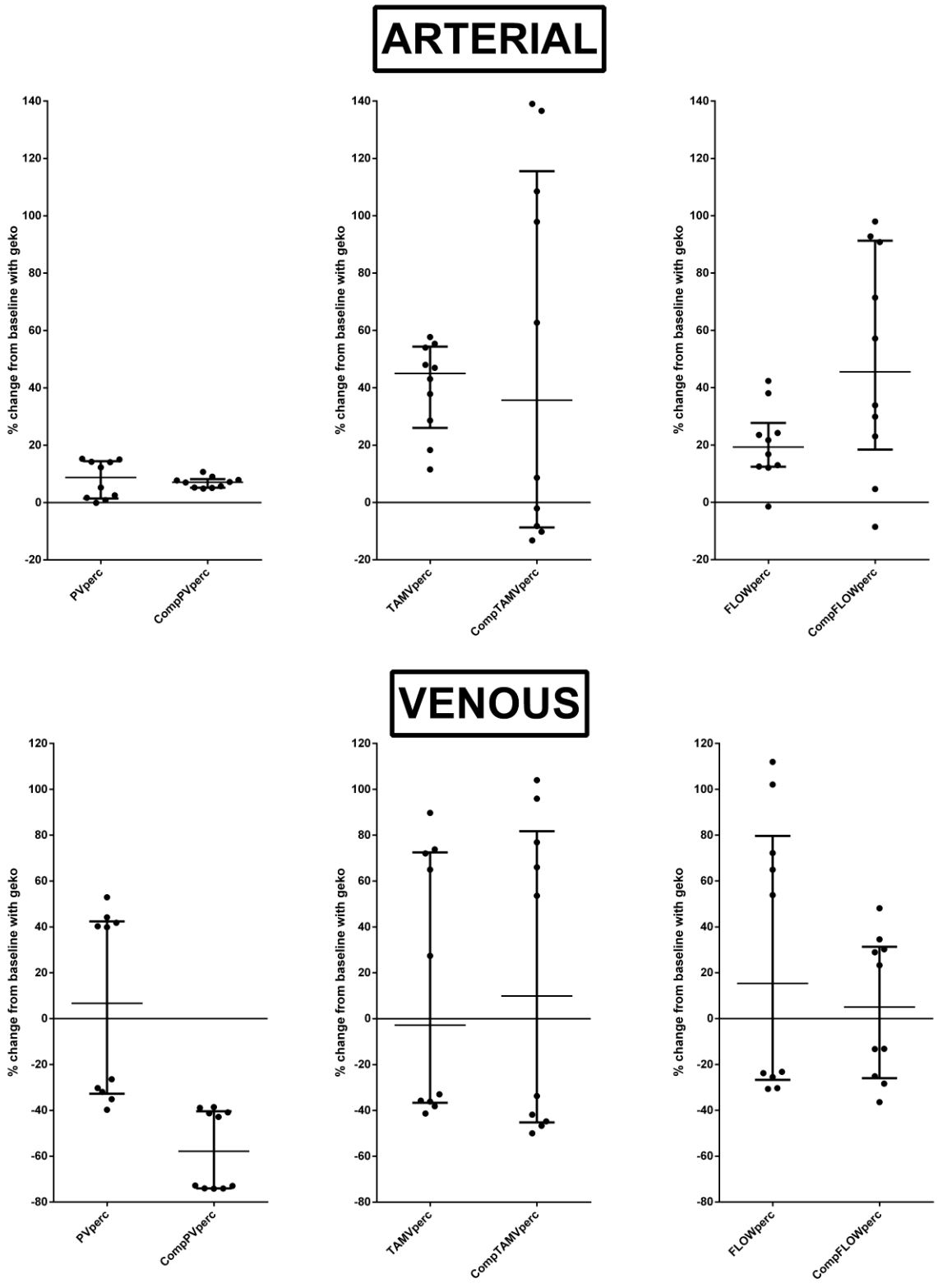


Figure 46 - Percentage changes to arterial and venous haemodynamic parameters with geko usage, with and without compression

(PV – peak velocity, TAMV – time averaged maximum velocity, comp – graduated compression applied)

6.4.3 Discussion

In this very small bench-test trial of five subjects, the NMES device significantly increased arterial PV, TAMV and volume flow. The addition of grade 2 graduated compression stockings increased mean values for TAMV, and doubled volume flow compared to no compression, but did not reach statistical significance. Use of the NMES device caused mean increases in venous parameters, but did not reach statistical significance. The addition of compression hosiery reduced recorded peak velocity values compared to baseline, and percentage changes with compression were significantly more negative than without compression.

With application of NMES, absolute change in arterial inflow was 29ml/min, and venous outflow 45ml/min. When compression is applied, the changes from baseline with NMES to arterial and venous volume flow measurements were 57ml/min and 1ml/min. Despite the lack of statistical significance this small sample, the effect on venous outflow of the stockings appears large. Application of the stockings at rest could have effected changes in the venous reservoir capacity via compression of the venous plexi of the calf and foot (Somerville *et al.* 1974; Gjores *et al.* 1977; O'Donnell *et al.* 1979; Evander *et al.* 1984; Partsch 1984; Christopoulos *et al.* 1990). Each muscle contraction may completely empty the underlying veins, but will shift much smaller volumes of blood. This may be reflected in the decreased venous velocities we detected at the level of the groin with compression. The use of air plethysmography to measure venous ejection fraction would be suited to measure the efficacy of the NMES device with relation to resting blood volume of the leg.

It has been noted in the literature that the application of compression garments to the lower leg causes compression of underlying veins, but the effect differs between the superficial and deep systems. Downie et al used MRI to illustrate the decreased cross-sectional areas of the

deep veins (64%) compared to the superficial (39%) in prone healthy volunteers (Downie *et al.* 2007). Uhl et al explained this deep vein preferential narrowing using intramuscular pressure measurements, where the interface pressure applied to the subcutaneous tissues acts in an additive fashion to resting muscle pressures. At the skin interface and around the superficial veins, pressures are near 0mmHg, whilst the muscle compartments rest at approximately 11mmHg). The application of 22mmHg compression results in a superficial pressure of 0+22mmHg, whilst deep structures are subjected to 11+22mmHg (Uhl *et al.* 2015). This is even more noticeable in the standing position, where resting intramuscular pressures can be as high as 34mmHg(Uhl *et al.* 2015). The action of NMES causes propulsion of blood cranially via compression of blood-filled veins. If the deep veins are compressed and under filled, redistributive effects between the deep and superficial systems may play a part in venous return routes to the body, and may affect the proportion of blood being measured at the level of the femoral vein. Blood returning via the great saphenous vein would be lost to measurement.

There may be changes to the microvascular system in the presence of compression. Laser doppler fluximetry measurements taken from the dorsum of the foot were increased with compression (59% versus 297%). This could represent changes in the effective capacity of the arterioles, capillaries and venules; either through reflexive or neuroendocrine vasodilatation, pressure dilatation, or recruitment of more numbers of open vessels. This could be further investigated using imaging techniques such as capillaroscopy, or dynamic contrast-enhanced ultrasound.

Ultrasound measurements of venous flow are prone to variation, both due to the second-to-second changes in venous vascular tone, external compression, and cardiac output and

respiratory intra-abdominal and thoracic pressures. Due to the high variability of venous measurements, and differing baseline values between subjects, this may spread values over a larger distribution, making statistical differences more difficult to elucidate. A small study of five subjects is prone to type 2 statistical error (failure to reject the null hypothesis).

Potential applications

Compression stockings are valuable in the management of pedal oedema and symptomatic chronic venous disease (O'Meara *et al.* 2012). Their action to reduce the venous volume of the leg, and reduce venous filling index might be advantageous when applying NMES in people with compromised venous systems (chronic venous disease). This may be reflected in greater response to device (reduction of tissue oedema between skin and common peroneal nerve) and possibly earlier symptom reduction and improvement in quality of life measures. Venous ulcers are often managed with dressings and four layer compression. There is the possibility that the use of NMES in conjunction with four-layer bandaging may enhance the blood supply to the skin of the foot, and by inference improve ulcer healing rates.

Graduated compression stockings have been recommended by the Cochrane Collaboration for use in the prophylaxis of venous thromboembolism in hospitalised patients (Sachdeva *et al.* 2014). It may be that increasing the number of muscle contractions with the use of NMES would decrease incidence of deep vein thrombosis by combatting stasis, but it is the feeling of the author that haemodynamic studies alone would be insufficient to support their use in this setting. Proper evaluation in a clinical trial with clinical and radiological diagnosis of VTE would be warranted.

The large increases in arterial inflow with the use of geko and compression may find utility in athletic recovery after training. The extra units of oxygen and carbohydrate per unit time

delivered to the muscles and tissues of the leg may reduce the time needed to recover after exertion. This hypothesis could be tested by a protocol using endpoints such as blood sampling for anaerobic metabolic products (e.g. lactic acid), objective performance measures (e.g. time to complete set of aerobic manoeuvres), and subjective measures of muscle pain.

6.5 Comparison of NMES with intermittent pneumatic compression

6.5.1 Intermittent pneumatic compression

Intermittent pneumatic compression (IPC) has established itself as a clinically useful tool for improving peripheral haemodynamic forces acting on veins, arteries, and lymphatics.

In 1812 Sir James Murray found that the application of differential amounts of pressure to different parts of the body caused changes in tissue perfusion. He championed the use of the "full body suction bath" in the treatment of sepsis secondary to cholera (Murray). Although unsuccessful, this was later taken up by Gibbon and Landis in 1930, where they used alternating positive and negative pressures to the limbs to effect "passive vascular exercises" (Herrmann *et al.* 1934; Landis *et al.* 1934). In the 1950's Allwood and Loane created what might now be recognised as the IPC machine – a rhythmically inflating and deflating pneumatic leg cuff, that was shown to increase xenon and iodine clearance from the foot (Allwood 1957).

The modern day IPC machine consists of a number of bladder reservoirs that are incorporated into the walls of a cylindrical sleeve garment (Figure 47). By using an air compressor to sequentially inflate and deflate the bladder reservoirs, intermittent pressure can be applied to the leg. By applying increased pressures distally, and lower pressures proximally, a pressure gradient can be applied which enhances venous emptying of the foot, calf and thigh. A garment deflation period allows venous refilling, although different machines use different combinations of times, pressures and programmes depending on indication. IPC machines used in the management of venous disease tend to operate over venous pressures (30-

50mmHg maximum), whilst arterial systems operate around systolic blood pressure (120mmHg maximum).

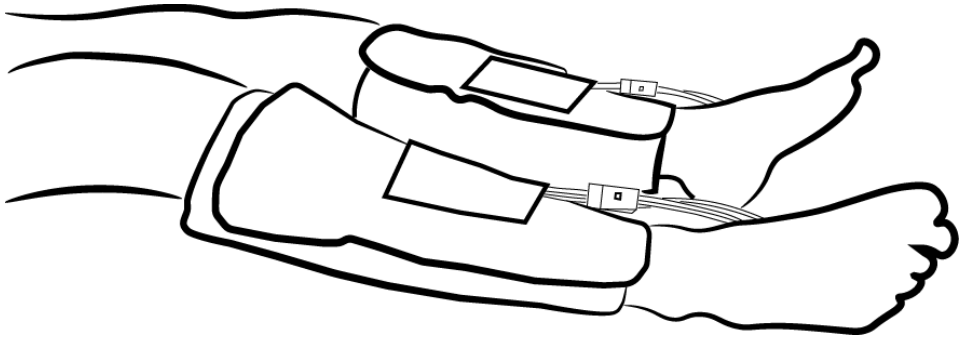


Figure 47 - Diagrammatic representation of an intermittent pneumatic compression device, applied to the lower limbs

IPC has been shown to be an effective treatment for a variety of circulatory disorders. There is strong clinical evidence demonstrating the efficiency of IPC in the treatment of lymphoedema (Morris 2008), prophylaxis against deep vein thrombosis (Urbankova 2005), enhancing the healing of venous ulcers (Comerota 2011) and improving arterial ischaemia (Delis *et al.* 2005), however the mechanisms of action of these devices are not yet fully understood. Reports have consistently shown that IPC devices enhance venous flow volume and velocity in both the femoral and popliteal veins (Morris *et al.* 2004). Significant increases in the popliteal artery flow were also reported during the application of IPC in patients with critical limb ischaemia (Labropoulos *et al.* 2005). The haemodynamic changes accompanying IPC use appear to correlate with its beneficial effects (Lurie *et al.* 2003; Labropoulos *et al.* 2005).

The limitations of IPC use include improper fitting, inappropriate use of device, peroneal nerve injury, discomfort, and excessive heat and sweating under the inflatable cuffs

(Lachmann *et al.* 1992). The size, weight and external power source requirements limit mobility and contribute to poor compliance, which restricts the efficacy of IPC devices (Comerota *et al.* 1992).

The hypothesis of the following study is that NMES via the common peroneal nerve will produce an improved haemodynamic effect equal to or greater than application of intermittent pneumatic compression.

6.5.2 Methods

Ethical approval was granted by National Research Ethics Committee (Brent), reference 11/LO/1292, and registered with www.clinicaltrials.gov under reference NCT01939288.

10 healthy volunteers were recruited and screened for evidence of cardiovascular disease. Inclusion and exclusion criteria are shown in Table 15.

An ankle-brachial pressure index (ABPI) was taken on all subjects. A urinary pregnancy test was performed in women of child-bearing age.

Table 15 - Inclusion and exclusion criteria for trial of NMES versus IPC

Inclusion criteria	Age over 18 years Body mass index 17-30 kg/m^2
Exclusion criteria	Past medical history of heart disease or respiratory disorder Current pregnancy History of peripheral vascular disease Cardiac pacemaker History of leg fractures or metal implants in the leg ABPI<0.8

DEVICE 1 (IPC): The SCD Express™ Compression System (Covidien, Ireland) consists of a controller, non-disposable tubing and medium size knee-length leg garments. The adjustable compression sleeve contains three bladders to deliver circumferential, sequential and gradient compression. The compression cycle delivers 11 seconds of 45mmHg pressure at the ankle and 40 mmHg at the calf (Kakkos, Griffin et al. 2005).

DEVICE 2 (NMES): The geko™ was fitted bilaterally, and device stimulation level set to the minimum level that can achieve upward and outward twitching of the foot when raised from the ground.

Haemodynamic ultrasound

Haemodynamic ultrasound was performed according to the standard protocol. Venous compression ultrasonography of the left leg on the day of study confirmed the absence of deep venous thrombosis and significant venous reflux (>0.5 seconds). Subjects were positioned semi-recumbent on an examination couch, and allowed to adjust position for comfort during the scanning protocol. All ultrasound measurements were taken from the left leg. The left superficial femoral artery and superficial femoral vein were identified and the position of the probe was marked on the skin for consistency of repeat measurements with the leg externally rotated at the hip to 35-40° and flexed at the knee according to subject comfort. An equilibration period of 10 minutes was observed before the first protocol scans were taken, during this time the subject was allowed to relax with eyes closed. Five repeated measurements of TAMV and volume flow rate were taken, recorded via an electronic screen shot, and an average of the five measurements was made later. Peak velocity (PV) of the greatest waveform amplitude of each screenshot was analysed at a later date using calibrated ImageJ software (National Institute of Health, USA, version 1.46R). As the activation cycle of

the IPC device is greater than the maximum screenshot time available, it is not possible for the equipment to conduct a single measurement and single calculation.

Therefore a compound equation using two measurements has been used.

$$Total\ volume\ flow\ (ml/min) = \left[\frac{tA(s)}{t(B(s)}\ x\ A(ml/min)\right] + \left[(1 - \frac{tA(s)}{tB(s)}\ x\ B(ml/min)\right]$$

where tA(s) and A(ml/min) respectively are the time and volume flow during the inflation part of the cycle, and tB and B are the time and volume flow during deflation and rest (Figure 48). The NMES device operates at 60Hz therefore the data capture during 15 seconds was adequate for direct measurement.

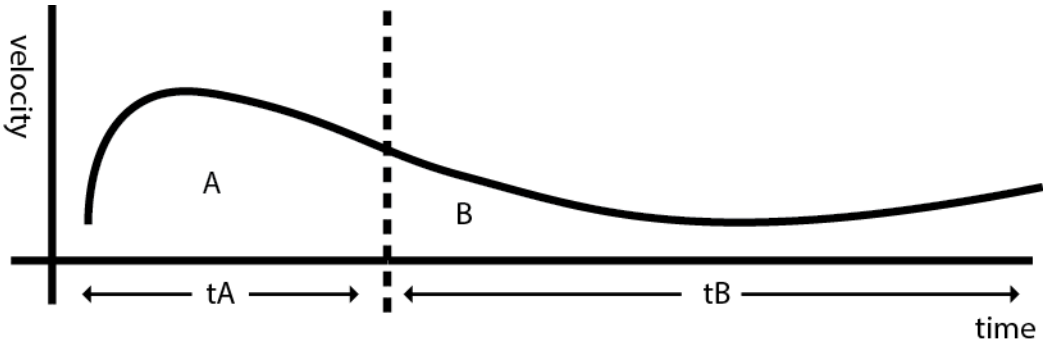


Figure 48 - Diagrammatic representation of intermittent pneumatic compression cycle for measurement equation

(area under curve is equal to volume flow rate)

Patients were randomised by a closed envelope method into two groups to create a cross-over interventional trial. Group A had device 1 applied to both legs and activated first, followed by removal and application of device 2 (Figure 49). In group B the order was reversed. Devices were applied according to manufacturer's instruction.

After an equilibration rest period of ten minutes, baseline haemodynamic measurements were recorded (arrows) and the first device was applied and activated for a 20 minute time period before repeat measurements were taken with the device on, off and 10 minutes post-cessation of device. The subject was allowed to rest for a 20 minute wash-out period. The second device was activated and, after 20 minutes, repeat haemodynamic measurements were taken as before.

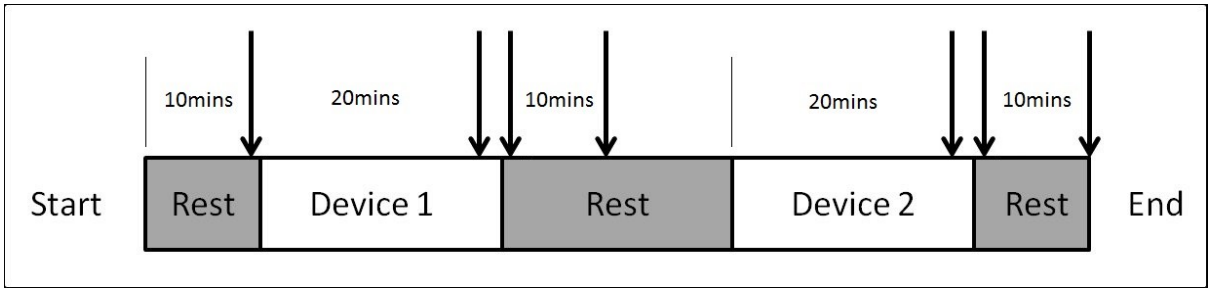


Figure 49 - Trial protocol for the comparison of neuromuscular stimulation and intermittent pneumatic compression

Tolerability of the device was rated by each user at the end of the protocol, using a verbal reported score (0-5; no pain to that experienced by inflating a blood pressure cuff to 200mmHg)

Results were analysed using Prism (Version 5, GraphPad Software Inc., USA), analysed for normal distribution, and then treated as parametric or non-parametric data as appropriate, with significance taken at 0.05.

6.5.3 Results

Demographics

10 healthy recruits were screened and enrolled onto the study (CONSORT diagram shown in Figure 50). There were 4 males, mean age was 27.1±4 years and BMI 24.8±4 kg/m^2. The absolute range of ABPIs was 0.9 to 1.1. Subjects denied any co-morbidities. One female had had 2 previous laparoscopies for endometriosis, and 2 females were on the oral contraceptive pill at the time of trial. All participants completed the study in full.

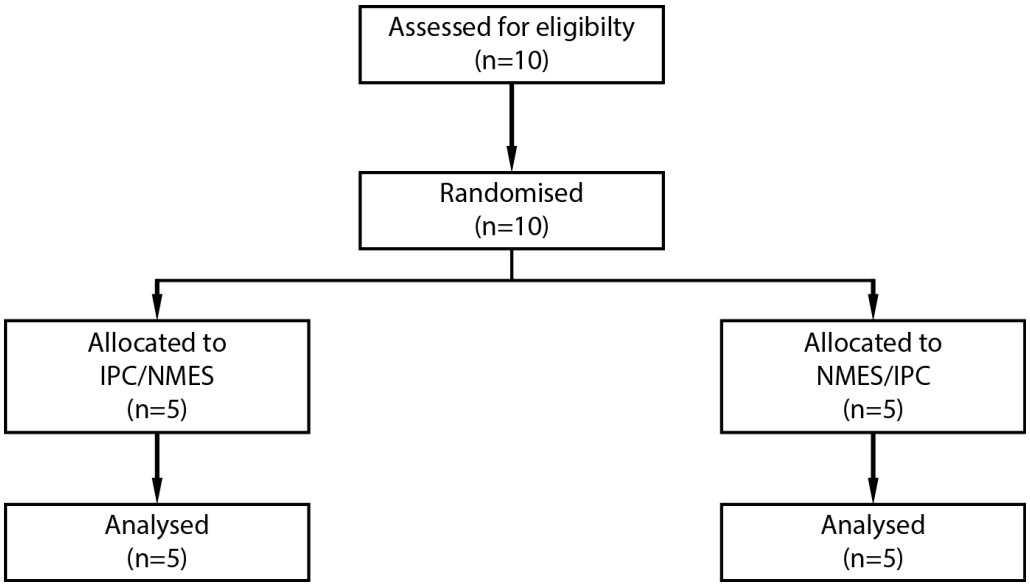


Figure 50 - CONSORT diagram for trial comparing intermittent pneumatic compression and neuromuscular stimulation

Haemodynamic

The ultrasound results are tabulated in the appendices (

Table 61, page 400).

- **Arterial**

IPC caused a median change of -8% (-5.2cm/s, not significant), -12% (-0.61cm/s, ns), -13% (-20ml/min, ns) in PV, TAMV and volume flow rates respectively, (Wilcoxon matched-pairs signed rank). NMES caused 11% (6.8cm/s, $p<0.05$), 84% (4.2cm/s, $p<0.05$), 75% (90ml/min, $p<0.05$) changes in the same parameters.

- **Venous**

IPC caused a median change of 51%(6.2cm/s, $p<0.05$), 5%(0.2cm/s, ns), 3%(4.2ml/min, ns) in peak velocity, TAMV and volume flow rates respectively; whilst NMES stimulation caused a 267%(37cm/s, $p<0.05$), 60%(2.1cm/s, $p<0.05$), 84%(108ml/min, $p<0.05$) change in the same parameters.

Results are presented graphically in Figure 51 and Figure 52.

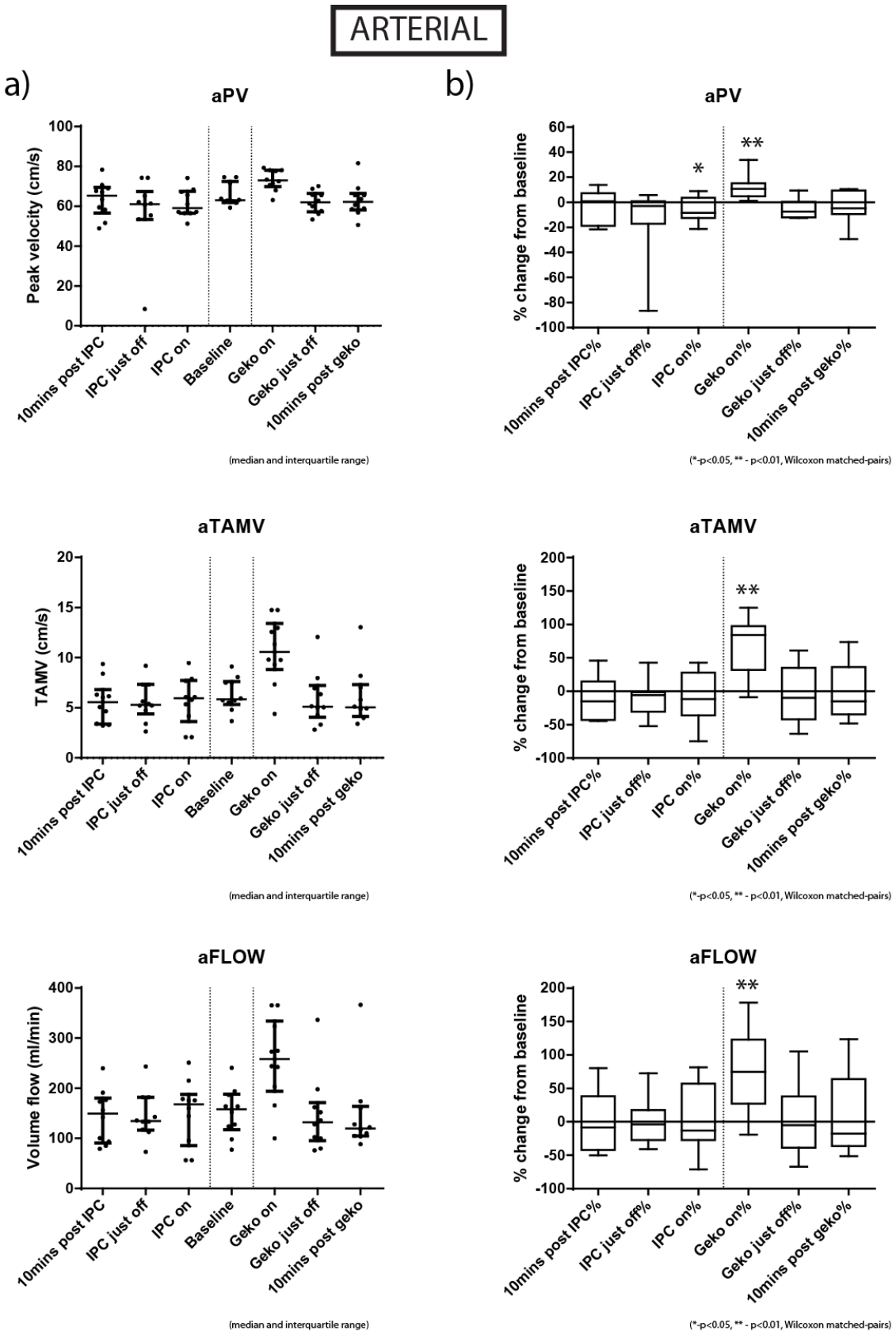
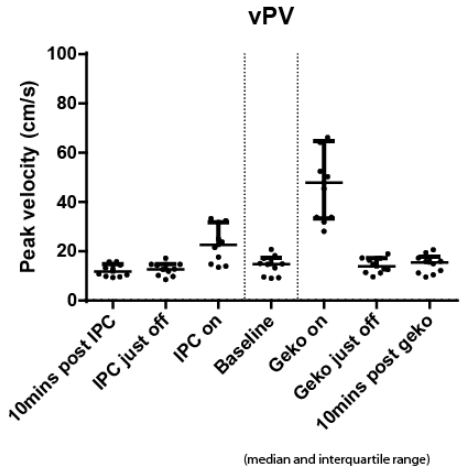


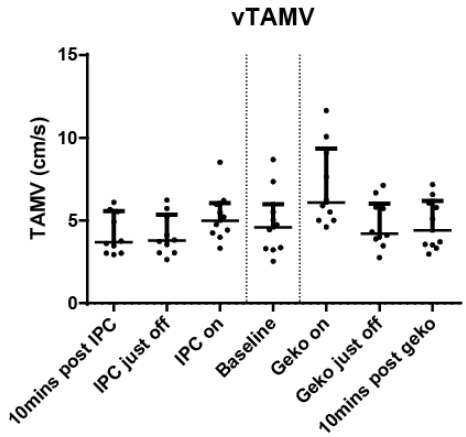
Figure 51- Graphs depicting arterial haemodynamic changes with the application of intermittent pneumatic compression and neuromuscular stimulation

VENOUS

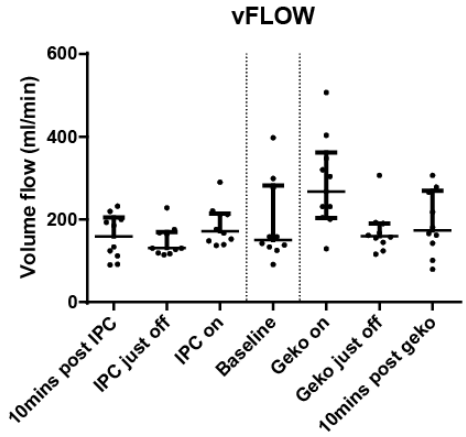
a)



(median and interquartile range)

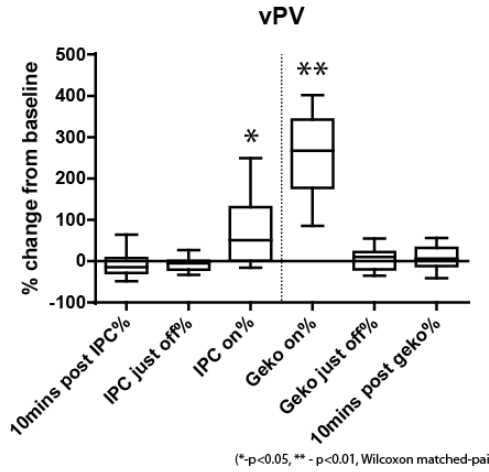


(median and interquartile range)

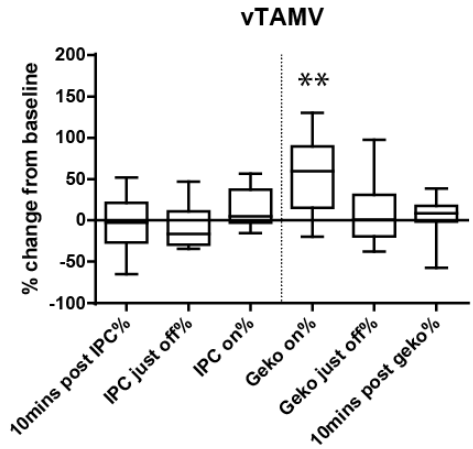


(median and interquartile range)

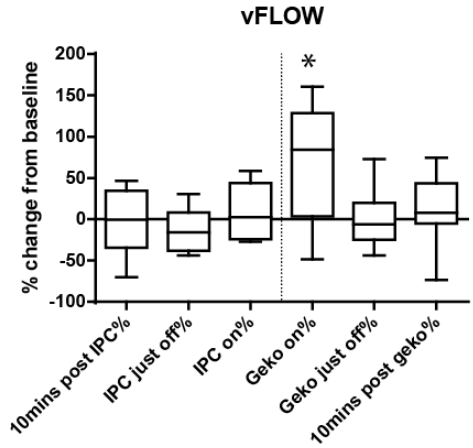
b)



(*-p<0.05, ** - p<0.01, Wilcoxon matched-pairs)



(*-p<0.05, ** - p<0.01, Wilcoxon matched-pairs)



(*-p<0.05, ** - p<0.01, Wilcoxon matched-pairs)

Figure 52 - Graphs depicting venous haemodynamic changes with the application of intermittent pneumatic compression and neuromuscular stimulation

Laser doppler fluximetry

Laser Doppler reading changes from baseline, taken from the leg, were not statistically increased by IPC but were significantly raised by NMES (Table 16). During operation of both individual devices there was a drop in signal from the ipsilateral hand, which returned to positive values after device cessation.

Table 16 - Laser doppler readings expressed as a change from baseline

	Leg (% change from baseline)	**Arm** (% change from baseline)
During IPC	5.3	-7.8
Post IPC	31.5	46.5
During NMES	251.8 **	-17.2
Post NMES	39.6	65.3

"Post" readings are taken immediately after device is switched off (one-way ANOVA, *= p<0.05, **=p<0.001)

Tolerability

All patients tolerated both devices and completed the trial. Subjects were asked to rate their experience of each device at the end of the study. On a linear verbal reported score, from 0 (painless) to 5 (as painful as a sphygmomanometer cuff inflated to 200mmHg), IPC was rated a mean of 1.5, and NMES as 2.8 (p=0.006, paired Student t-test).

Systemic response

Observations of systolic and diastolic blood pressure, pulse oximetry, and heart rate did not change significantly over the protocol.

6.5.4 Discussion

This study compared the use of NMES to IPC for the enhancement of the lower limb circulation. IPC is an established medical therapy for prevention and treatment of vascular disorders; and as regards increasing the blood velocity and flow rate in the leg neuromuscular stimulation compared favourably. The comparatively small size of the NMES device, coupled with its disposable battery-operated characteristics, may make it more convenient than IPC for some to use, and limit the impact on subject mobility during use.

Venous

This study demonstrated a significant increase in venous peak velocity, TAMV and flow rate with the use of NMES compared to both baseline and IPC. It has been shown previously that an increase in venous flow volume and velocity causes venous emptying of the leg and facilitates clearance within the calf vessels and valve cusps which are the most common sites of DVT formation (Flanc *et al.* 1968; Nicolaides *et al.* 1971). Trials of use of NMES as DVT prophylaxis are therefore indicated. Warwick et al noted similar results, and observed that the venous effect of NMES more closely approximated physiological effects of ambulation than IPC (Warwick *et al.* 2013).

Arterial

The results of this trial show NMES to be an effective measure for enhancing arterial TAMV and flow rates. NMES has been said to mimic the effect of exercise in the redistribution of arterial circulation (Liu *et al.* 1987). This may prove of use in the management of intermittent claudication, critical limb ischaemia and limb salvage, although caution must be exercised given the lack of clinical data for NMES in this patient cohort.

Laser Doppler fluximetry

The use of devices, such as NMES and IPC, have been shown to enhance microcirculation of the foot either during or immediately after usage. The differences between the two might be explained by their different mechanisms of action. The pneumatic squeeze of the IPC, whilst not inflated above arterial diastolic pressure, does reduce the arterio-venous gradient during the compression period, and releases this during deactivation. The much faster action of the NMES device is likely to increase this gradient, enhancing venous clearance through activation of the venous foot and leg pumps, and allowing faster arterial inflow against a lower venous back pressure. The increase in microcirculatory perfusion has been quantified by Tucker et al as up to a 25-fold increase in Laser Doppler fluximetry and increased skin temperature from baseline with use of the geko™ device at all settings (Tucker *et al.* 2010).

The fact that the changes in the arm signal either lag behind that of the leg, or rise as a result of device cessation, is suggestive of the release of endogenous cytokines (such as nitric oxide, vascular endothelial growth factor, or some other metabolite) that affect downstream vascular tone or sphincter behaviour, over a local pressure effect on recruitment of arterioles and capillaries. This would agree with the observation that arm-crank training in claudicants improves performance status of the lower limbs as measured by claudication and maximal walking distances (Treat-Jacobson *et al.* 2009). In experiments on ischaemic muscle in rats and humans, Brown et al, and Oldfield et al showed that repeated muscle stimulation in ischaemic human limbs improved nutritive perfusion without modifying endothelial signalling pathways, suggesting that endothelial dysfunction is not changed by electrical stimulation (Brown *et al.* 2005; Oldfield 2005). A unifying theory is missing and consensus is lacking.

Therapies which increase arterial and microvascular circulatory markers are needed for the treatment of intermittent claudication, non-reconstructable peripheral arterial disease and threatened diabetic limbs. The big question will be whether patients with microvascular disease, or a mix of large and small vessel pathology, will respond in a like-for-like fashion to healthy subjects. There is a paucity of literature on this subject, and further trials are indicated.

Tolerability

All subjects rated both devices as more comfortable than a sphygmomanometer cuff on the arm inflated to 200mmHg, although the IPC device did score significantly better for tolerability overall. Depending on the indication and length of use of the device this may limit the acceptability of NMES to patients. No adverse events occurred during the protocol, or were reported to us afterwards.

Limitations

The NMES cycle of 1Hz is applying a compressive force more frequently than the slower cycle of the IPC system, and whilst this may be said to be a strength of the NMES device, it may also have biased our haemodynamic measurement results, although we attempted to address this through the use of a venous flow estimation equation.

There are a variety of different IPC systems, operating over different pressures and inflation/deflation cycles, which may compare differently to the one tested.

Measurements taken after a longer period of device inactivity may be clinically useful, as would studies into repeated and prolonged usage of the device in this context. The long term clinical effects of NMES have yet to be evaluated, whereas the track record and safety data

for IPC is well established. It is not necessarily true that the magnitude of device effect on the haemodynamics of the circulation will be reflected in clinical endpoint indicators such as lower rates of DVT, or faster healing of vascular ulcers. However given the findings of this study, and the possible economic saving of treatment with NMES, further research in this area is warranted.

The NMES device used in this study enhances venous flow and peak velocity in the legs of healthy subjects, and is equal or superior to IPC. This warrants further clinical and economic evaluation for DVT prophylaxis, and exploration of the haemodynamic effect in venous pathology. It also enhances arterial TAMV and flow rate, which may prove to be of clinical use in the management of peripheral arterial disease. The effect on the microcirculation, as evidenced by laser doppler fluximetry may reflect a clinically beneficial target in microvascular disease, such as in the diabetic foot.

6.6 NMES compared to graduated compression stockings for management of oedema

Previously, the efficacy of graduated compression stockings (GCS) has been investigated in the preventative management of occupational oedema (Jungbeck *et al.* 2002; Partsch *et al.* 2004; Blättler *et al.* 2008; Mosti *et al.* 2013). The pressure applied by GCS is thought to increase the efficacy of the calf muscle pump. Some neuromuscular stimulation devices on the open market have been advertised for use in the reduction of leg swelling (Actegy).

In this study, the effect of GCS on leg swelling is compared to two NMES devices. Changes in great saphenous vein (GSV) diameter, and its correlation with leg swelling were also be monitored, as this has been linked to severity in chronic venous disease (Navarro *et al.* 2002; Lane *et al.* 2013). Subjective measures of comfort and tolerability will also be noted.

6.6.1 Methods

Ethical approval was obtained from the Imperial College Research Ethics Committee (ICREC 13/6/5).

Participants

This pilot interventional crossover study trial included 10 participants (10 legs). Participants were recruited from staff at Charing Cross Hospital, and written consent was obtained. Eligibility criteria are shown in Table 17.

Table 17 - Eligibility criteria for inclusion in trial comparing NMES to graduated compression in the management of occupational oedema

Inclusion criteria	Age eighteen or over. Ability to provide informed consent.
Exclusion criteria	A history of peripheral vascular disease. Renal/hepatic/cardiac failure. The subject needed to walk or cycle to another work site during the testing period.

Measurement tools

Ultrasound: A clinical ultrasound system (Phillips iU22 xMATRIX) and an L9-3 vascular venous linear transducer with venous protocol were used. The GSV of the right leg was identified, and reflux (>0.5s) was excluded using a series of calf squeezes with colour Doppler evaluation. A section of vein two fingers breadth below the knee joint was identified and marked. If this section was extra-fascial then the left leg was used. Two orthogonal diameter measurements were taken, and an average calculated.

For the protocol, three repeat sets of measurements of were recorded and averaged, as per Abraham et al (Abraham *et al.* 1994). The trial room conditions were kept as constant as possible, without the facility for climate control.

Perometer: Type 35 S,Pero-system Messgeräte GmbH, Germany, using PeroPlus 2000 software. Subjects were seated on a standard office chair, positioned with their leg extended and foot placed flat on the footplate. Care was taken to minimise space between sole and footplate, and relax the leg as much as possible to minimise leg muscle contraction. A baseline scan was taken and the length of footplate to middle of patella noted. All successive measurements of volume used this length value as a standardised cut-off, particular to that

trial subject. One observer took all perometer scans. For the protocol, five repeat measurements were taken for each scan and averaged.

Devices

"Graduated compression stockings" - Compression stockings (Active Compression Socks, Mediven™, UK). These grade 2 graduated below-knee stockings were fitted according to manufacturer's instructions, on the appropriate test day, applied immediately after the morning scans. They were removed at the time of the evening scan.

"NMES 1" - The geko™ T-1 (Firstkind Ltd, UK). A device was worn on each leg for four hours at the minimum level to stimulate definite dorsiflexion movement of the foot, as per manufacturer's instructions.

"NMES 2" - Revitive IX™ device with Isorocker™ (Actegy Ltd, UK). Stimulation intensity was zeroed, and then increased until muscle contraction occurred (motor threshold). The pre-programmed stimulation cycle of thirty minutes was applied during the trial subject's lunch break, at double motor threshold intensity (or if not tolerated, then at the maximum tolerated level).

Protocol

The first scan of the day was performed in the morning after arrival at work, followed by a second set of readings six hours later, or before leaving work. Subjects positioned standing upright with a non-weight bearing heel, and GSV diameter measurements were taken. Subjects were then seated, and the volume of the same leg was measured. The first day was taken as a control. Over subsequent days different interventions were applied in different orders (subject-to-subject) in an effort to reduce order bias (Table 18).

Table 18 - Order of device application in occupational oedema trial

Subject	1	2	3	4	5	6	7	8	9	10
Day 1	Control	Control	Control	Control	Control	Control	Control	Control	Control	Control
Day 2	GCS	NMES1	NMES1	NMES2	GCS	NMES1	NMES2	NMES2	GCS	NMES1
Day 3	NMES1	GCS	NMES2	GCS	NMES2	GCS	NMES1	GCS	NMES1	NMES2
Day 4	NMES2	NMES2	GCS	NMES1	NMES1	NMES2	GCS	NMES1	NMES2	GCS

(GCS: Graduated compression stockings, NMES1: Bilateral geko application, NMES2: Revitive)

Preference questionnaire

On completion of the protocol subjects were asked to rate the three devices in order of preference in the following domains: comfort, ease of use, and perceived effectiveness; scale 3 (best) to worst (1). This was then scaled to a percentage.

Statistical analysis

All statistical analysis was performed using Prism 6 (Graphpad Software Inc., version 6.01), on an intention-to-treat basis, with significance taken at $p<0.05$.

6.6.2 Results

Four females and six males were recruited, screened and completed the protocol in full. Participants had a mean age 29.2 years, and BMI 25.3kg/m^2. Time between scans varied according to subject workload and schedule, mean 371 minutes +/- 43 (SD).

In one subject NMES1 was unable to elicit dorsiflexion, despite multiple fitting attempts. This could not be linked to any anatomical variation seen on ultrasound, or known physiological abnormality.

Morning measurements of leg volume and GSV diameter varied over four days, maximum coefficients of variation 1.58% and 19.5% respectively. Median absolute control leg volume changes over the day were 49.4ml (IQR 15-75, $p<0.05$, Wilcoxon signed-rank). Absolute change in GSV diameter over the control day was -0.4cm (-1 – 0.4, $p=0.32$).

Compared to control, compression stockings, NMES 1 and NMES2 reduced swelling further by 60ml ($p<0.05$), 15ml (not significant), and 12ml (ns). This is rendered graphical in Figure 53.

Correlation of percentage changes in GSV diameter and leg volume was poor (Spearman $r=0.2$, $p=0.2$), and is shown graphically in Figure 54.

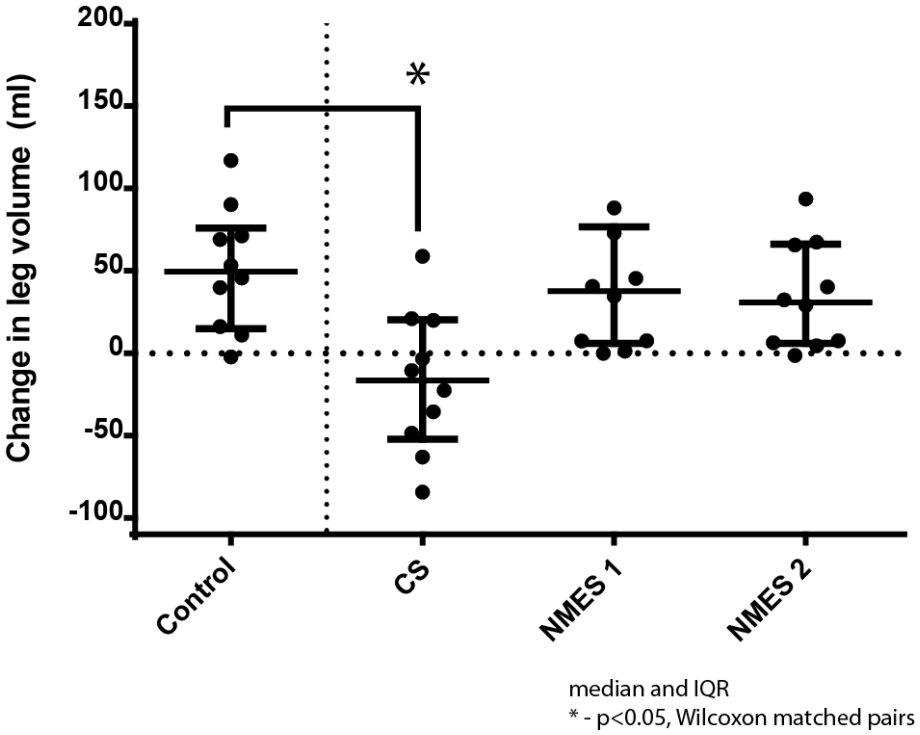


Figure 53 - Changes in leg volume over testing protocol, comparing graduated compression stockings and NMES devices

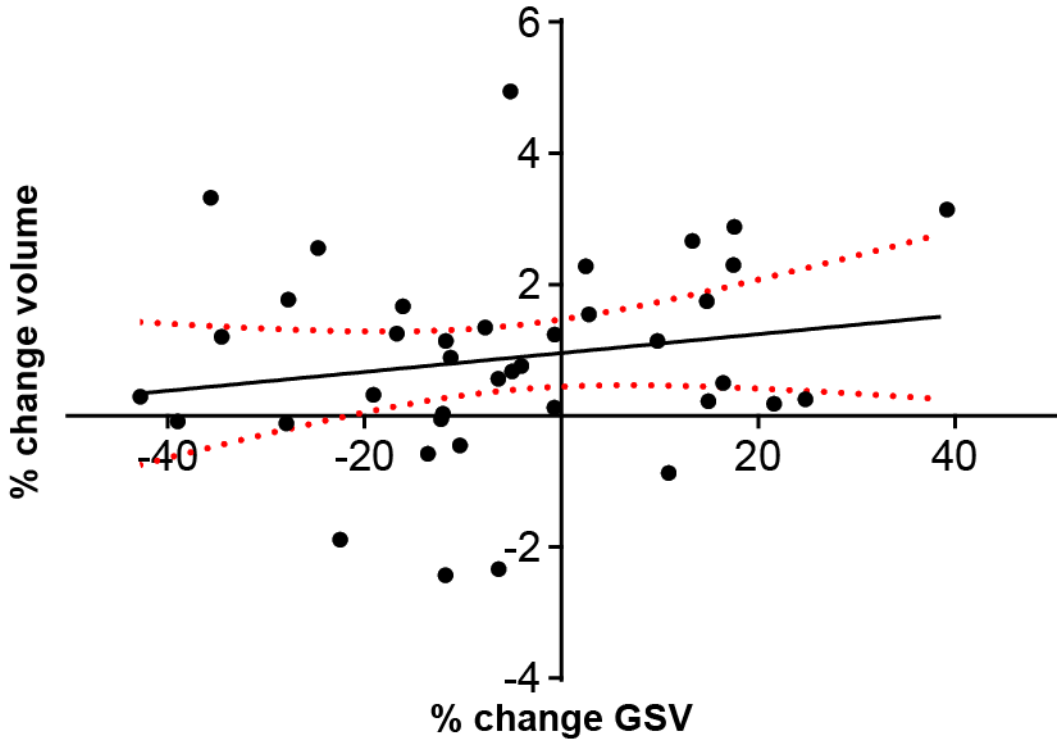


Figure 54 - Percentage changes in great saphenous vein diameter plotted against percentage changes in leg volume.

Line of best fit is shown with 95% confidence intervals.

The results of preference questionnaires are shown in Table 19. GCS were preferred for subjective effectiveness and comfort, whilst NMES1 was felt to be easiest to apply and operate.

Table 19 - Summed subject preference scores for the devices

(expressed as percentages)

	Comfort	Ease of use	Effectiveness
GCS	47	32	47
NMES1	28	36	28
NMES2	25	32	25
TOTAL	100	100	100

6.6.3 Discussion

This study has shown that over a representative sedentary office-based working day, there is a significant change in leg volume in healthy individuals, reproducing results from other studies (Blättler *et al.* 2008). In the absence of disease or musculoskeletal injury, this can be attributed to decreased movements of the leg with insufficient calf muscle pump activity (Jungbeck *et al.* 2002; Hirai *et al.* 2013).

Occupational oedema can be managed using medical devices such as compression stockings and NMES devices. They both promote calf muscle pump efficacy, thereby reducing ambulatory venous pressures, interstitial fluid volume and leg volume (Partsch *et al.* 2004; Tucker *et al.* 2010; Williams *et al.* 2014; Zhang *et al.* 2014)

Compression stockings were most effective at reducing leg volume increase, with NMES devices reducing swelling but not reaching statistical significance in this trial.

NMES devices cause muscular contraction of the calf muscle pump, above what would normally occur in an average sedentary working day, but body position may affect the efficacy of such measures. Flexed hips and knees may increase resistance to blood and lymph flow from the lower extremities. Compression stockings apply graduated pressure from the ankle to knee, both increasing interstitial space pressures, and assisting resistance to muscle expansion with contraction (Morris *et al.* 2004). This allows the calf muscle pumps to perform more efficiently, but relying on the wearer to activate the leg muscles. In an office-based environment, we expected NMES to outperform GCS due to increased muscle activity. Clear guidance on the most efficacious use of the NMES devices has not yet been published, and a different NMES protocol may give different results. The protocol itself may have put the devices at a significant disadvantage, as the Revitive^{TM} device was only operated for the 30min programmed cycle, and this was compared to a device like the GCS which was applied for the full protocol.

In this study, there is little to no correlation between GSV diameter and leg swelling, nor does it change significantly in any of the interventions compared to control. In our pilot trial, subjects were healthy, whereas some of the literature has shown relationships between GSV diameter and severity of clinical symptoms in populations with venous disease (Navarro *et al.* 2002; Lane *et al.* 2013). Barros *et al* have shown a strong correlation between severity of reflux (analysed using air plethysmography), and GSV diameter (Barros *et al.* 2006). When measurements of patient reported quality of life are used as endpoints, the correlation with GSV diameter in patients is poor or absent (Gibson *et al.* 2012). The clinical impact of GSV

diameter on clinical symptoms of chronic venous disease, such as swelling, remains contentious. In many first world countries, medical insurance companies stipulate that treatment for venous disease be rationed according to criteria that include GSV diameter. Whilst this is the case, academic interrogation of data and its possible biases is important.

Differences in tolerability of the devices did not lead to poor compliance, and all subjects completed the whole protocol. A preference for compression stockings in healthy subjects over NMES is noted, although this did vary from person to person.

Efforts were taken not to restrict normal subject activity over the working day, in an effort to produce results applicable to the general population. Common tasks carried out during the day involved sitting at a desk typing, clinical duties in outpatient clinic (taking histories, clinical examination, dictation), with minimal walking. Distances walked were not recorded. Fluid intake, bathroom breaks, environmental temperature and subject working position were not controlled, and this could have skewed our results. 40% of our sample was female, and variation in hormonal profile (particularly progesterone) may have had a confounding effect. This pilot study small sample size also makes us prone to both type 1 and 2 statistical errors.

There are significant differences between the three medical devices tested here, and may be more suitable for certain lifestyles or population groups. GCS need to be properly fitted to be efficacious, whereas the Revitive™ device can be used straight from the box. The geko™ can take a few minutes to position properly over the appropriate anatomical landmarks, and did not work in one of our healthy trial subjects. The reason for this is unknown. The Revitive™ requires the user to be stationary in a chair for 30 minutes, whereas the geko™ and GCS allow full mobility throughout. The two NMES devices work through slightly different mechanisms, one working anterograde through the common peroneal nerve, the other retrograde through

the plantar muscles and plexus of nerves. This may or may not be significant in different populations. GCS reported compliance in chronic disease has been poor, often being described as uncomfortable and restrictive (Raju *et al.* 2007; Ziaja *et al.* 2011). The long term tolerability of NMES device such as the geko^{TM} and Revitive^{TM} has yet to be evaluated. From small pilot trials using NMES in healthy subjects, it does seem that the maximum tolerated level is efficacious for producing maximum positive results whilst limiting discomfort (Zhang *et al.* 2014). It has also been shown that exposure over time to neuromuscular stimulation leads to tolerance of higher stimulation settings and experience of lower levels of discomfort (Corley *et al.* 2012). However in this trial we did not explore a range or grade of stimulation settings, allowing subjects to select their own intensity settings. This may have affected the comparison with GCS.

Previous studies combining GCS and NMES (DUO Stim or BMR Neurotech) have shown an additive effect on haemodynamic flow measurements (Lyons *et al.* 2002; Corley *et al.* 2012). This may translate into added clinical benefit with combined use of NMES devices with GCS in future interventional trials.

6.7 Changes in autonomic activation with NMES of the legs

Data so far has confirmed that electrically induced contraction of the muscles in the legs augments blood flow. It has been assumed that this is largely due to a build-up of hydrostatic pressure by the increased pumping action of the muscle pump units in the leg, but the effect of electricity on the nervous system has not been explored.

The sympathetic and parasympathetic arms of the autonomic nervous system work in parallel to fine-tune homeostatic mechanisms depending on environmental stressors. They consist of cholinergic pathways that modulate complex neuronal networks in target organs, such as smooth muscle in enteric and vascular structures (Guyenet 2006). Changes in blood pressure are controlled by these mechanisms by altering cardiac output and vascular resistance, and selective blockade can treat hypertension.

It might seem logical that NMES effects not only downstream muscles and nerves, but may also modulate upstream elements, such as activation of the different components of the autonomic nervous system. This may be apparent in changes in parameters such as blood pressure, heart rate, skin responses, and vascular tone.

A short trial was designed to evaluate the effect on local and systemic measures of the autonomic nervous system, before, during and after electrical stimulation of the leg.

6.7.1 Methods

Ethical approval was granted by Imperial College Research Ethics Committee (ICREC 15/IC/2529). 10 healthy subjects were recruited from Imperial College Medical School

(Inclusion and exclusion criteria shown in Table 20). Verbal and written consent was taken. Screening was performed via a short medical history and examination, including ABPI. A urinary pregnancy test was performed on all females.

Table 20 - Inclusion and exclusion criteria for "Autonomic Activation" trial

Inclusion	18 years or older Current good health
Exclusion	History of cardiovascular disease Diagnosis of cystic fibrosis Pregnancy Non-English speaker ABPI<0.9

Physiological parameters

Intermittent readings of blood pressure, pulse and oxygen saturations were measured from the left arm with standard equipment. Laser Doppler adhesive skin electrodes were attached to the right hand and foot to measure changes in skin perfusion throughout the protocol.

Ultrasound (Philips iU22 vascular system)

Blood velocity and speed were measured at the level of the groin vessels on the right leg of the subject, according to standard protocol. The subject was positioned on an examination couch, semi-recumbent with attention to subject comfort. The leg was exposed from groin downwards, and the rest of the subject kept warm. Traces were saved to disk and analysed offline.

Sympathetic skin response (SSR)

SSR was taken from the plantar and palmar surface of the left hand via adhesive skin electrodes during the "rest" part of the protocol (see Figure 55). The response to a nociceptive stimulus was measured and compared across time-points. The preferred stimulus was a loud noise, but technical difficulties regarding reproducibility and technological integration precluded this. An electrical shock (set at twice sensory threshold in amperes) was delivered to the contralateral hand (DS7A HV, Digitimer Ltd, UK), and the exact timing relative to other recordings automatically logged. Isolated amplifiers (ISODAM, World Precision Instruments) were set with a gain of x 100 and band pass filter 0.1–100 Hz. Amplified signals were sampled at 200 Hz (CED 1401+, Cambridge Electronic Design) and stored on a PC. Signals were be recorded for 0.5s prior to-, and 12s after-, the triggering stimulus. The baseline was monitored on an oscilloscope so that the activating stimulus can be given at a stable recording point and interference potentials could be avoided. Further discussion of the SSR can be found in section 5.3.2.2, page 136.

Device Stimulation

The geko™ T-1 device was applied to the right leg, and stimulation titrated to an intensity level that caused comfortable dosiflexion of the foot. Baseline measurements were recorded, and then stimulation commenced for 5-mins. The device was switched off, and repeat ultrasound and SSR measurements were taken. After this the device was activated for another 5min section, with repeat measurements recorded at the end of the period with device off. The protocol is illustrated in Figure 55 and Figure 56.

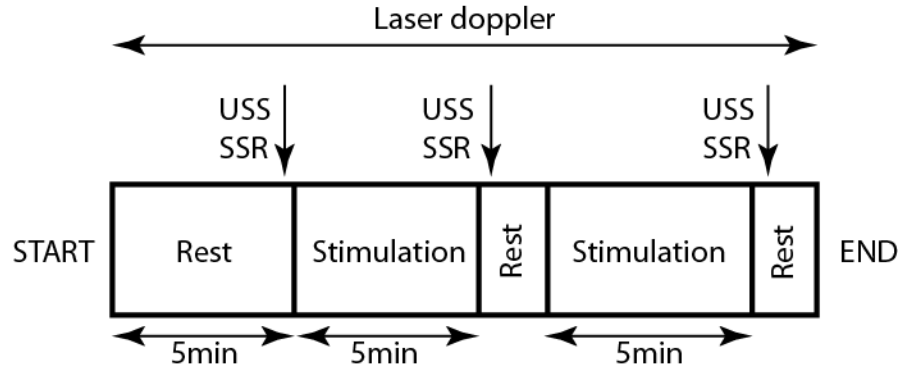


Figure 55 - Protocol for assessment of "Autonomic Activation" outcome measures

(USS – ultrasound scan, SSR – sympathetic skin response)

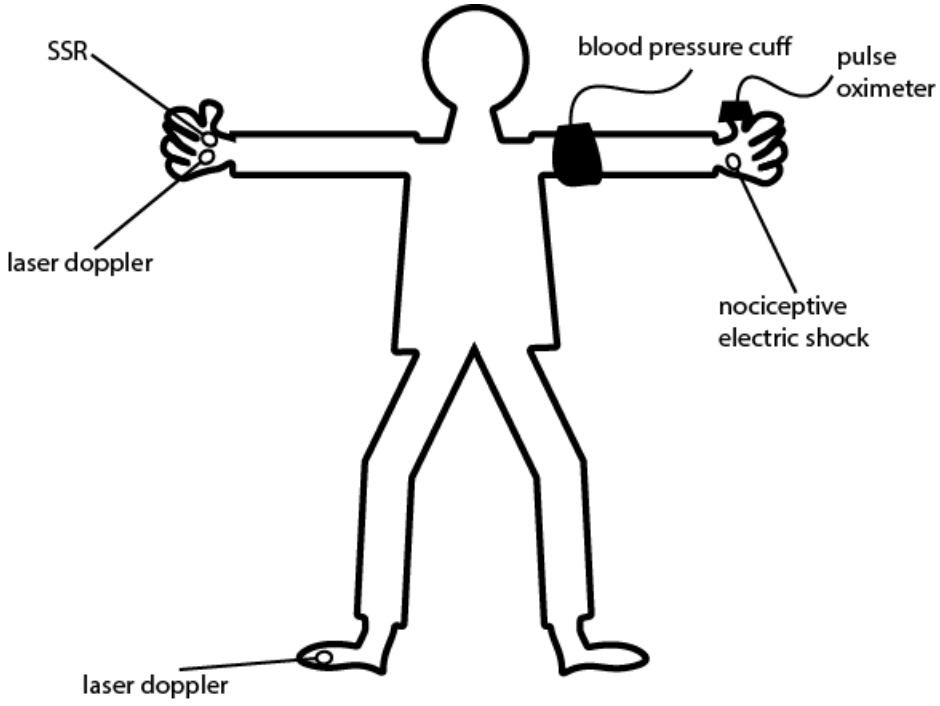


Figure 56 – Equipment application to subject during "Autonomic Activation" protocol

(SSR – sympathetic skin response machine)

6.7.2 Results

Demographics

Nine subjects completed the protocol in full, 3 males, with mean age 23.4±4 years and BMI 23.8±3 kg/m^2. Three subjects failed to complete the protocol due to equipment failure (SSR machine).

Physiology

Arterial blood pressure (brachial artery), pulse oximetry and heart rate were measured as per the protocol. Results are displayed in Figure 57 and Table 21. There were no statistically significant differences between resting and activation phases in heart rate, oxygen saturation, or systolic blood pressure. There was significant lowering in diastolic blood pressure with NMES device activation (76.4±6 mmHg to 74.5±6 mmHg; mean ± SD; $p<0.05$, Wilcoxon matched-pairs). This is shown in Figure 57B.

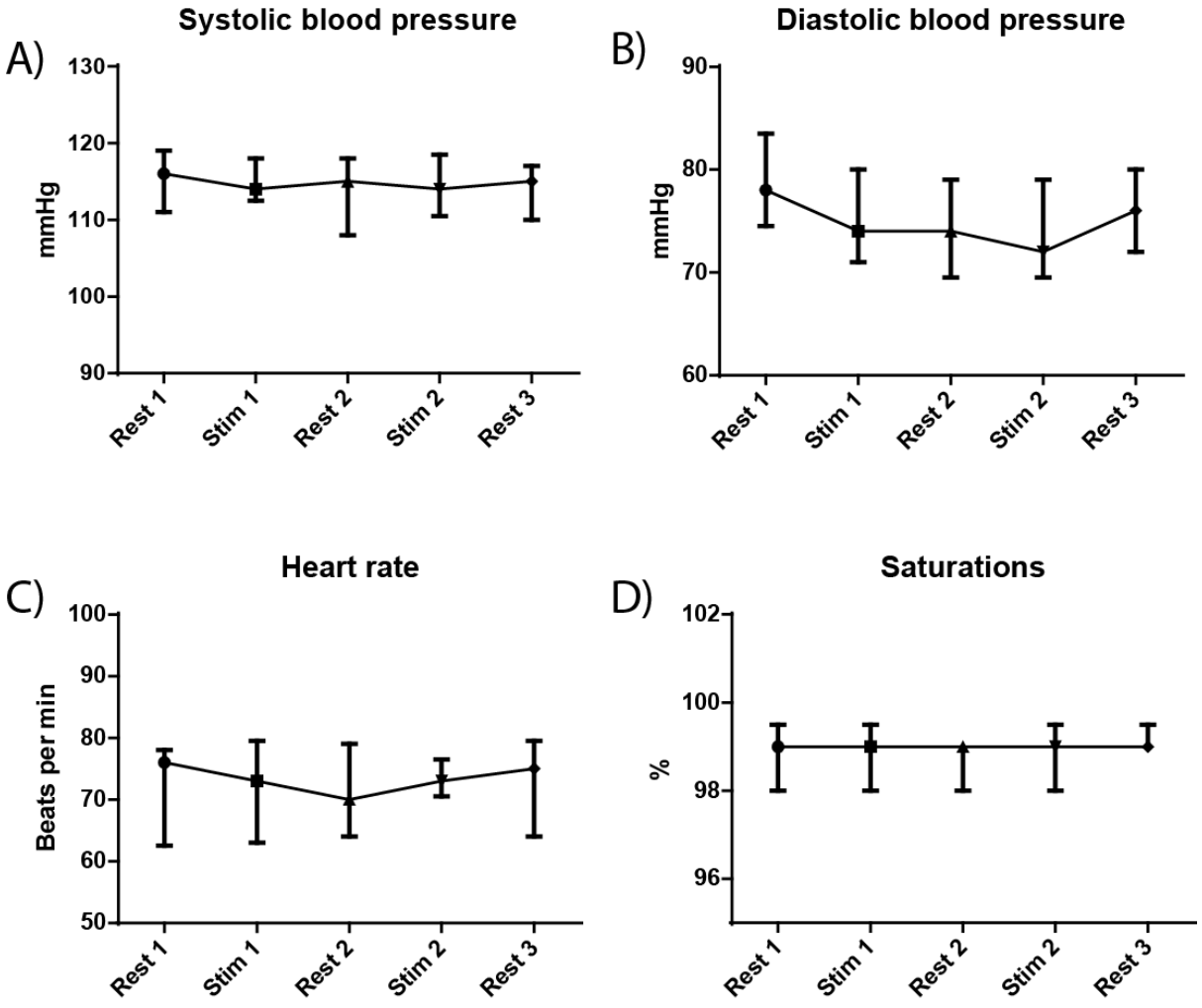


Figure 57 - Physiological changes over time with neuromuscular stimulation

("stim" = stimulation period)

Table 21 - Physiological changes with neuromuscular stimulation

	Rest	Device on	P value (Wilcoxon matched-pairs)
Systolic blood pressure (mmHg)	115 (109-118)	114 (112-118)	0.7
Diastolic blood pressure (mmHg)	76 (74-82)	73 (70-79)	0.04
Heart rate (per min)	74 (65-78)	73 (68.5-77.3)	0.2
Pulse oximetry saturations (%)	99 (98-99)	99 (98-99.3)	0.6

Median and interquartile range reported

Sympathetic skin response

Median amplitude of SSR in the hand at baseline was 1.24mV (IQR 0.7-1.88). Friedman analysis of changes over time was significant ($p<0.05$). Median changes in SSR amplitude were -0.5mV for the first cycle ($P<0.05$ compared to baseline, Wilcoxon matched-pairs) and -0.3mV for the second cycle compared to baseline ($p<0.05$). Median latency before NMES intervention was 1.4secs (1.3-1.5). Friedman analysis of changes over time was not significant. Median changes in latency from baseline at 5 and 10 minutes were 0secs and 0.1secs. Data is presented graphically in Figure 58.

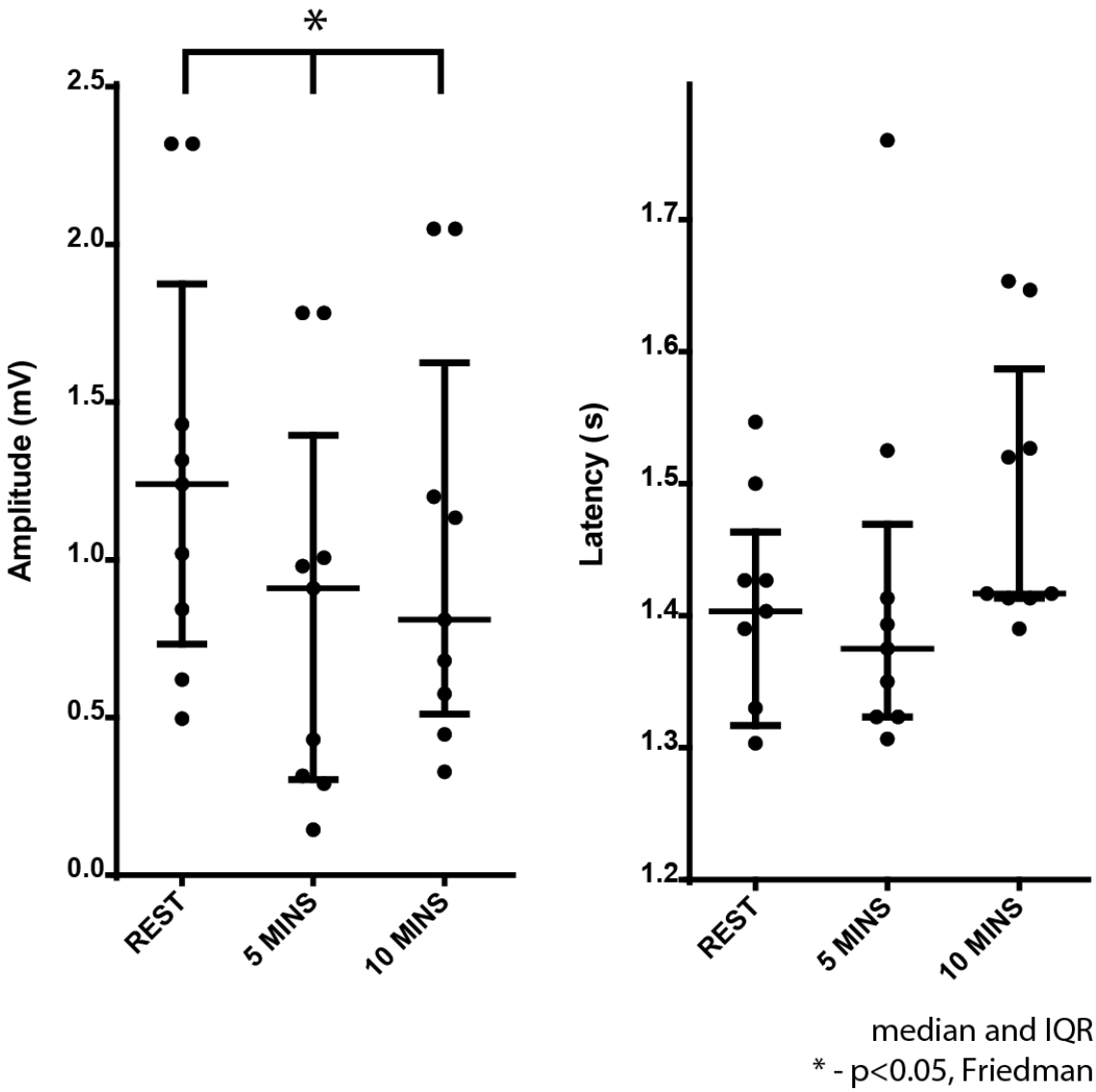


Figure 58 - Changes in sympathetic skin response with "Autonomic Activation" protocol

(IQR – interquartile range)

Haemodynamic ultrasound

Haemodynamic changes from baseline with device activation were calculated as absolute and percentage values. Changes in arterial peak velocity, TAMV and volume flow were +3.6cm/s (not significant, Mann-Whitney U; 4%), +3.3cm/s ($p<0.05$; 33%) and +99.6ml/min ($p<0.01$; 49%). Changes from baseline in venous peak velocity, TAMV and volume flow were -0.5cm/s (ns; -1%), +1.3cm/s (ns; 9%) and +13.1ml/min (ns; 7%). Data is presented graphically in Figure 59 and Figure 60. There were no significant differences between readings at 5 and 10 minutes of any parameters. Median changes in arterial and venous diameters from baseline to 5 minutes were +3mm ($p<0.05$, Wilcoxon matched-pairs) and -1mm (ns).

Laser doppler fluximetry

Fluximetry and skin temperature changes in the hand and foot with device activation were recorded and analysed. Fluximetry changes of -16% and -9% were detected in the hand and foot respectively, which were not statistically significant. Results are displayed in Figure 61.

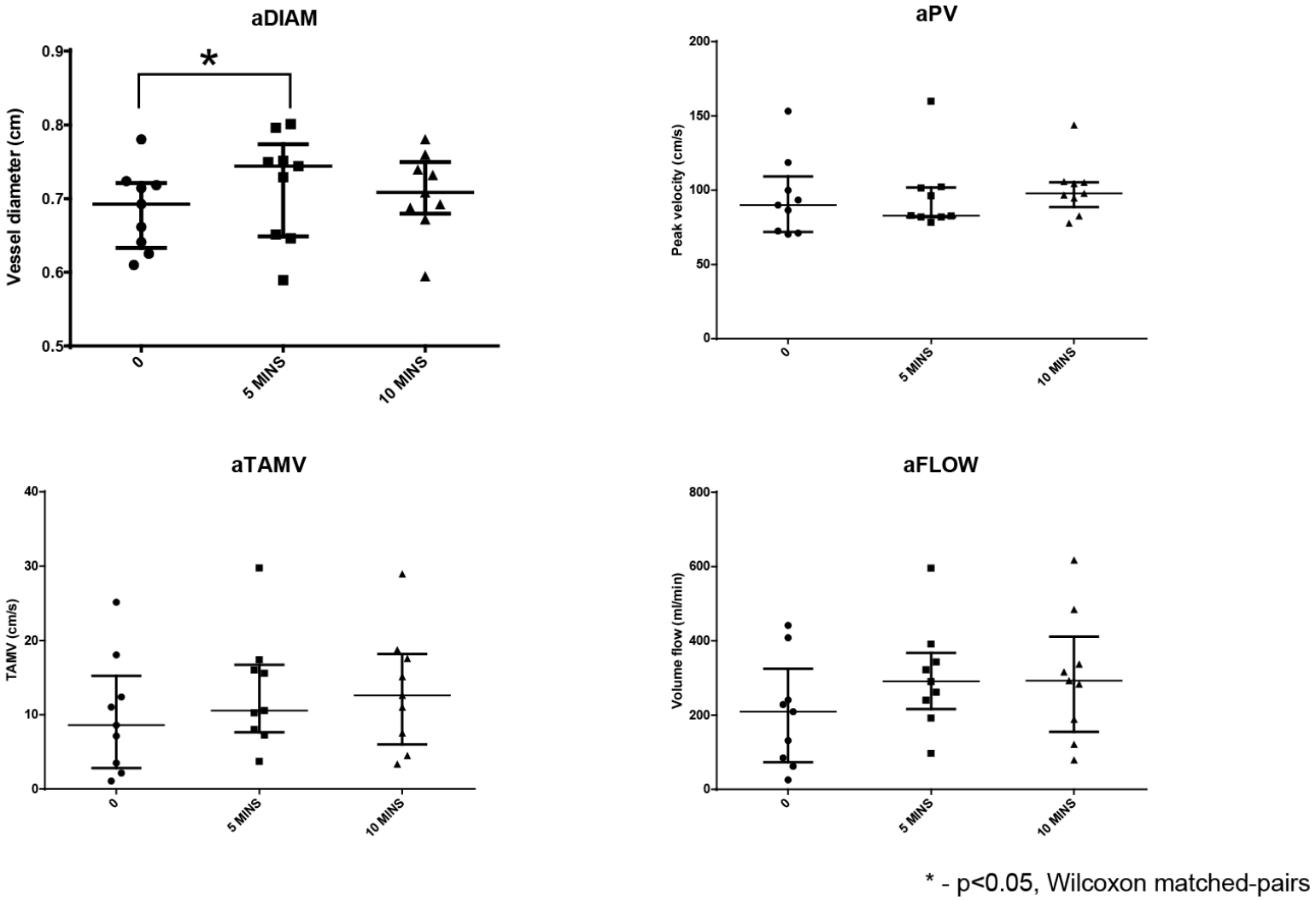


Figure 59 - Changes in superficial femoral artery haemodynamic parameters with "Autonomic Activation" protocol

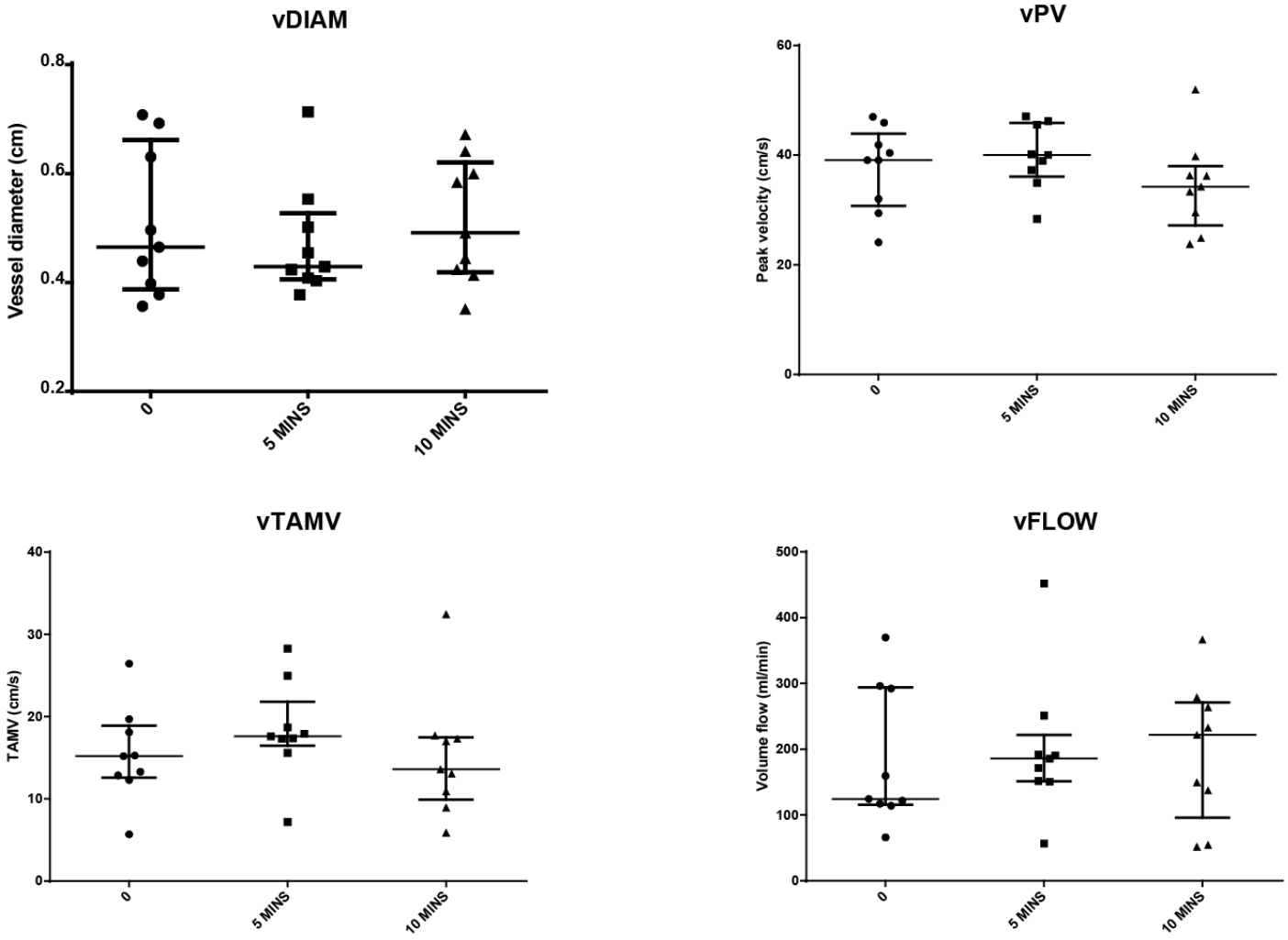


Figure 60 - Changes in femoral vein haemodynamic parameters with "Autonomic Activation" protocol

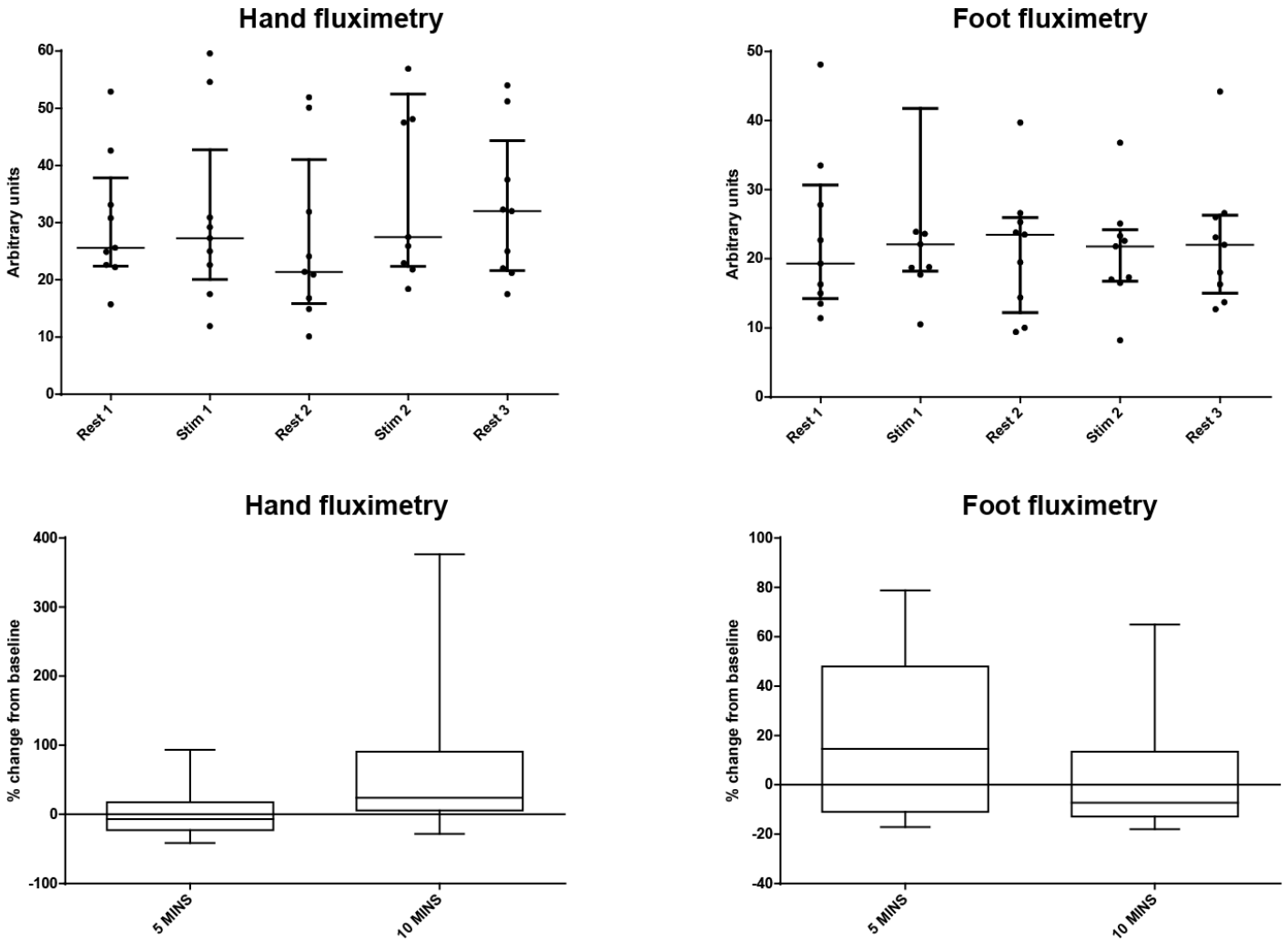


Figure 61 - Changes in fluximetry parameters of the dorsum of the hand and foot with "Autonomic Activation" protocol

6.7.3 Discussion

This small pilot trial has attempted to evaluate the effect of NMES stimulation of the leg on the autonomic nervous system.

Physiological parameters such as heart rate and diastolic blood pressure were not affected, however during NMES activation, the diastolic blood pressure was lowered by statistically significant levels. Over the course of the protocol it can be seen (Figure 57) that measured pressures trended downwards with stimulation and plateaued during stimulation cessation. This may be a result of changes in myocardial compliance, contractility, or ventricular capacity, all of which are affected by venous return and venous smooth muscle tone, both of which are under sympathetic control (Guyenet 2006).

Changes in the sympathetic vascular tone of resistance arterioles (the main component of total peripheral vascular resistance) can affect diastolic blood pressure. 90% of postganglionic axons in nerves projecting to muscle have vasoconstrictive properties, and are under powerful inhibitory control by arterial baroreceptors. Activity in these nerves is synchronous with both the cardiac and respiratory cycle (Macefield *et al.* 1999; Macefield *et al.* 1999). Activation causes small arteriolar vessels to constrict and blood pressure to rise. However, there is a small population of sympathetic postganglionic nerves (<20%) projecting to certain hairy areas of the skin that behave as vasodilators. They are decoupled to baroreflex, and instead respond to pain. Stimulation of local nociceptors causes reflex arteriolar dilatation and increased blood flow in adjacent skin areas – the " Loven effect" (Loven 1866; Nordin *et al.* 1995). Physiologically this makes sense, allowing the body to direct blood clotting factors and immunologically active particles to damaged tissues. This has been described in both animal models, and in humans. Mild painful intraneural electrical stimulation of the

superficial common peroneal nerve at the ankle has been shown to increase blood flow to the skin of the foot and leg adjacent to the path of the nerve (Blumberg *et al.* 1987). It had a similar but smaller magnitude effect on the skin on the contralateral limb. Both vasomotor responses were abolished by proximal pharmacological nerve block. In our bench experiment we did see what could be a trend in the laser doppler fluximetry reading, rising with stimulation, and then falling with rest. This was seen in both the hand and the foot, but did not reach statistical significance. Separating this out from the pressure effect of limb blood inflow increase and increased pressure effect is not possible, however it is suggestive that blood velocity and flow in the femoral vessels did not significantly change over this short time period.

Changes in the sympathetic skin response were measured both before and after NMES treatment. This is the result of reflex activation of the sudomotor sympathetic efferent fibres, generated in the deep layers of the skin (Elie *et al.* 1990). We were able to show a significant decrease in amplitude from baseline to both time-points measured. All recordings were taken as the mean of three recordings, spaced more than 60secs apart, and the sets of measurements were spaced >5mins apart. In this way we have tried to limit the effects of habituation to the response stimulus. According to the literature, an evoked SSR would be expected to increase in amplitude over 10minutes, before starting to decrease. Elie et al found that the amplitude at 60 minutes, after repeated burst stimulation every 2 minutes, was roughly half that recorded at baseline (Elie *et al.* 1990). This has been duplicated by Fagius et al, and correlated with direct physiological nerve impulse readings using microneurography (Fagius *et al.* 1980). How our findings are interpreted in this context is unclear. The most simple answer must be that the repeated shock from the NMES produces a repeated sympathetic response that then starts to reduce in amplitude as the system is saturated with

the corresponding stress hormones (such as catacholamines and prostaglandin) and then stores depleted. This may be clinically useful in the management of disorders where modulation of the autonomic nervous system alleviate symptoms. Whether NMES would make the pathological condition better or worse may be debateable. Such an example might be in the treatment of ischaemic rest pain, where lumbar sympathectomy has been shown to decrease symptoms and increase limb salvage rates in some (not all) cases.

Limitations

It is noted that the recorded changes in diastolic pressure were smaller than the measured standard deviation, and may be a result of type 1 error.

Technical problems were experienced with the recording equipment, which unfortunately nullified the protocols of three of our subjects. With a small sample size of 9 it is hard to envisage any statistically significant results. A power calculation was not feasible due to the lack of known data regarding the sympathetic response, and the broad range of stimuli that have been used to elicit it (loud noise, inspiratory gasp, bladder manipulation, dermal injection, and magnetic stimulation) (Vetrugno *et al.* 2003; Kucera *et al.* 2004). If this experiment were to be repeated, there is considerable advantage in utilising auditory stimuli to evoke the SSR, in order to separate the effect from that of the NMES device. It would be helpful to physically correlate this with microneurography.

6.8 Summary of findings from bench testing and small trials

1. The inter-subject variability in stimulation intensity for contraction, electrical stimulation tolerance, and haemodynamic effect of NMES is large.
2. Differences in skin impedance, muscle mass and composition, and anatomical variation may limit the standardisation of devices and protocols.
3. NMES via the common peroneal nerve or footplate can increase femoral flow rates and velocities.
4. NMES of the leg increases fluximetry readings of the dorsum of the foot, and may be of benefit in skin dysfunction situations (e.g. ulceration). NMES has a systemic effect that can be measured using fluximetry in the hand.
5. The main source of discomfort reported with NMES usage originate at the electrode-skin interface.
6. Repeated NMES over time may have additive effects, but the risk profile is unknown.
7. Grade 2 graduated compression stockings may enhance the effect of the geko when reducing excess tissue fluid, and may increase the efficacy of the calf muscle pump. Air plethysmography testing is recommended.
8. The geko device is non-inferior to venous-setting IPC with respect to femoral blood flow, foot fluximetry, and comfort.
9. NMES may have an effect on diastolic blood pressure. This has yet to be experimentally reproduced, or empirically quantified.

7 Pilot trials of clinical efficacy

7.1 NMES in the management of chronic venous disease (VeINS)

The flow of blood around the body in maintained by the effective pumping of the heart, and this is augmented in the limbs by venous muscle pumps. The venous hypertension and stasis in CVD contributes to clinical symptoms (Williams *et al.* 2014). Venous ulcers are a significant health burden (Baker *et al.* 1991). Standard treatments aim to reduce venous stasis by improving the peripheral pump system. Compression hosiery or layered bandaging aids the compressive ability of the muscle pump to squeeze. Extrinsic compression of the leg can be achieved with intermittent pneumatic compression (IPC), and has been shown to be effective in the treatment of CVD (Vowden 2001; Comerota 2011; Nelson *et al.* 2011). If appropriate, surgical procedures to the deep or superficial venous systems can reroute blood through competent veins. Activation of the venous pump muscles themselves can be achieved through neuromuscular electrical stimulation (NMES), and has been shown to have an equivalent haemodynamic effect to IPC in healthy subjects (Williams *et al.* 2015). We have further investigated this in the context of CVD.

7.1.1 Methods

Ethical approval for a controlled interventional trial was obtained from National Research Ethics Committee (13/WM/0027). Group 1 (10 subjects) were healthy subjects with no clinical venous disease.

Subjects with venous disease were recruited into one of three groups. Group 2 (10 subjects) had superficial venous incompetence; Group 3 (10 subjects) had deep venous incompetence; Group 4 (10 subjects) had deep vein occlusion. Duplex ultrasound was performed on all recruits to confirm presence or absence of disease. All subjects were over 18 years old. Subjects were excluded if they had a history of peripheral arterial disease, leg fracture or metallic implant, or other systemic causes of limb swelling (heart, lung or renal failure).

Healthy subjects were recruited by word of mouth, and venous patients from vascular clinic and the imaging laboratory. Written informed consent was recorded. Screening included a medical history, physical examination, pregnancy test if female, and ultrasound examination of the lower limbs.

The device

The NMES device used in was the geko™ T-1 (Firstkind Ltd, UK). The minimum pulse width to achieve dorsiflexion was used. Devices were fitted bilaterally, and worn for 4-6 hours, 5 days a week, for 6 weeks. A usage diary was collected from each subject at the end of the testing period.

Initial duplex ultrasound screening was performed with subject standing, using iU22 xMATRIX ultrasound system with L9-3 transducer and venous protocol (Philips, Seattle, WA, USA). After

confirmation of presence or absence of venous disease, ankle-brachial pressure index (ABPI) was taken.

Measurement of haemodynamic variables

Prior to haemodynamic assessment, subjects were allowed to quietly rest for 10 minutes, semi-recumbent on an examination couch. The right femoral vein was marked for repeat measurements, 3-5cm from the saphenofemoral junction, as per Williams el al (Williams *et al.* 2015). Five measurements of venous parameters were taken from the femoral vein, and a mean calculated. Laser Doppler fluximetry was recorded from the dorsum of the left hand and foot. Baseline measurements will be taken before activation of both devices, and 20 minutes after activation of the device.

Leg volume (V) was calculated by measuring ankle and maximal calf diameters, the vertical distance between the two points (h), deriving ankle and calf radii (R1 and R2), and applying a truncated inverted cone model. This was performed bilaterally.

$$V = \frac{\pi h}{3}(R1^2 + R1R2 + R2^2)$$

Six Week Device Usage

Participants were trained in how to apply the NMES device themselves and given a supply to use at home. After week 6 NMES was discontinued. Physician- and patient-reported quality of life assessments (VCCS, VDS, AVVQ, EQ5D, VAS, CES-D, SF-12) were performed at weeks 0, 6, and 8. An unsupervised diary of device usage was completed by each participant.

Analysis

Data were analysed using IBM SPSS Statistics version 21. Physical parameters were assessed comparing healthy to diseased subjects, and subdivided according to disease aetiology. Quality of life scores were assessed subdivided according to disease severity.

7.1.2 Results

Ten healthy subjects and thirty venous patients were recruited and grouped. There were 24 men, mean age was 49.4+/-18 years, BMI 29.0+/-7kg/m2, calf circumference 40.4+/-6cm, and unilateral leg volume 1927.2+/-826ml. Five patients in group three had mixed deep and superficial disease, two patients in group four had mixed superficial and deep disease. Demographic and clinical characteristics of each group are shown in Table 22, with anatomical segments affected shown in Table 23. Previous superficial venous surgery had been performed in 6 subjects in the superficial reflux group, and 5 in the deep reflux group. Figure 62 illustrates recruitment and follow-up for the trial.

Table 22 - Demographic data of recruited subjects to VeINS trial

	Healthy subjects	Superficial venous insufficiency	Deep venous insufficiency	Deep venous obstruction	ANOVA
Number	10	10	10	10	
Age (years)	30.7±10	55.0±15	58.6±18	53.2±16	<0.001
Sex	50% M	40% M	80% M	70% M	0.04
BMI (kg/m^2)	23.6±4	26.4±6	30.1±3	34.8±6	<0.001
CEAP clinical severity score	0.8±1	3.1±1	4.4±1	4.0±1	<0.001
Unilateral leg volume (ml)	1451.1±574	1730.2±390	2025.8±1029	2501.7±814	<0.001

(mean ±standard deviation)

Table 23 - Anatomical segments affected by venous disease in VeINS trial patients

Vein segment affected	Superficial venous insufficiency	Deep venous insufficiency	Deep venous obstruction
Venae cava	0	0	1
Iliac	0	0	4
Femoral	7	9	6
Popliteal	6	8	1
Below knee (including perforators)	9	8	3

(Disease may affect more than one segment in any one subject, therefore total not equal to participant number in each group.)

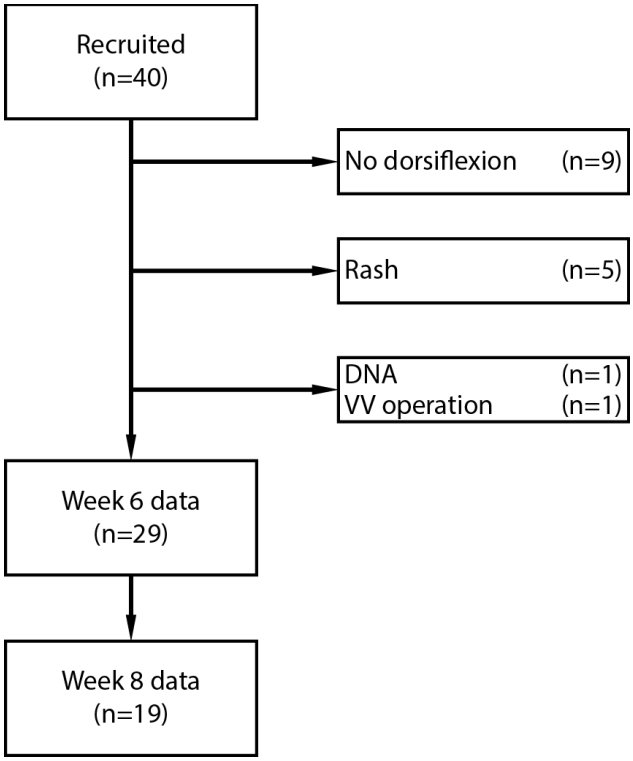


Figure 62 – VeINS trial diagram showing recruitment and follow-up numbers

(DNA – did not attend follow-up, VV – varicose vein)

The NMES device was ineffective (unable to elicit a twitch) in nine patients, with a mean BMI of 35.5±5kg/m2, calf circumference 45.9±6cm, CEAP C4.1±0.6, and AVVQ 31.3±14.

Kolmogorov-Smirnov analysis of endpoint parameters concluded the majority of data was not normally distributed. Therefore all data was treated as non-parametric.

Haemodynamic results

Median changes with device activation in PV, TAMV and volume flow in healthy subjects were 34%, (6.5cm/s, IQR -1 – 12, p=0.01, wilcoxon signed-rank), -14%, (-1.0ml/min, -2 – 2, p=0.9) and -23% (-29.7ml/min, -64 – 51, p=0.6). Median changes in PV, TAMV and volume flow in those with venous disease were 41%, (3.4cm/s, IQR -1 – 13, p<0.001), 25%, (0.4, -1 – 2, p=0.02) and 20% (12.7ml/min, -17 – 51, p=0.01). Gains were less in subjects with deep venous disease (see Table 24). Regular use over 6 weeks enhanced all stimulation venous parameters in both health and disease, which was statistically significant for TAMV in healthy subjects, and PV in venous disease (Figure 63).

Mean increases in healthy subjects arm and leg fluximetry are shown in Table 25. Increases in leg fluximetry signal were attenuated in deep venous disease. Changes in fluximetry signal with device activation were not affected by repeated use over 6 weeks.

Table 24 - Haemodynamic changes in the femoral vein, with use of NMES in VeINS trial, "device off" compared to "device on"

% change from baseline	*Group*							
	Healthy subjects		Superficial venous insufficiency		Deep venous insufficiency		Deep venous obstruction	
	Median	*IQR*	*Median*	*IQR*	*Median*	*IQR*	*Median*	*IQR*
Peak velocity	*34.8***	*-4 - 81*	*62.8***	*25 – 138*	*9.0*	*-10 – 84*	*14.8*	*-8 - 51*
TAMV	*-14.2*	*-30 – 55*	*28.1***	*-2 – 111*	*28.2*	*-30 – 66*	*-5.1*	*-28 - 35*
Volume Flow	*-22.5*	*-40 - 40*	*37.5**	*-10 - 172*	*17.4**	*1 - 49*	*5.9*	*-11 - 21*

(*-p<0.05, **-p<0.01, wilcoxon signed-rank) IQR – interquartile range

Table 25 - Laser Doppler fluximetry changes in the hand and foot with use of NMES in VeINS trial, "device off" compared to "device on"

Percentage change from baseline	**Healthy subjects**	**Superficial venous insufficiency**	**Deep venous insufficiency**	**Deep venous obstruction**
Leg flux	274.8 ± 279**	264.9 ±283*	69.3 ± 126	46.9 ± 50*
Arm flux	84.0 ± 126**	71.3 ± 76*	23.7 ± 57	106.5 ± 115*

(*-p<0.05, **-p<0.01, wilcoxon signed-rank)

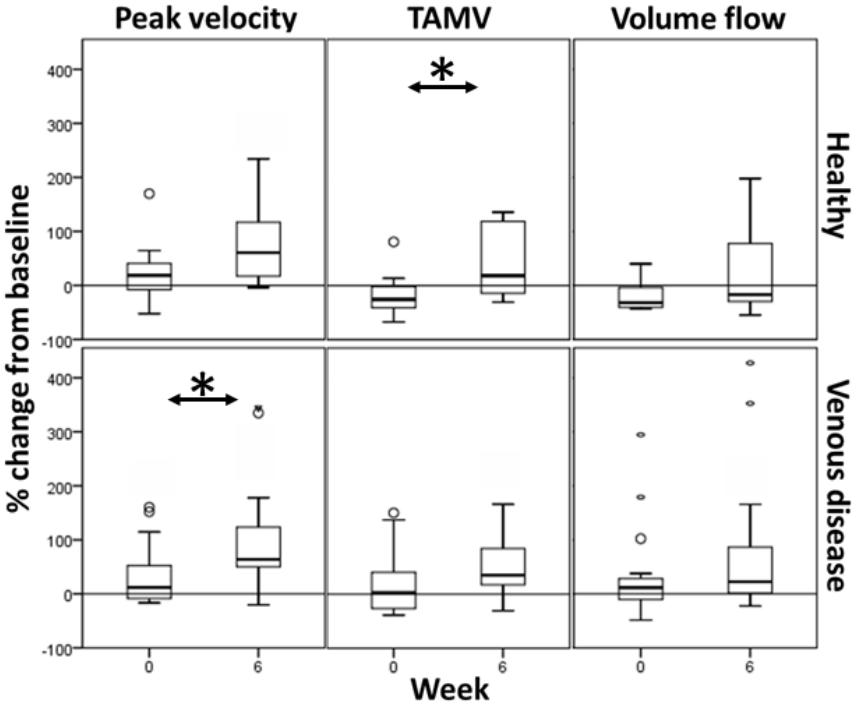


Figure 63 - Haemodynamic changes in the femoral vein with neuromuscular electrical stimulation in VeINS trial, taken at weeks 0 and 6

(*-p<0.05, wilcoxon matched pairs signed-rank)

Leg volume changes

Over 6 weeks median unilateral leg volume decreased by an average of 0.2% (26.3ml, p=0.61) for healthy subjects, and 11.6% (225.4ml, p=0.03) for those with symptomatic venous disease. This is shown in Table 26.

Table 26 - Changes in unilateral leg volume in VeINS trial

	Healthy	Venous disease
Week 0 (ml)	1451.1 ± 574	2085.9 ± 840
Week 6 (ml)	1552.2 ± 580	1797.2 ± 491
Significance (wilcoxon signed-rank)	*0.61*	*0.03*

Quality of life measurements

In those with venous pathology, scores for disease-specific and generic quality of life questionnaires improved (Table 27). Those with C4-6 disease benefitted the most, with improvements in VDS score of 1, AVVQ of 6, and SF-12 of 10. The only statistically significant change was in VDS score from week 0 to 6 (p=0.03, Mann-Whitney U test). After two weeks of NMES abstinence, the improvements in venous clinical scores were sustained. Healthy subjects, and those with mild clinical symptoms, saw no improvements in generic quality of life over 6 weeks.

Table 27- Quality of life scores over time in VeINS trial

	CEAP C0-3			CEAP C4-6		
	Week 0 N=17	Week 6 N=16	Week 8 N=11	Week 0 N=18	Week 6 N=14	Week 8 N=13
VCSS	2.9	2.9	3.4	9.6	6.5	7.4
VDS	0.69	0.71	0.78	1.9	1.1**	1.3
AVVQ	9.6	8.0	9.2	26.6	22.4	23.6
EQ5D	0.81	0.86	0.90	0.7	0.7	0.7
VAS	82	83	79	66	73	73
SF-12	99	102	94	85	95	87
CES-D	8.9	7.3	10.6	12.4	11.1	12.5

(-p<0.01, Mann-Whitney U, week 0 to week 6. VCSS-venous clinical severity score, VDS-venous disability score, AVVQ-Aberdeen varicose vein questionnaire, VAS-visual analogue score, SF-12- short form 12).**

Device usage and diary

Devices were activated for a patient-reported mean time of 5.56±1.6 hours per day, 5 days a week. Devices were well tolerated by subjects. Some chose to activate the devices during the day, others at night. No subjects reported use of the device interfering with their activities of

daily living, or mobilising. Skin irritation occurred in five subjects, and the device was discontinued where further use made it worse. Mean device usage prior to cessation was 122.7 hours, for mean of 4.9±1 hours per day. No link was identified between length of use and incidence of skin irritation.

7.1.3 Discussion

Morbidity and mortality from chronic venous disease, DVT, venous thromboembolism, and post thrombotic syndrome are significant (Beebe-Dimmer *et al.* 2005; Baldwin *et al.* 2013). A device that supports the efficacy of venous circulation in the legs could aid thromboprophylaxis in healthy individuals, and perhaps help manage symptoms in those with pre-existing venous disease.

This study has shown that the geko^{TM} NMES device can increase venous peak velocities in the healthy leg, which is concordant with existing literature (Tucker *et al.* 2010; Williams *et al.* 2015). NMES may be useful in the management of stasis-related disease (e.g. venous thromboembolism), where the speedy transition of blood through the valves of the deep venous plexi of the leg is seen to be important (Nicolaides *et al.* 1971). This study did not attempt to measure thrombosis rates in our population. In addition it also enhances the circulation in patients with chronic venous disease, even those with obstructive pathology. The benefits of repeated use include relief of leg oedema, and possible improvements in quality of life (AVVQ) of those with clinically severe venous symptoms. The study was underpowered to statistically evaluate quality of life score effects. Laser Doppler fluximetry is increased in the chronic venous foot, which may indicate that blood flow to the skin of the foot is enhanced. This may be very helpful in the management of venous ulcers, especially where compression is not tolerated or contra-indicated. The mechanism for an enhanced

effect with repeated use has not been elucidated. The authors postulate that this may be due to synergistic local and systemic factors (e.g. muscle tone with exercise, neuroendocrine modulation, or functional modulation of capillary networks). The significant changes in arm laser Doppler flux may support the systemic roles that exercise and enhanced venous return have on the circulatory system.

There were large variations in age and BMI between groups which may have affected activity levels, and therefore venous symptoms, over the course of six weeks. We did not control for activity, fluid intake or balance, or menstrual cycle in females, which may have affected our results. We did not assess perforator competency in isolation as part of our protocol, which has been shown to modify the haemodynamics of the leg (Delis *et al.*). The effect of calf perforators on the response to calf muscle pump action has not been explored, but may have had a detrimental effect on therapy when present. We did not covertly monitor adherence to the six week protocol, so are evaluating the effects of the device on an intention-to-treat basis. The correlation between reported and actual compliance is not known, therefore the effect of the device on all parameters, including side-effects, may have been underestimated as a result. The trial was underpowered to fully evaluate quality of life changes from using the device. For this reason we have not attempted to perform any health economic arguments. However, the changes in AVVQ, SF-12, and CES-D could be described as clinically significant. The effect size demonstrated here may be used in the future for the purposes of power calculation.

The non-invasive nature of transcutaneous stimulation means that some subjects remain recalcitrant to its effects. In the authors' experience, inability to elicit dorsiflexion renders treatment impossible. In 23% of our subjects, all with severe venous disease and a larger than

average BMI, maximum device stimulation was inadequate. If oedema is a contributing factor, prior limb elevation may help. Otherwise IPC and exercise programs may be viable alternatives (Kan *et al.* 2001; Padberg Jr *et al.* 2004; Comerota 2009; Nelson *et al.* 2011). Skin irritation occurred in five subjects, which was treated with simple emollients. We have raised this issue with the manufacturer of the device, and other device adhesives are currently under investigation.

NMES enhances the peripheral venous circulation of both healthy subjects and those with chronic venous disease, and may be helpful in managing clinical symptoms. It may be a useful adjunct in the prevention of VTE, particularly in those patient groups at high risk with contraindications to anticoagulation.

7.2 Adjuvant NMES in the management of peripheral arterial disease (PAD)

To evaluate the effect of NMES on patients with peripheral arterial disease, a study was devised. As there is a rich supply of claudicants to outpatient clinic in the local outpatient department, it was decided to concentrate on this cohort. If there is sufficient evidence that it is useful, then further studies in critical limb ischaemia may be appropriate.

7.2.1 Methods

A controlled interventional trial was devised, and ethical approval was granted by the National Research Ethics Committee (14/LO/0854). Subjects were recruited from vascular outpatient clinic, and vascular scanning laboratory. Eligibility criteria are presented in Table 28. Written informed consent was obtained.

Table 28 - Eligibility criteria for PAD trial

Inclusion criteria	- 18+ years old - BMI 17-30kg/m2 - Clinical diagnosis of stable intermittent claudication (>6 months) with confirmation by medical imaging - ABPI<0.9
Exclusion criteria	- Pregnancy - Cardiac pacemaker - Previous lower limb major amputation - Endovascular or surgical intervention planned in the next 8 weeks

BMI – body mass index; ABPI – ankle brachial pressure index

Screening

Screening evaluation included a medical history, physical examination, and pregnancy test if appropriate. Ankle-brachial pressure index (ABPI) and

Protocol

The group was split into two cohorts using a closed envelope technique (blinded randomisation). The control group were treated according to local and national guidelines (e.g. lifestyle advice, exercise class referral, smoking cessation advice, and pharmacological intervention as appropriate). The device group, in addition to best medical treatment, were allocated to use a protocol of neuromuscular stimulation. (Figure 64).

All trial subjects completed both generic and disease-specific quality of life questionnaires (EQ-5D, SF-12, CES-D, ICQ). Exercise treadmill test was performed according to protocol (2mph, 0% grade increased by 3.5% every 3 minutes to a maximum of 15%) (Hiatt *et al.* 1995).

Arterial ultrasounds performed as part of normal clinical care were analysed for distribution of disease. Stenosis greater than 50% in a claudicant was deemed significant, and was divided into iliac, femoral, and below knee sections. Scans performed within 6 months of the trial start date were available for all subjects.

Supervised exercise

Referral to supervised exercise programmes (SEPs) for patients with intermittent claudication is recommended by TASC II, NICE and SIGN guidelines (TASC2 2007; NICE 2014). The local supervised exercise classes are run by the physiotherapy department, for an hour a week (30 minutes exercise time), for 6 months(SF-36.org). Referrals from vascular clinic are usually

seen within 2 weeks for primary assessment and induction. Attendance is registered by the physiotherapists.

Neuromuscular stimulation devices

In those randomised to device, subjects were taught how to apply the geko™ device over the common peroneal nerve bilaterally, according to manufacturer's instructions. Subjects were asked to adjust the stimulation level to the minimum level that caused outward and upward twitching of the foot when raised from the ground. They were asked to activate the devices for 4-6 hours daily, 5 times a week. Subjects were asked to keep a daily diary of how often they used the device. Subjects were reviewed at 3 weeks, given a fresh supply of devices, their diaries collected and fresh diaries provided.

Haemodynamic ultrasound

Haemodynamic measurements were performed on all subjects allocated to device. Vascular ultrasonography was performed using a Philips ultrasound system with vascular protocol and transducer (Philips, Seattle, WA, USA). Multiple ultrasound measurements of blood flow were taken at the groin, and a mean calculated. Blood flow measurements were taken from the most symptomatic leg, with subject seated. Resting ultrasound haemodynamic measurements were compared to that with device turned on, and expressed as a percentage change. A laser Doppler probe was attached to the dorsum of the foot to measure changes in skin perfusion and temperature throughout the protocol.

Treadmill

Treadmill performance was measured using standard treadmill equipment and protocol, as specified in Hiatt et al. Subjects were verbally encouraged to push themselves as hard as

possible. Intermittent claudication distance (ICD) was taken at the first reported muscle pain, brought on by the treadmill exercise. Absolute walking distance (AWD) was the maximum distance covered before treadmill exercise was terminated, either at subject request or protocol termination.

At 6 weeks, all measurements were repeated and compared to baseline values.

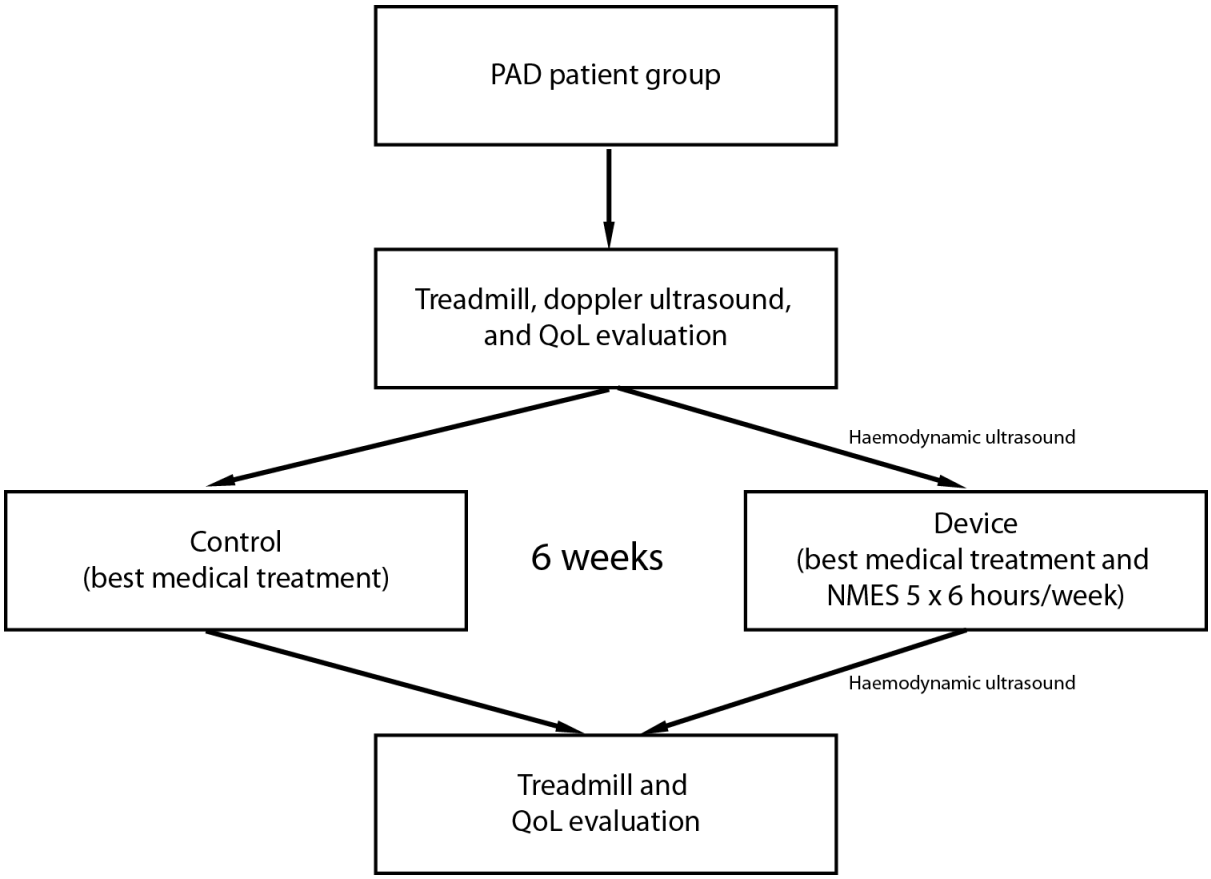


Figure 64 – Protocol for the use of neuromuscular stimulation in peripheral arterial disease

QoL – quality of life; NMES – neuromuscular electrical stimulation

Statistical analysis

Data was analysed using SPSS© Statistics version 21 (IBM).

7.2.2 Results

35 subjects were recruited and randomised, with 19 in the control group, and 16 in the device group. Two subjects in the device group did not stimulate appropriately with the device. One of these subjects had a history of renal impairment, diabetes, COPD (FEV_1 51%), an aorto-bi-iliac arterial bypass with surgical revision, and a claudication distance of 50 yards. After a few seconds on the treadmill he requested to withdraw from the trial. In the second subject, no twitch could be elicited despite all our technical efforts. This was presumed due to significant pedal oedema (thigh high), secondary to congestive heart failure. A consort diagram is shown in Figure 65.

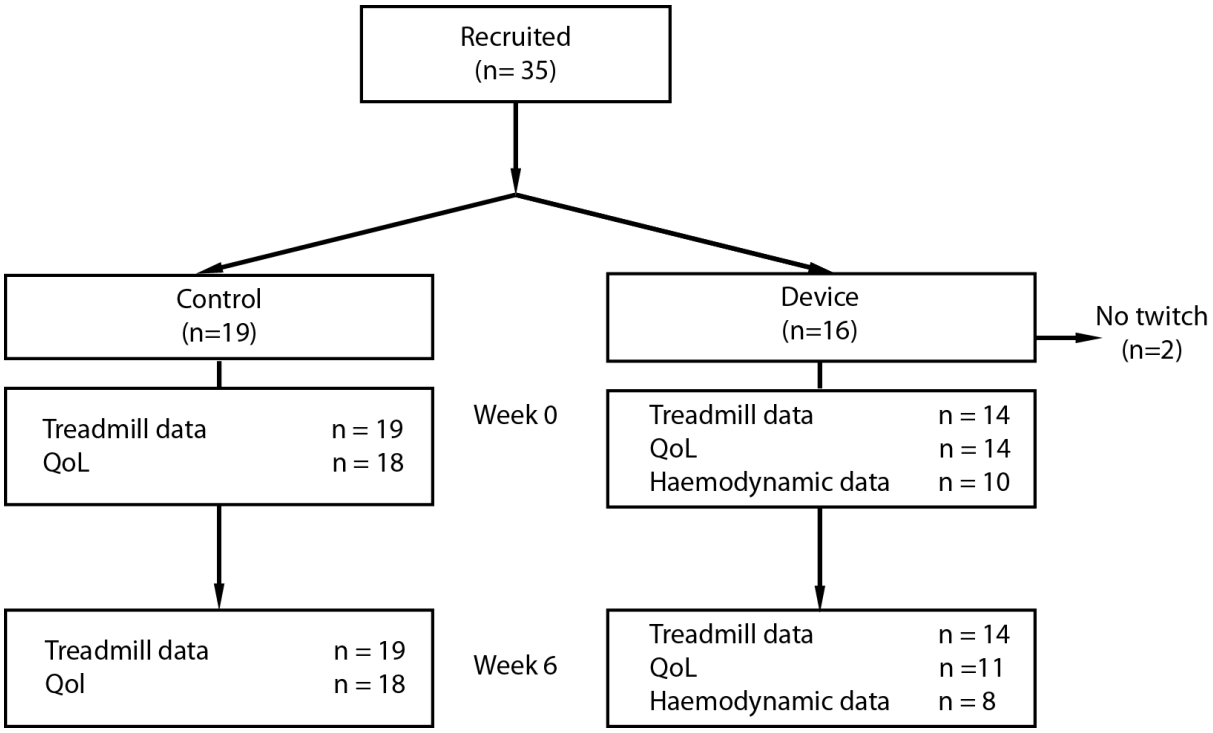


Figure 65 - CONSORT diagram for the PAD trial

Subject descriptors are shown in Table 29. The control population was significantly older than the device group, and contained more subjects that were diabetics, and ex-smokers. The device group had more current smokers. Stenotic disease distribution, as measured by doppler ultrasound, was mainly found in the femoral vessels in both groups. Percentage disease visible on ultrasound was higher in the device group, but this was not statistically significant. Baseline absolute walking distances were significantly longer in the device group distance, whilst claudication distances were not significantly different.

At the start of the trial period, >80% of both cohorts had already enrolled onto the SEPs course. Attendance was 52% and 48% for the control and device groups respectively. Subjects seemed to be divided into either very good attenders (<80% attendance) or poor/non-attenders (<20% attendance).

Diary recorded device hours showed good protocol adherence, with subjects using the device for mean (SD) 5.15 (+/-1.6) hours.

Walking distances at the beginning and end of the PAD protocol are shown in

Table 30. ICD and AWD changes for the control and device groups were 30.3m/91.9m (49%/122%) and 35.8m/124.3m (40%/231%). Both groups increased their walking distances by statistically significant amounts. Comparing the two interventions, there was no statistically significant difference between SEPS, and SEPS with adjuvant NMES (Table 31).

Table 29 – Table of subject descriptors for PAD trial

	CONTROL		DEVICE		P value	
	Mean	SD	Mean	SD		
n	19		16			
Age	73.0	12.5	63.6	8.7	0.02	$
Sex (F, %)	15.8		25.0		0.52	^
Smoker – never (%)	10.5		37.5		0.00	^
Smoker – current (%)	26.3		62.5			
Smoker – ex (%)	63.2		0.0			
DM (%)	5.3		37.5		0.03	^
BMI	27.6	4.4	31.0	9.1	0.15	$
DISiliac (%)	10.5		37.5		0.90	^
DISfem (%)	15.8		68.8		0.39	^
DISbelowknee (%)	5.3		37.5		0.36	^
ABPI	0.8	0.1	0.7	0.1	0.10	$
ICD (m)	76.8	72.4	163.2	280.0	0.27	$
AWD (m)	125.1	97.7	301.2	298.1	0.07	$

F – female; DM – diabetes mellitus; BMI – body mass index; DIS – ultrasound evidence of >50% stenosis in arterial segment specified; ABPI – ankle brachial pressure index; ICD – intermittent claudication distance; AWD – absolute walking distance; $=Mann-Whitney U, ^=paired t-test

Table 30 - Walking distances for subjects at the beginning and end of PAD protocol

	Control			Device		
	Week 0	Week 6	P value	Week 0	Week 6	P value
	n=19	n=19		n=14	n=14	
ICD (m)	68.4	110.0	<0.001	163.0	255.0	0.001
AWD (m)	129.0	203.0	<0.001	301.0	425.0	0.002

ICD – intermittent claudication distance; AWD – absolute walking distance; significance Wilcoxon signed-rank

Table 31 - Change to walking distances with PAD trial over 6 weeks

	CONTROL (n=19)		DEVICE (n=14)		
	Mean	SD	Mean	SD	P value
Change to baseline ICD (m)	30.3	34.3	91.9	128.9	0.5
% change ICD	49.4	47.9	122.2	136.1	0.6
Change to baseline AWD (m)	35.8	32.9	124.3	136.2	0.4
% change AWD	39.8	29.8	113.0	230.7	0.9

ICD – intermittent claudication distance; AWD – absolute walking distance; significance Wilcoxon signed-rank

Graphs of walking distances measured during the protocol are illustrated in Figure 66, Figure 67, and Figure 68.

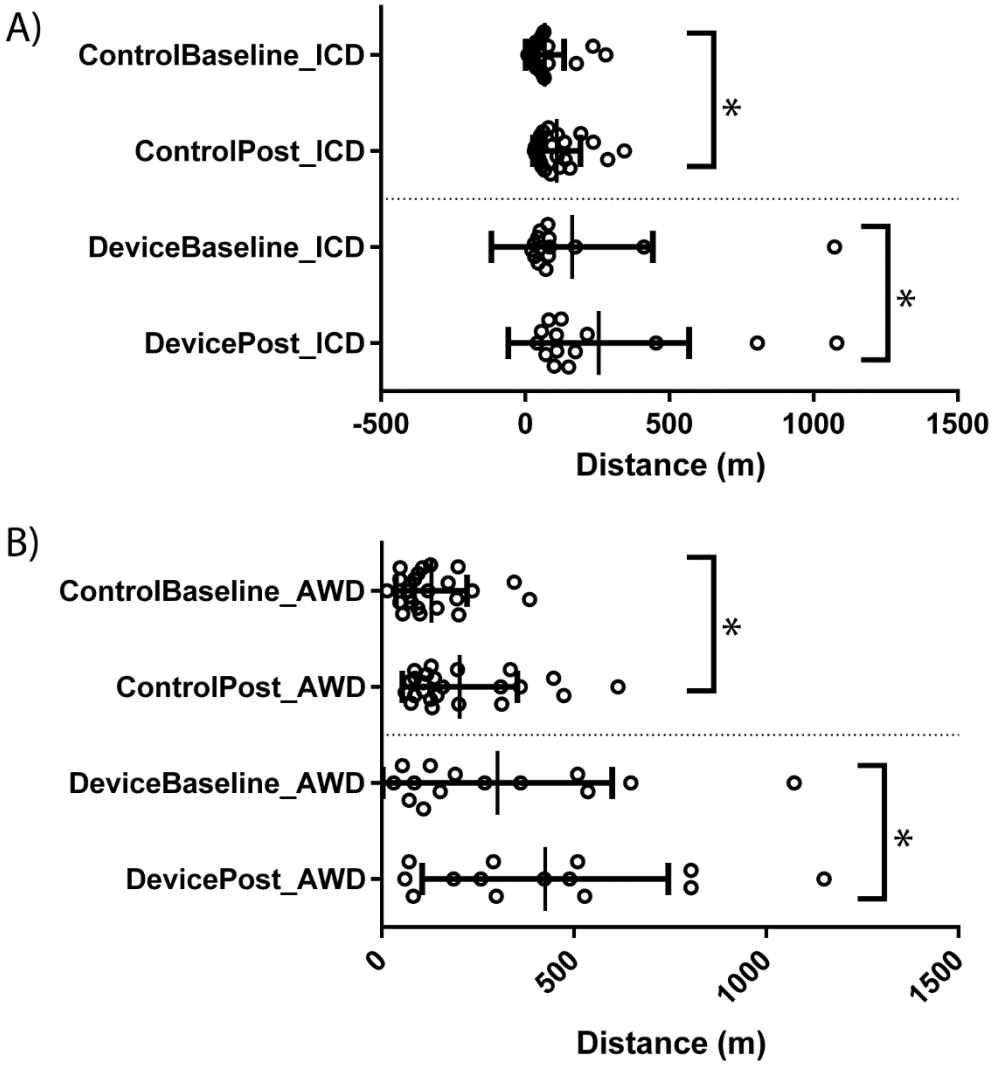


Figure 66 - Mean walking distances for subjects in the PAD trial

A) intermittent claudication distance, B) absolute walking distance; error bars +/- 2 SD

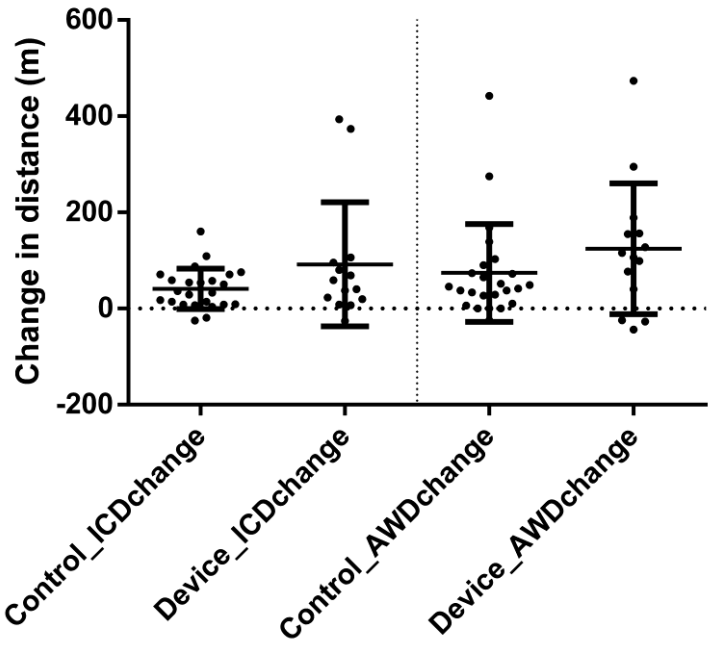


Figure 67 - Absolute changes in walking distances during PAD protocol

ICD - intermittent claudication distance; AWD – absolute walking distance; error bars +/- 2 SD

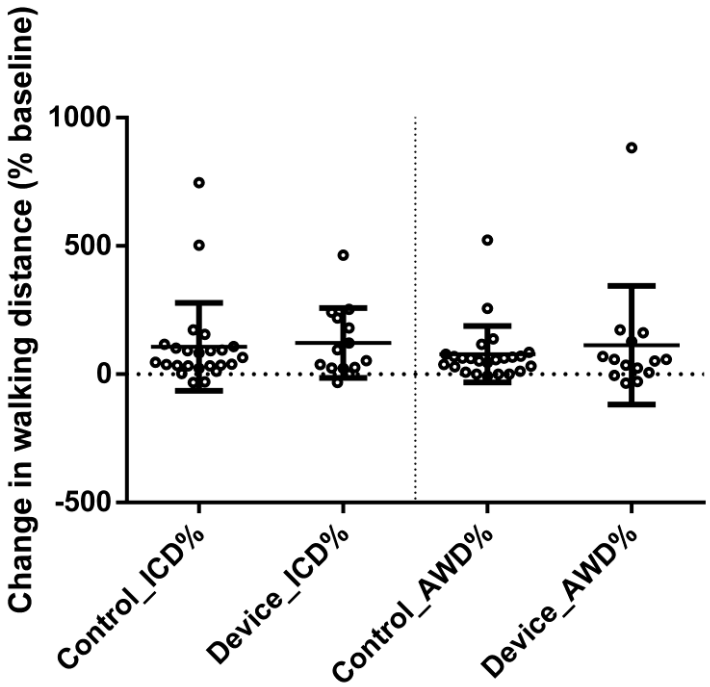


Figure 68 - Percentage changes in walking distances during the PAD protocol

ICD - intermittent claudication distance; AWD – absolute walking distance; error bars +/- 2 SD

With activation of the NMES devices, the percentage increases in arterial TAMV (104%) and volume flow (118%) were statistically significant. The percentage change in venous peak velocity (43%) was also statistically significant. This is shown in Table 32.

Over six weeks of PAD protocol, there was no statistically significant increase in response to stimulation in any parameter, except venous peak velocity. This rose from 8% to 86%. Results are tabulated in Table 33 and Table 34.

Table 32 - Absolute and percentage changes to haemodynamic variables in PAD subjects

n=18		PV (cm/s)			TAMV (cm/s)			VF (ml/min)		
		Mean	SD	Sig	Mean	SD	Sig	Mean	SD	Sig
Arterial	Change from baseline	6.23	27.57		2.16	5.71		56.93	152.14	
	%	25.8	66.0	0.30	104.0	241.8	0.02	117.5	212.6	0.01
Venous	Change from baseline	5.09	16.51		1.07	5.70		23.89	138.57	
	%	42.8	84.3	0.06	31.6	97.4	0.37	39.5	150.4	0.78

PV – peak velocity; TAMV – time averaged maximum velocity; VF – volume flow; Sig - p value, Wilcoxon signed rank

Table 33 - Percentage change from baseline with NMES in PAD subjects (arterial)

Week	PV			TAMV			VF		
	0	6	Sig	0	6	Sig	0	6	Sig
N	10	8		10	8		10	8	
Mean (%)	25.1	26.7	0.46	56.9	163.0	1.00	88.0	154.4	0.90
Std. Deviation	49.1	86.6		92.9	351.8		141.7	284.9	

PV – peak velocity; TAMV – time averaged maximum velocity; VF – volume flow; Sig - p value Mann-Whitney U

Table 34 - Percentage change from baseline with NMES in PAD subjects (venous)

Week	PV			TAMV			VF		
	0	6	Sig	0	6	Sig	0	6	Sig
N	10	8		10	8		10	8	
Mean (%)	8.4	85.8	0.04	-0.8	72.2	0.15	-0.9	90.1	0.17
Std. Deviation	52.9	99.2		49.7	128.4		56.4	213.5	

PV – peak velocity; TAMV – time averaged maximum velocity; VF – volume flow; Sig - p value Mann-Whitney U

Temperature and fluximetry changes with stimulation are shown in the appendices (Table 62). They are summated in Figure 69. Percentage changes from baseline in limb temperature and fluximetry were both significant ($p<0.05$, wilcoxon signed rank). When looking at individual subjects, the effect on device activation seemed to become more potent over time,

with percentage increases in skin blood flow increased from week 0 to week 6 (Figure 70). However, this was not statistically significant.

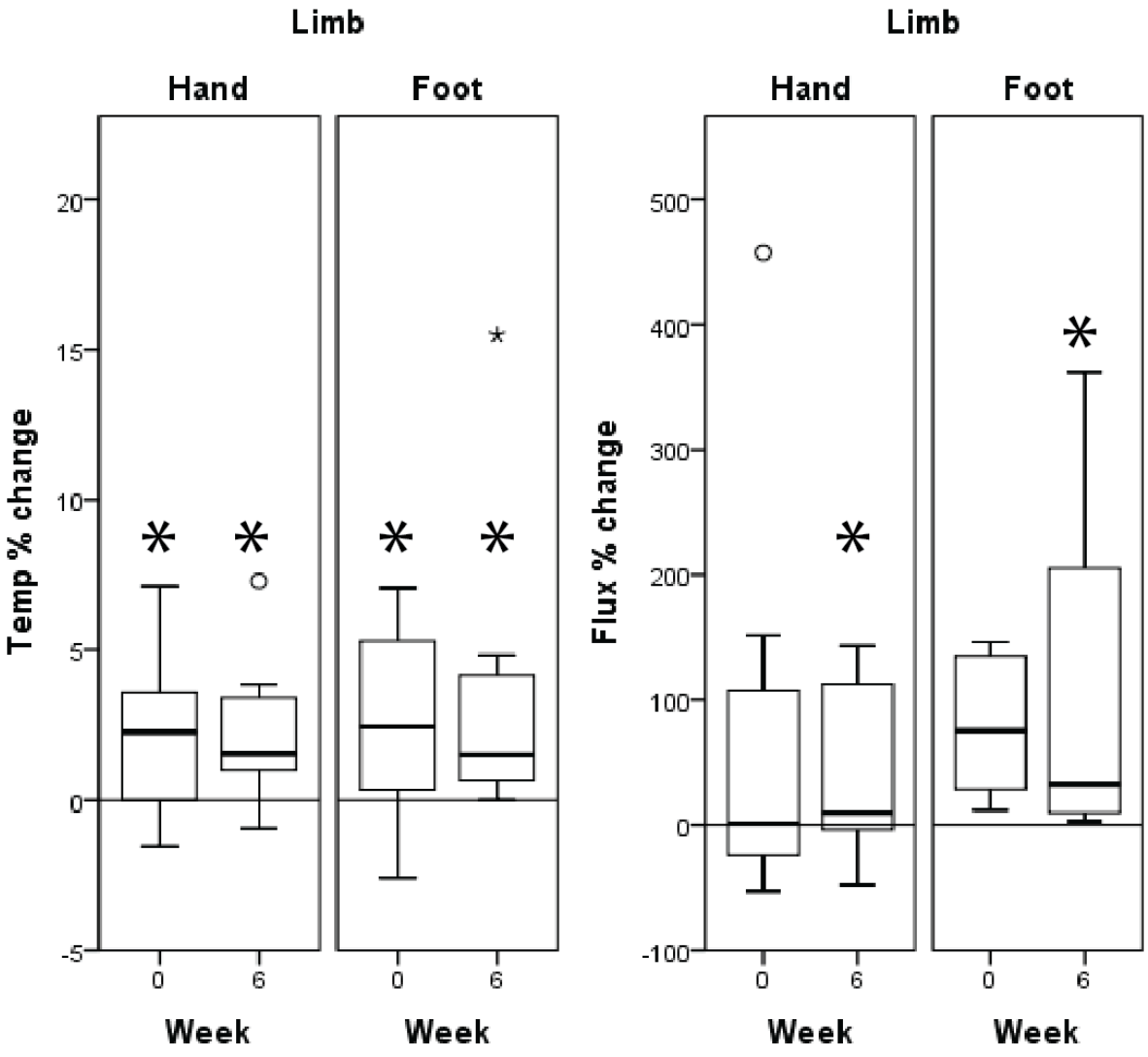


Figure 69 - Temperature and fluximetry changes from baseline, with activation of the neuromuscular stimulation device during PAD protocol

***- p<0.05, Wilcoxon signed-rank**

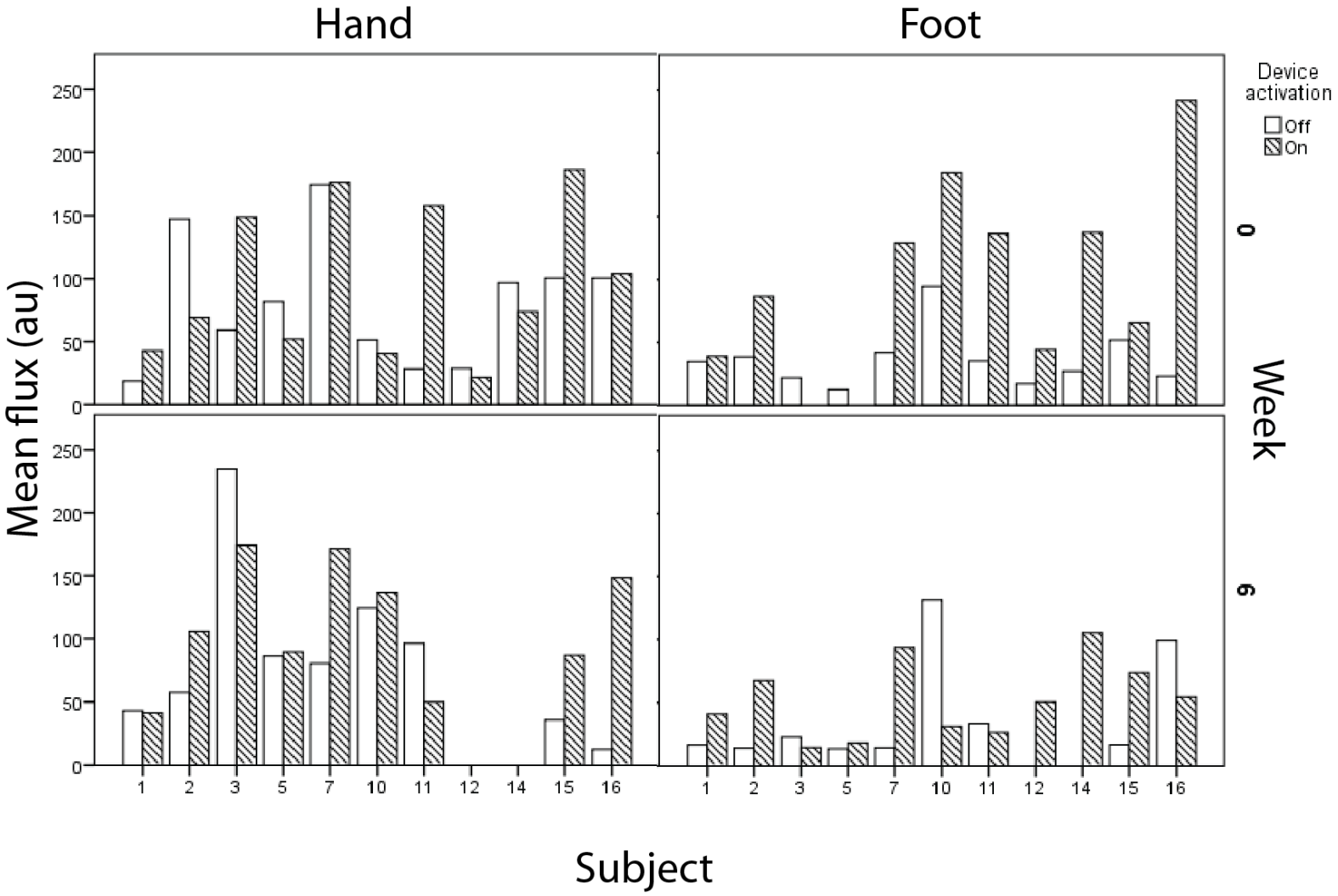


Figure 70 - Graph showing individual responses to NMES activation in PAD trial

Disease severity was scored using the intermittent claudication questionnaire, a higher score indicating more severe symptomatology. Scores in both groups decreased (not significant). The EQ-5D and the Visual Analogue Scale were used to evaluate generic quality of life, a higher score indicating a better quality of life. Scores in both groups increased (not significant). Results are shown in Table 35, Figure 71, Figure 72, and Figure 73.

Table 35 - Disease severity and quality of life questionnaires for subjects in the PAD trial

	CONTROL				P value	DEVICE				P value
	Week 0 (n=18)		Week 6 (n=18)			Week 0 (n=14)		Week 6 (n=11)		
	Mean	SD	Mean	SD		Mean	SD	Mean	SD	
ICQ	44.2	19.9	33.7	17.9	0.09	48.5	14.2	40.5	20.5	0.31
EQ-5D	0.5070	0.22	0.6149	0.13	0.18	0.3911	0.34	0.5657	0.28	0.12
VAS	64.7	24.9	64.7	24.9	0.96	66.1	23.0	71.6	16.3	0.54

Mann-Whitney U

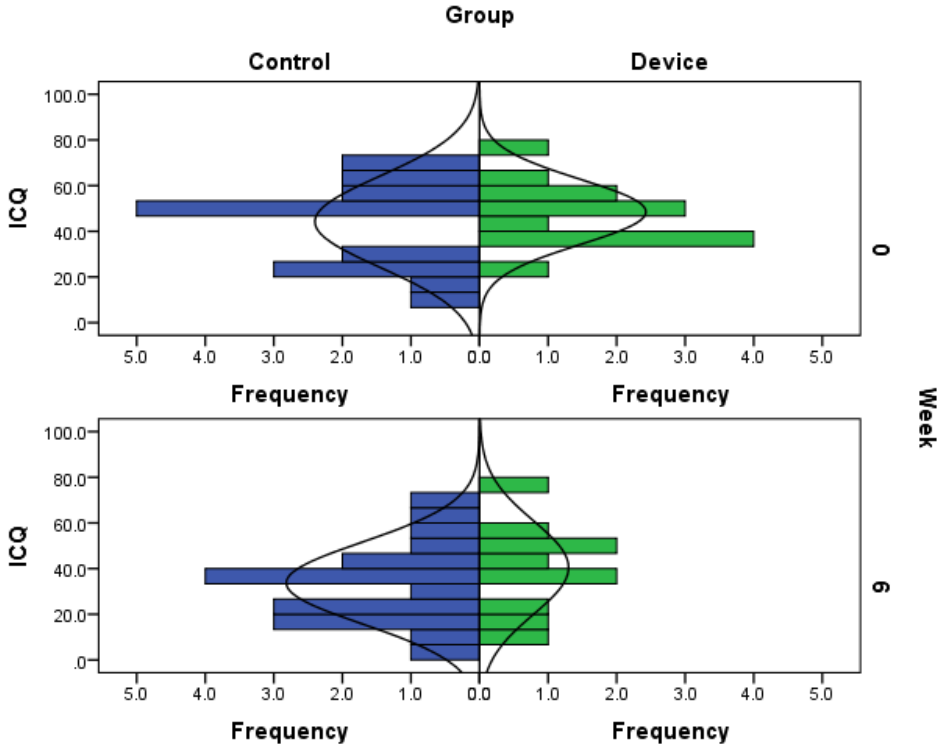


Figure 71 - Scores over time for intermittent claudication questionnaire in PAD trial

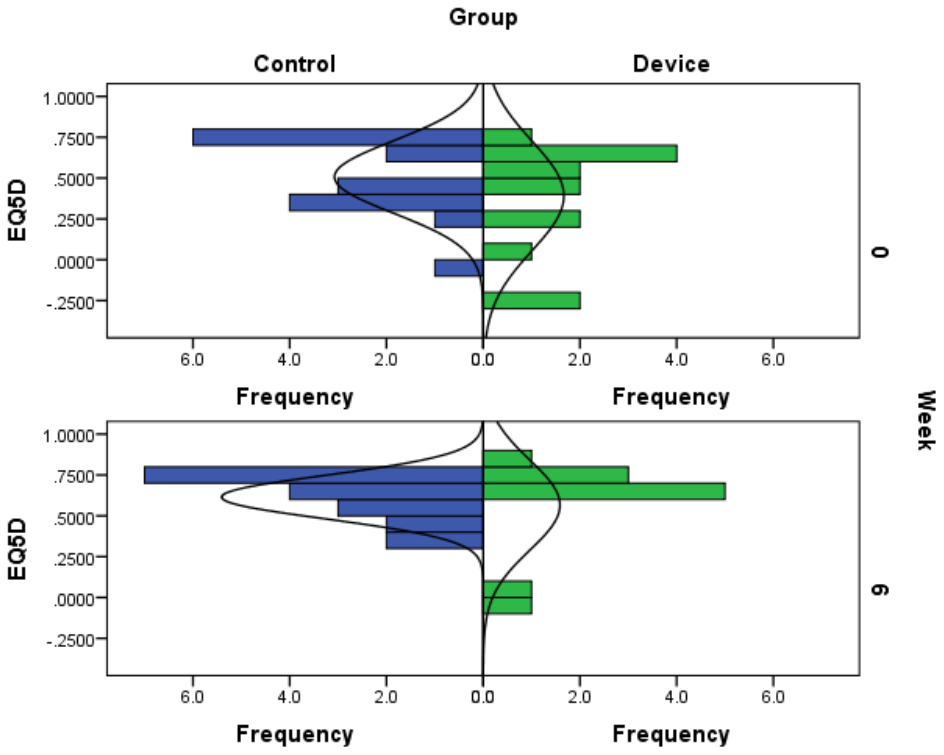


Figure 72 - Scores over time for EQ-5D questionnaire in PAD trial

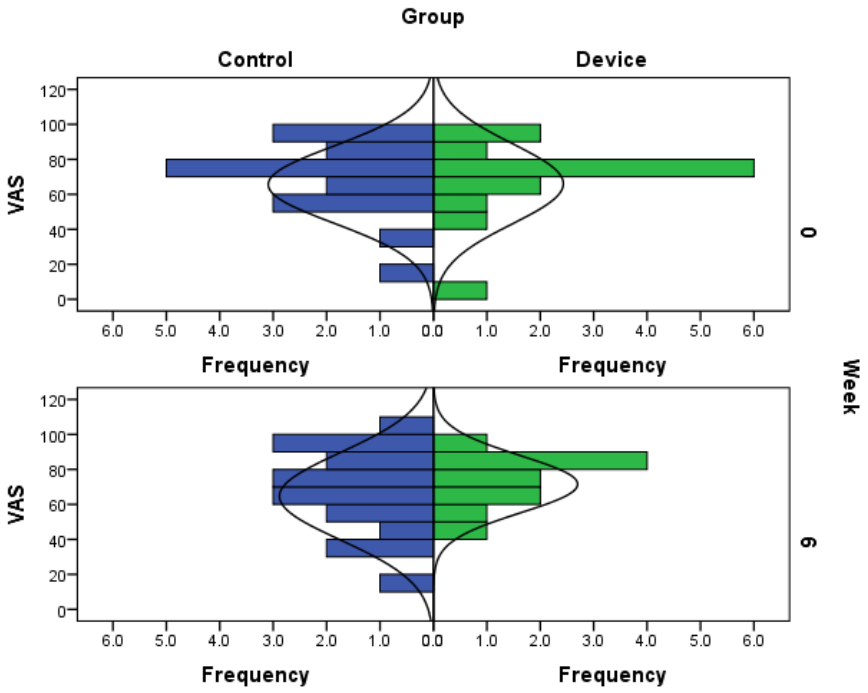


Figure 73 - Visual analogue scores over time for PAD trial

No adverse events, device malfunctions, or skin reactions at electrode sites were reported by trial subjects, either by diary or by direct questioning.

7.2.3 Discussion

This trial has shown convincing evidence that the use of both supervised exercise and neuromuscular stimulation increase walking distances in claudicants. Despite small sample size, statistically significant improvements in tangible outcomes can be seen.

The primary outcome for this trial was walking distances, in which improvements can be seen in both groups. However, the absolute and percentage increases were of a much greater magnitude in the group which used stimulation as an adjunct (triple the increase in ICD and AWD). A mean increase in both pain free walking and absolute walking distances will mean a lot for patients, and should limit the impact of their symptoms on their activities of daily living. If this were to hold true, one would expect this to be reflected in the quality of life data. Measurements of claudication symptoms (ICQ) decreased by a mean of 11 in the control group, and by 8 in the device group (24% and 16% respectively). Generic quality of life scores also improved (mean 0.11 in the control group, and 0.17 in the device group; 21% and 45%). The clinical significance of the magnitude of these changes is uncertain, but most subjects subjectively felt better and more positive about life when questioned. One gentleman in the device group had started to wear shoes again after an improvement in his peripheral neuropathy, and one gentleman who had had a small non-healing laceration on his anterior shin for two months finally granulated and closed his wound. The questionnaire score changes did not reach statistical significance, but were a larger scale trial to be considered, it would be pragmatic to power the study according to these results.

The population demographics were very different in both cohorts, with smokers and diabetics unevenly distributed, and a significantly older population in the control group. It is readily apparent from Figure 66 that baseline walking distance characteristics were different between groups, which may have impacted on progress with the protocol. However, despite having longer baseline distances in the device group, their baseline disease symptomatology as measured by the ICQ was more severe than the controls. The device group also had a poorer baseline generic quality of life, as measured by EQ5D. This may have been affected by more severe patterns of disease in the device group, and a higher prevalence of diabetes.

The haemodynamic ultrasound data is very encouraging, in that it supports an increase in limb and muscle blood flow as playing a part in the increased walking function and improved symptomatology of the subjects using the device. A doubling of femoral artery TAMV and flow rate with use of the device may well help not only with recovery from exercise, but perhaps also with muscle repair and growth. This could perhaps be investigated with the use of pre- and post-protocol muscle biopsy and/or NIRS. The increased response to stimulation at week 6 may be a result of vascular modification and improved collateralisation compared to baseline (as is known to occur with exercise programs), enabling more flow to the capillary beds with metabolic demand. (Hamburg *et al.* 2011).

Two subjects failed to stimulate with the geko device. One may be put down to increased skin impedance with their significant oedema, preventing sufficient transcutaneous electricity passage. The other subject had significant co-morbidities, with severe peripheral sensory loss and anoxia of tissues. Whilst they did not suffer with critical limb ischaemia at the time of testing, they have had previous episodes, necessitating major bypass grafts, and long term nerve damage is a reasonable assumption. Whilst the use of electrical stimulation as an

adjunct to exercise may be beneficial, it is not a viable option for those with significant amounts of pedal oedema at the knee, or those with large amounts of nerve damage. The precise threshold nerve function necessary for successful device use is not yet known.

There were no reported adverse events during the trial, which may be a reflection of how safe these therapies are. No one reported any skin irritation at stimulation site, and none was found on examination at the half way point or at the final debrief. The favourable side effect and interaction profiles make both exercise and neuromuscular stimulation attractive, particular in a population where polypharmacy is common.

Future work could include powering for quality of life outcomes, especially if the evidence is to be used to propose change to clinical practice. Cost effectiveness calculations could be performed, and would be especially important in this cohort given how cost-effective stand-alone SEPS programmes can be (Lee *et al.* 2007). One paper estimates the cost per quality adjusted life year (QALY) gain over conservative medical therapy to be £1780 at one year (Shalhoub *et al.* 2009). To put this in context, the threshold in the UK for cost per QALY is £20000.

The underlying biological changes could be investigated with blood sampling for blood gases (oxygen, carbon dioxide), lactate, glucose, tissue plasminogen activator, nitric oxide, and nitric oxide synthase (Dai *et al.* 2000). Simultaneous sampling from both the leg and upper body may reveal different regional profiles in biological millieau (Lattimer *et al.* 2015). Tissue sampling of muscle, skin or nerve may show growth changes over the protocol, although this would be particularly invasive and potentially unpleasant for trial subjects. NIRs traces taken from the gastrocnemius/soleus muscles during rest and exercise would give more information on the metabolic and perfusion performance under physiological conditions.

7.3 NMES in the management of diabetic foot ulcers (ULCERS)

The diabetic foot poses a uniques management problem, with diabetic foot ulcers being extremely challenging and multifactorial. As discussed in the early part of this thesis, a non-invasive adjunct to regular clinical care, that had tangible measureable impact on healing rates and limb salvage, would be welcome.

To investigate the effect of NMES on the rate of healing of diabetic foot ulcers, a trial was devised which would measure wound healing, pain, and quality of life.

7.3.1 Methods

Ethics approval was obtained (NRES 14/SC/0084). Patients fitting the inclusion criteria were recruited from diabetic foot and vascular clinic, and the vascular imaging laboratory (Table 36). Written informed consent was obtained.

Table 36 - Eligibility criteria for ULCERS trial

Inclusion criteria	18+ years old Type 2 diabetes, confirmed by a Diabetologist, on best medical therapy. Active foot ulceration, present <3 months
Exclusion criteria	Pregnancy Pacemaker Metal implants in the legs (below knee)

Screening evaluation included a medical history, physical examination, pregnancy test if female, and ultrasound examination of the lower limbs (venous duplex and arterial Doppler).

Subjects confirmed their diagnosis of diabetes, and compliance with best medical therapy was assessed using patient notes and clinical letters.

Protocol

The trial protocol is illustrated in Figure 74. Subjects were recruited from a tertiary diabetic foot unit at Imperial College NHS Trust. Recruitment target was 20 subjects, divided into two groups. Randomisation of subjects into two groups was performed using a closed envelope technique. One group acted as a "control", and received regular NHS care only (e.g. advice and guidance for improved diabetic control, wound debridement, antibiotics if clinically indicated, etc.). The other group received regular care in addition to a six week program of self-administered neuromuscular stimulation ("device"). Trial endpoints are presented in Table 37.

Table 37 - Endpoints used in the ULCER trial

Primary	Ulcer assessment – time to 50% healing
Secondary	Tolerability of the device Haemodynamic changes in the femoral vessels (baseline values, and response to stimulation) Quality of life scores Need for surgical intervention

Ulcer size was assessed using the Eykona 3D camera (Fuel 3-D, UK) at each outpatient appointment after any clinically necessary wound debridement.

Both specific and generic quality of life questionnaires were performed at the first visit, and repeated at the end of the six week protocol (PAID, DQOL, VAS, EQ-5D, SF-36).

The device

Those allocated to the device group wore a geko™ device on the ulcerated limb only. They were trained to apply the device themselves. The stimulation level was set by the subject to the minimum level that achieved distal twitch.

Devices were activated for 4-6 hours per day, 5 days a week, and they were asked to record a diary of device usage. With their diary keeping, any changes to medication, hospital visits, and unexpected events were recorded.

Haemodynamic ultrasound

Haemodynamic measurements were performed on all subjects allocated to the device group. Vascular ultrasonography was performed using a Philips ultrasound system with vascular protocol and transducer (Philips, Seattle, WA, USA). Multiple ultrasound measurements of blood flow were taken at the groin, and a mean calculated. Blood flow measurements were taken from the stimulated leg, with subject seated. Resting ultrasound haemodynamic measurements were compared to that with device turned on, and expressed as a percentage change. A laser Doppler probe was attached to the dorsum of the foot to measure changes in skin perfusion and temperature throughout the protocol.

At 6 weeks, all measurements were repeated and compared to baseline values.

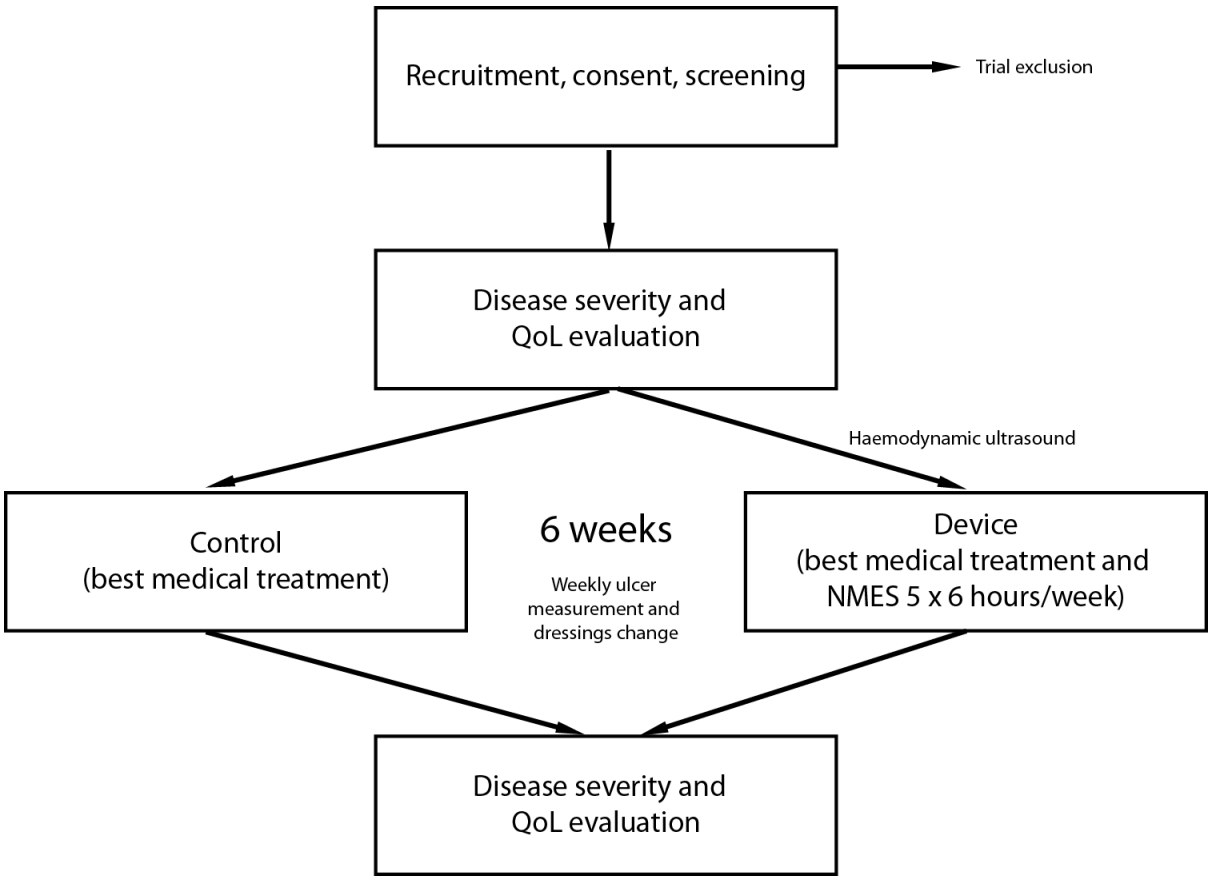


Figure 74 - Protocol for ULCERS trial

Data was analysed using IBM SPSS Statistics (version 21). As this is a small dataset, data was treated as non-parametric.

7.3.2 Results

The trial was suspended due to lack of staffing in September 2015. Eight patients were recruited, and the characteristics of trial recruits are shown in Table 38. No subjects declined inclusion in the trial.

The device was well tolerated, with all patients completing the protocol. No adverse events were reported, although one subject did pass away six weeks after the end of the trial. This

subject had been bed-bound with Alzheimer's dementia for over a year, and had multiple severe physical comorbidities. After discussion with his GP and diabetologist, this was not considered to be linked to the trial. Examination of trial diaries suggested that devices were being worn and activated continuously, five to seven days a week. In some subjects the device was applied and then leg bandages applied over the top. On inspections this did not result in any localised skin reactions.

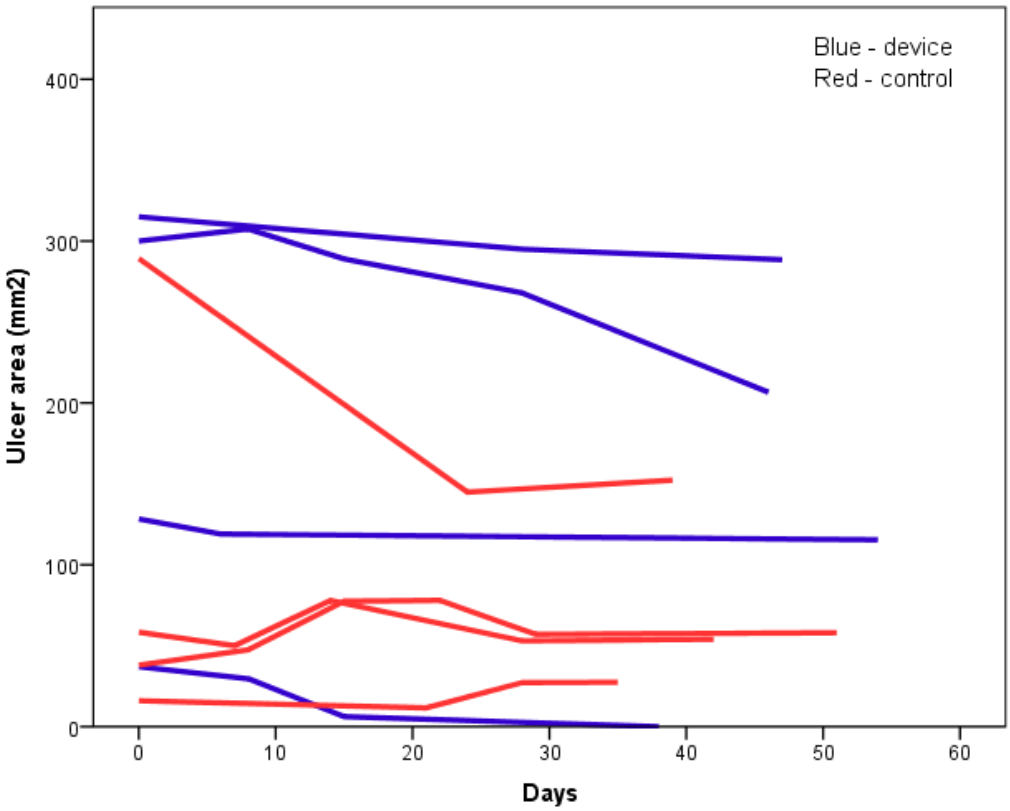
Table 38 - Subject characteristics for ULCERS trial

	Control		Device	
Number	4		4	
Age (years)	65	(56-76)	69.5	(49-87)
BMI (kg/m^2)	26.5	(25-31)	25.1	(23-36)
Smoker (current or ex)	75%		75%	
Insulin-dependant	50%		75%	
ABPI	1.1	(0.4-1.4)	1.2	(1.1-1.6)
On antibiotics during trial	100%		75%	
Baseline ulcer area (mm^2)	58.3	(16-289)	214.2	(37-315)
Baseline ulcer volume (mm^3)	65.2	(5-425)	278	(23-1005)
MNSI	14.5	(4-16)	17	(15-18)

Figures shown as percentage, or median and range.
MNSI – Michigan Neuropathy Screening Instrument

Compared to control, there were more smokers with higher neuropathy scores in the device group. The distribution of age, BMI and ABPI were comparable between groups. The median wound area and volume was 58.3mm^2 and 65.2mm^3 in the control group, and 214.2mm^2 and 278mm^3 for the device group. In this small analysis, differences between group measurements did not reach statistical significance ($p>0.05$, Mann-Whitney U).

Graphs in Figure 75 and Figure 76 show changes in ulcer area and volume over time. Percentage changes are also shown, with interpolation lines for both the device and control groups. Points underneath the Y-origin imply healing, whilst points above the origin indicate increasing size. Neither of the extrapolated lines of best fit (% area versus time) cross the 50% healing mark in the trial period. When looking at percentage volume healing, the device group achieved 50% healing at approximately 30 days, whilst the control group achieved this at approximately 55 days. Kaplan-Meier analysis was not attempted due to the low number of subjects that achieved 50% healing in area and volume measurements.



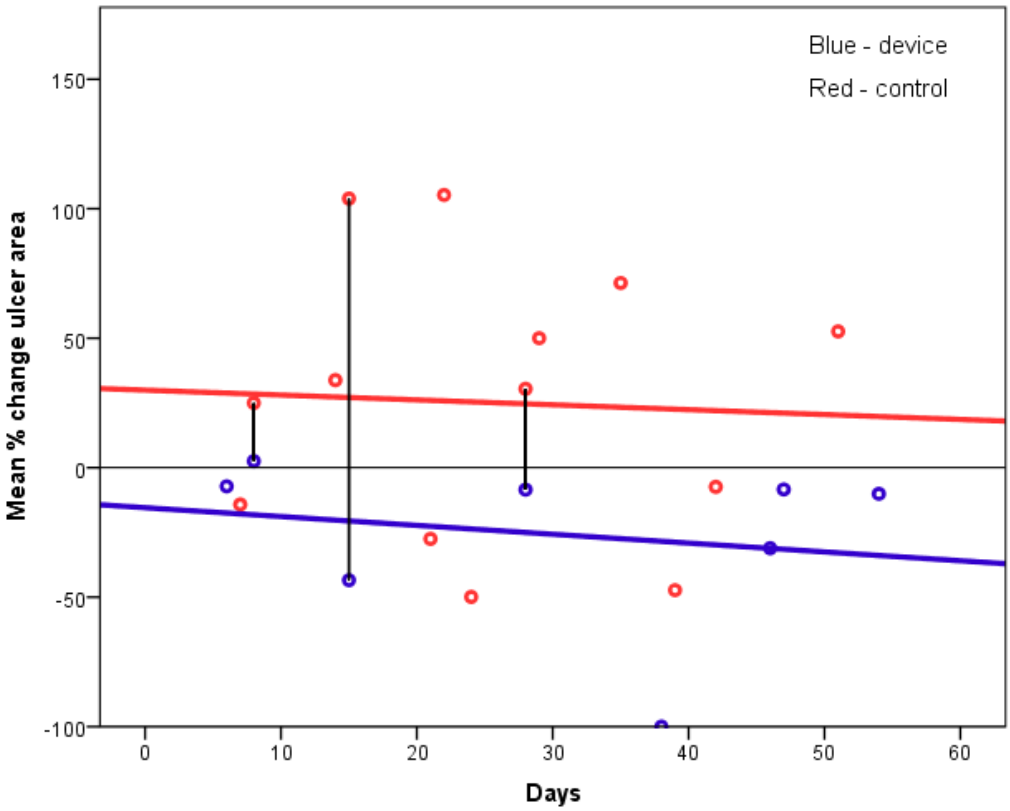


Figure 75 - Graphs illustrating results for wound surface area healing in ULCERS trial

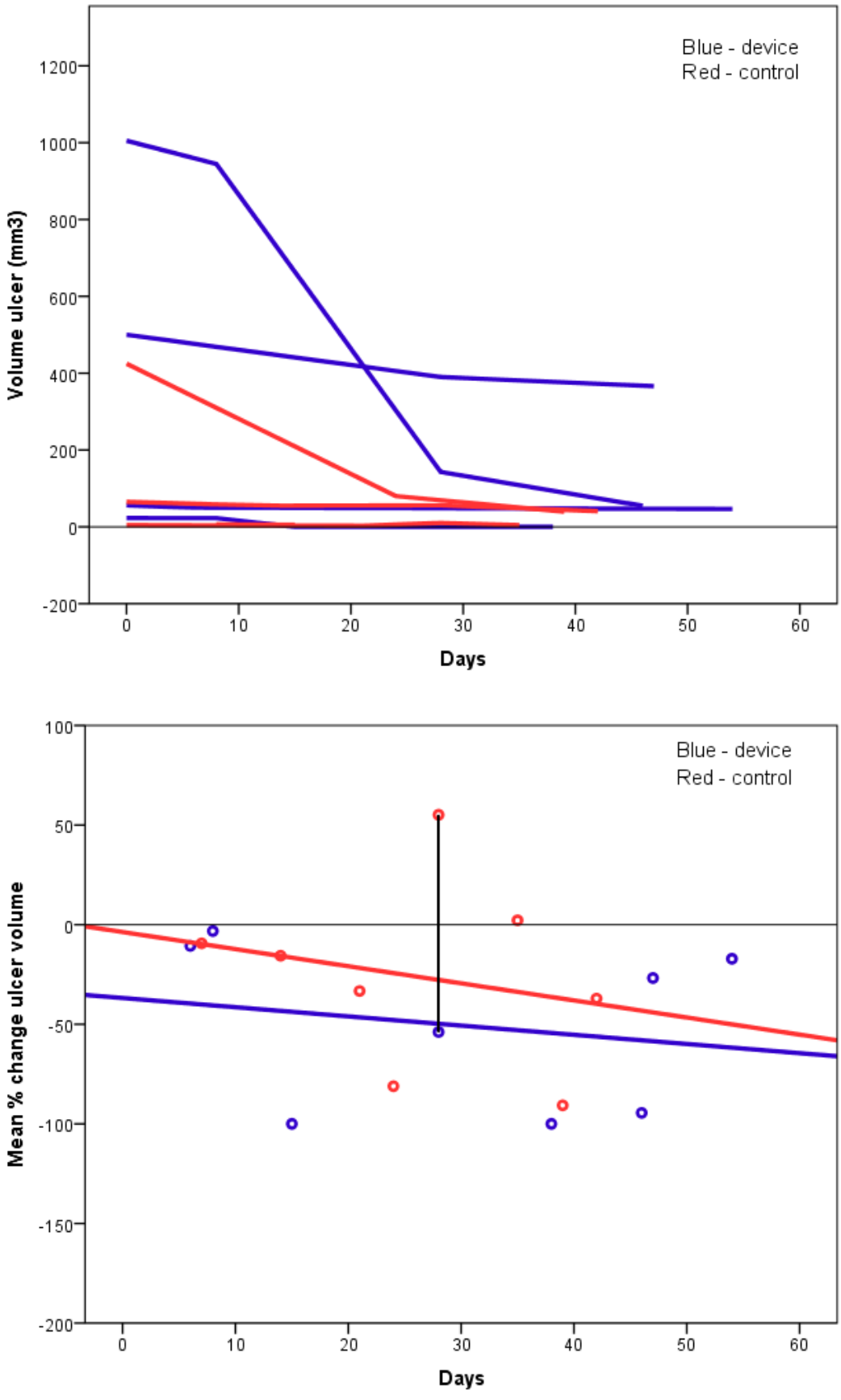


Figure 76 - Graphs illustrating results for wound volume healing in ULCERS trial

Disease severity and generic quality of life questionnaire scores are shown in Table 39, both at baseline and at week 6. Disease severity scores decreased across the protocol for both groups, by mean 9.8 in the control group, and 4 in the device group. Generic quality of life questionnaires EQ-5D, VAS and SF-12 scores changed by mean +0.16/+0.22 (control/device), +2/+18, and -15.1/+6 respectively. Due to the low number of subjects in each group, no statistical analysis was performed. Histograms of results are shown in Figure 77.

Table 39 - Questionnaire scores for subjects in ULCER trial, between beginning and end of trial (6 weeks)

	Control		Device	
	Week 0	**Week 6**	**Week 0**	**Week 6**
PAID	22.8 ±14	13 ±1	21.3 ±7	17.3 ±14
EQ5D	0.54 ±0.4	0.70 ±0.2	0.18 ±0.4	0.40 ±0.4
VAS	58 ± 13	60 ±14	37 ±38	55 ±23
SF-12	82.1 ± 12	67 ±9	66 ±14	72 ±11

Data presented as mean ± standard deviation.
PAID – Problem Areas in Diabetes; EQ5D – EuroQol 5D; VAS – Visual Analogue Score;
SF-12 – Short Form 12

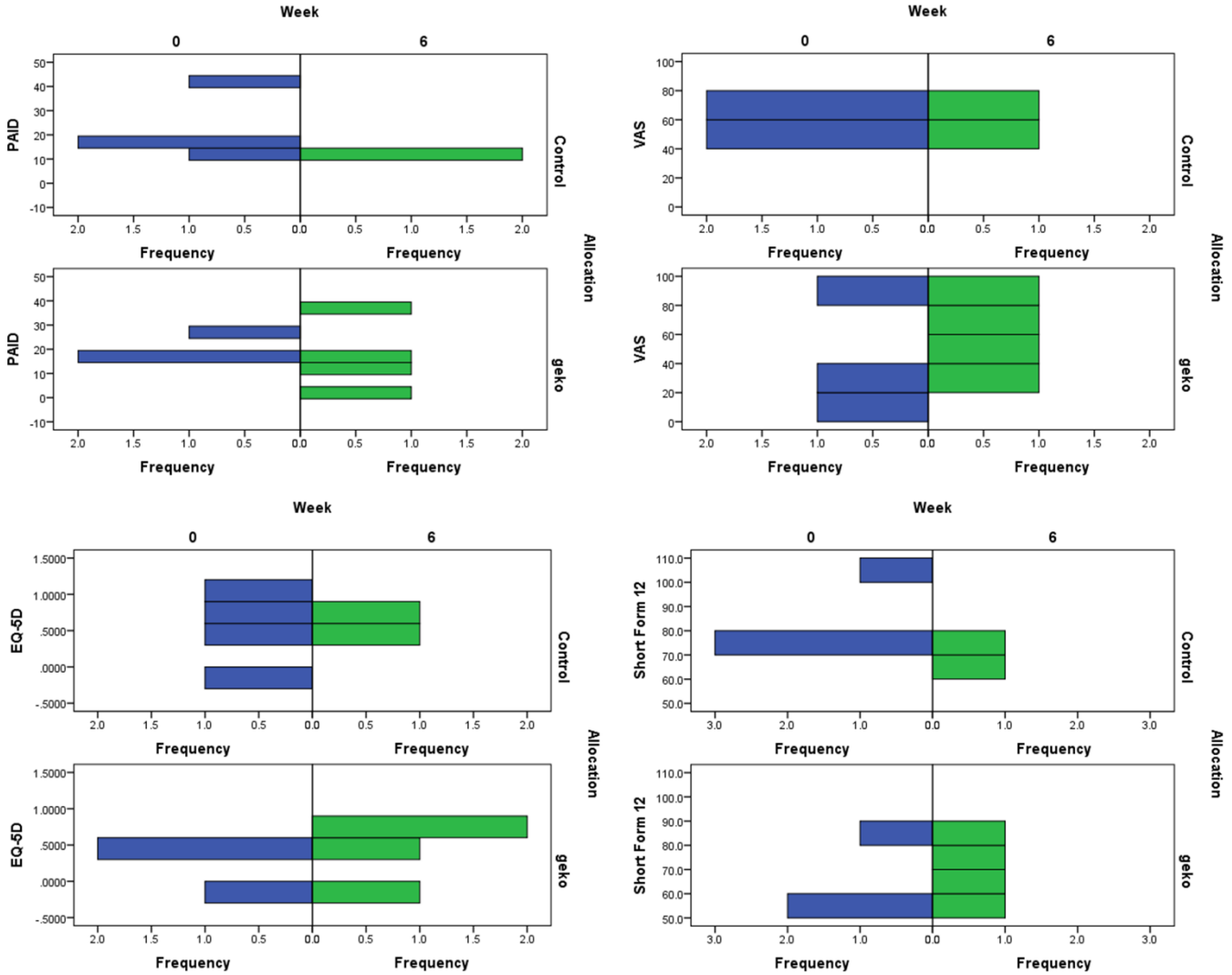


Figure 77- Histograms of questionnaire responses for ULCER trial

The haemodynamic changes in the femoral vessels with activation of the device are shown in Table 40 and Table 41. Subject 1 was unable to transfer to the scanning couch (in a wheelchair with significantly painful pressure sores), therefore 3 subjects are included in the analysis. Haemodynamic responses to stimulation varied widely. In one subject arterial TAMV increased by 120%, in another it hardly changed at all. Venous parameters changed very little, no more than ±40% from baseline.

Table 40 - Arterial haemodynamic changes with geko device activation (ULCERS trial)

		A	B	C
Peak velocity (cm/s)	Baseline	83.4	104.3	41.0
	Device activation	86.0	95.3	79.9
	% change	**3**	**-9**	**95**
TAMV (cm/s)	Baseline	13.2	11.6	4.5
	Device activation	18.1	11.3	10.5
	% change	**37**	**-3**	**121**
Volume flow (ml/min)	Baseline	387.0	171.3	24.4
	Device activation	535.7	191.7	43.1
	% change	**38**	**12**	**77**

Table 41 - Venous haemodynamic changes with the geko device (Ulcers trial)

		A	B	C
Peak velocity (cm/s)	Baseline	16.3	18.2	14.7
	Device activation	17.9	23.4	14.5
	% change	**10**	**28**	**0**
TAMV (cm/s)	Baseline	4.6	5.8	4.9
	Device activation	5.4	4.7	3.7
	% change	**17**	**-20**	**-25**
Volume flow (ml/min)	Baseline	167.7	196.0	145.7
	Device activation	136.3	110.4	99.4
	% change	**-19**	**-44**	**-32**

Laser doppler fluximetry changes with device activation compared to baseline were 9% on the dorsum of the ulcerated foot, and 50% on the dorsum of the hand.

7.3.3 Discussion

This was a very small study, and required many resources in terms of time and travel for both the trial subjects and the investigators. The physical incapacity encumbant with diabetic foot ulcers meant that most trial weeks needed several home visits, to be present at the time of district nursing dressing changes for wound photography to be performed. Funding for a weekly clinic and patient transport would have been helpful, and needs to be factored in should this trial be considered for repetition. The results of this trial therefore are very much just a snapshot of a small group of patients, and not necessarily representative of the diabetic foot ulcer population.

The two cohorts for comparison were poorly matched. The subjects allocated to device tended to have a greater degree of ulceration, and more severe neuropathy scores. Any changes in these parameters must be interpreted with this in mind.

Interpretation of wound healing is always difficult in the context of small numbers, as individual variations in disease process, physiology, activity, diet and environmental factors will skew averaged results. It is noted that measurements of wound surface area changed by smaller percentages than measurements of wound volume, which may reflect an increased sensitivity of healing detection. The primary endpoint of 50% healing (volume rather than surface area) was reached much quicker by the device group (30 days) than the control group (55 days). This is potentially very exciting, as closure of a wound in a diabetic foot is a significant event in the life of a diabetic patient, and reduced the potential for infection and incapacitation. It must be noted that during the course of the trial only two people achieved complete wound closure, on in each group. These wounds were very small to begin with, but were in load-bearing areas, significantly harder to treat than ulcers in non-weight bearing areas (Levin 1995; Snyder *et al.* 2002).

Generic quality of life scores increased much more in the device group than the control group, as measured by the VAS and SF-12. In the latter there was a mean discrepancy of 21/100 between the two cohorts. This may be due to the reduction in size of the proportionately large ulcers, or may be a result explained by confounding factors. The EQ-5D seems to have changed by proportional amounts in both groups, which may point to the fact that functional tasks were not improved significantly by addition of the device. Diabetes severity scores for both groups decreased by similar amounts, and there are limits as to how this may reflect ulcer improvement progression. The effects of diabetes on every day life are far-reaching and

multi-modal. The changes in these scores may be used in the future to power future trial work, and aid future health care cost effectiveness analysis.

Haemodynamic data are difficult to interpret with so few subjects. In isolation it can said that the device activation increased femoral arterial TAMV and volume flow in three subjects. This varied by large amounts from subject to subject. Venous parameters did not appear to have changed much, and in some cases decreased. This may be an artefact caused by microvascular disease (microvascular hardening, calcification and occlusion), or by multiple vascular sites of disease, both peripheral and central. It has been shown that diabetics are more likely to suffer from ischaemic heart disease, stroke, nephropathy, retinopathy, and peripheral neuropathy (Amos *et al.* 1997; Currie *et al.* 1998; Donnelly *et al.* 2000; Stratton *et al.* 2000). This is certainly confirmed in our cohort, who suffered with most if not all of these co-morbidities. It is puzzling however, that the haemodynamic mechanism of action in this group of patients does not appear to be activated via venous clearance, as was presumed in the healthy subjects earlier in this thesis.

7.4 NMES in the management of diabetic peripheral neuropathy (NERVES)

Exercise and NMES both increase cardiovascular parameters in the lower limbs. It is postulated that NMES may be beneficial in this cohort.

It has been noted clinically that vascular patients with peripheral neuropathy, having used the device, have declared symptomatic benefit. These benefits to patients have not yet been formalised. It is hypothesised to work either by increasing blood flow to the limb and therefore the nerves themselves, or that the electric current is having a direct effect on the peripheral nervous system itself.

The short- and longer-term effects of neuromuscular stimulation on diabetic peripheral neuropathy was subjected to preliminary evaluation as a therapeutic intervention.

7.4.1 Methods

Ethical approval was granted by London (Dulwich) NRES committee, reference 13/LO/1844. Recruitment was initiated at both the local neurophysiology laboratories at Imperial College London, and the diabetic foot outpatient clinic at St Marys Hospital, Imperial College NHS Trust. A total of twenty subjects was thought to represent an attainable number for a pilot study, and to give an idea of effect size for a future power calculation. Eligibility criteria are tabulated in Table 42.

Table 42 - Eligibility criteria for NERVES trial

Inclusion criteria	18+ years old A diagnosis of diabetes as defined by WHO diagnostic criteria, on best medical therapy Diabetic peripheral polyneuropathy present, confirmed by nerve conduction testing
Exclusion criteria	Pregnancy Pacemaker Metal implants in the legs (below knee)

The length of the trial was set at 10 weeks, as this is the shortest time that we reasonably expected an objective change in peripheral neuropathy to occur between groups. A small exercise study showed an improvement in neuropathic symptoms in 10 weeks (Kluding *et al.* 2012).

Screening evaluation included a medical history, physical examination, blood tests, and pregnancy test if female. Compliance with best medical therapy was assessed by the trial recruiter through subject interview and review of hospital notes and clinic letters. The definition of diabetes for the purposes of this trial will conform to that of the World Health Organisation. It is diagnosed either:

1. On the basis of history (i.e. polyuria, polydipsia and unexplained weight loss) plus a random venous plasma glucose concentration ≥ 11.1 mmol/l
2. By a fasting plasma glucose concentration ≥ 7.0 mmol/l
3. By a plasma glucose concentration ≥ 11.1 mmol/l two hours after 75g anhydrous glucose in an oral glucose tolerance test

Diabetic peripheral neuropathy for the purposes of this trial will conform to the American Diabetes Association definition: the presence of symptoms and signs of peripheral nerve dysfunction in patients with diabetes after exclusion of other causes. Blood tests for HIV,

syphilis, thyroid function, B12 and folate were performed, and abnormal results disqualified participants. An alcohol history was elicited through direct questioning, and physician suspicion of chronic alcoholism also disqualified subjects.

Ultrasound examination of the lower limbs was carried out as deemed necessary by the treating physician (diabetologist or vascular surgeon).

Outcome measurements

The primary outcome, measured at baseline and at 10 weeks, was nerve conduction velocity. This was performed using standard protocol in the neurophysiological laboratory at Charing Cross Hospital by a consultant neurophysiologist. Nerve conduction speed has been shown to be a sensitive measure of change in diabetic peripheral neuropathy (Brown *et al.* 2004). Amplitudes and latency were also recorded. Lowest recorded values were used, and repeat values at the end of the protocol taken from the same laterality.

Secondary outcomes were haemodynamic ultrasound measurements taken in the femoral vessels (baseline and device on), quality of life questionnaires (PAID, MNSI, NTSS-6, EQ-5D, SF-36), tolerability scores (VAS, VRS), and device usage diary. Candidates were asked to notify the team of any clinical deterioration during the trial period. Surgical intervention during the trial period was recorded.

Protocol

At the time of recruitment, all subjects were asked to fill out quality of life questionnaires. If not already performed within the last 4 weeks, appointments for neurophysiology testing with a consultant neurophysiologist were made at this point. No interventions were commenced until after initial nerve conduction testing.

Subjects were randomised to “device” versus “no device” using a closed envelope technique, according to order of recruitment. Subjects allocated to “device” were taught how to use the geko™ T1 device (Firstkind Ltd, UK), and asked to apply it bilaterally. The device stimulation level was set to the minimum level that can achieve outward and upward twitching of the foot when raised from the ground. Baseline and stimulation values of blood flow in the femoral vessels were taken at this time, according to protocol (section 5.6.1). Subjects were asked to wear the devices for 4 or more hours per day, 5 times a week, and asked to keep a diary of usage. Subjects allocated to “no device” continued with management regimes as prescribed by their physician.

All subjects were seen at week five and asked how they were finding the protocol, in an effort to identify problems, address adverse events, and replenish device stocks. Device diaries were collected and reissued.

After ten weeks of the protocol, subjects filled out quality of life questionnaires and had repeat nerve conduction studies. Subjects in the “device” group submitted their device usage diaries, and repeat femoral ultrasound measurements taken at baseline and during stimulation. All subjects were directly asked about antibiotic use, changes in analgesia, attendances to hospital or general practice, and need for surgery.

The protocol is shown in Figure 78.

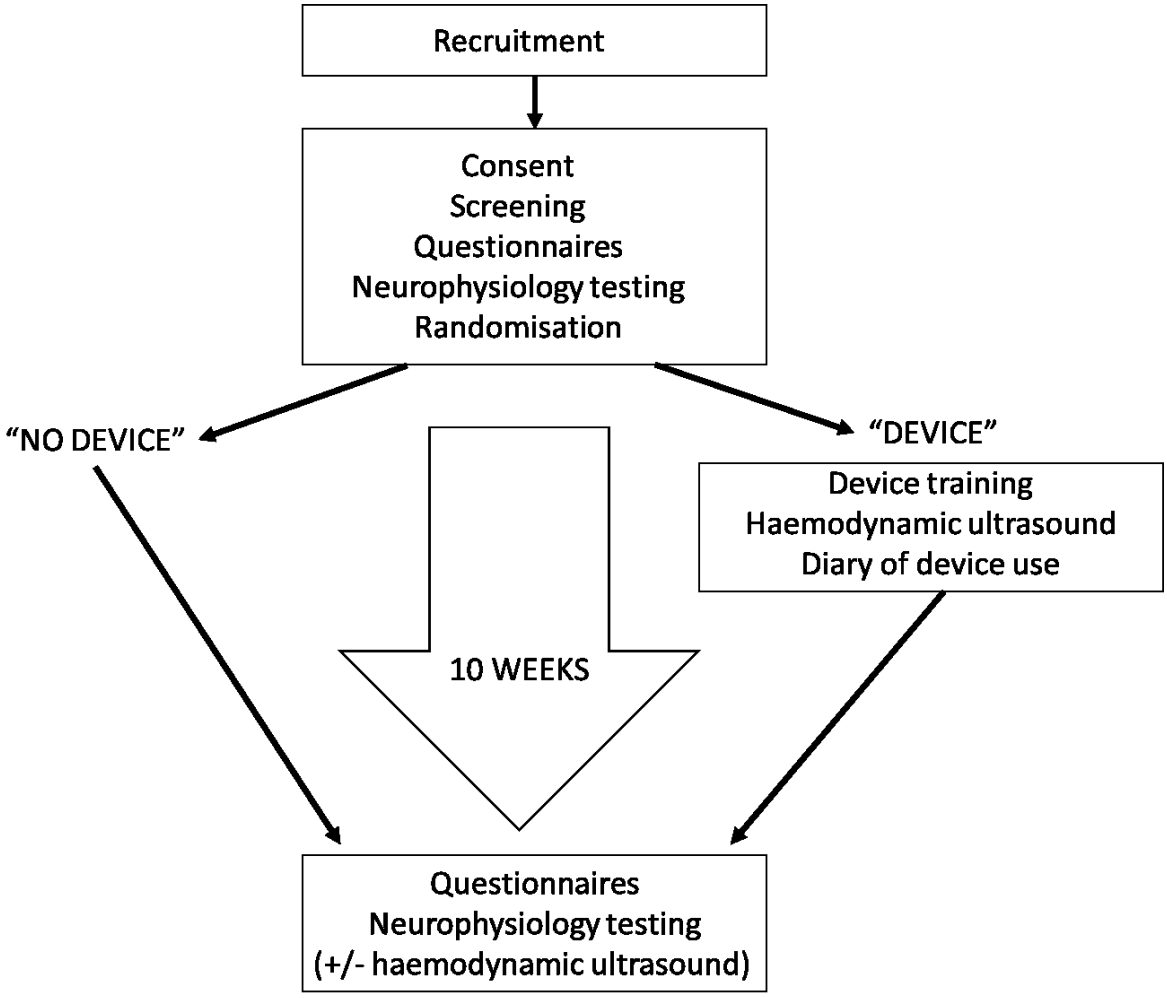


Figure 78 - Protocol for NERVES trial

Data was analysed using both Prism (Graphpad , version 6) and SPSS Statistics (IBM, version 21), significance taken at 0.05.

7.4.2 Results

14 diabetic subjects with clinically confirmed peripheral neuropathy were recruited and consented. Recruitment was halted at 14 due to time constraints on the trial (PI finished research term). Subjects were split according to protocol, and population characteristics are presented in Table 43. All screening tests for other causes of peripheral neuropathy were negative. Data was analysed on an "intention-to-treat" basis.

Table 43 - NERVES population characteristics

	Device (n=9)	Control (n=5)
Age (years)	65.8	70.6
Female sex	11%	80%
Smoker	22%	20%
Insulin dependence	56%	80%
Ischaemic heart disease	67%	20%
BMI (kg/m2)	27.1	31.7
ABPI	0.98	1.1

5 subjects (56%) allocated to device were unable to elicit a twitch to stimulation (subjects 4, 7, 10, 12 and 14). No defining characteristics of age, sex, smoking history, insulin dependence or presence of peripheral arterial disease were identified as significant between those that responded and non-responders.

Nerve conduction studies

The availability of repeat nerve conduction studies at the end of the protocol depended on subject attendance at scheduled appointment. Non-attenders were offered one more appointment for neurophysiological assessment, but data is missing for 6 of the 14 subjects. Results of nerve conduction studies are shown in the appendix (Table 63 - Table 65) and in

Table 44. The conduction velocities (primary outcome measure) for weeks 0 and week 10 for each nerve is shown graphically in Figure 79. No significant differences were shown between time points for either the “device” or “no device” groups (Mann Whitney U).

Table 44 - NERVES nerve conduction studies paired samples, comparing "device" to "no device"

		NO DEVICE			DEVICE		
Outcome parameter		**N**	**Mean**	**SD**	**N**	**Mean**	**SD**
Sural SNAP amplitude (µV)	Week 0	4	0.78	1.6	3	1.33	2.3
	Week 10	4	2.38	1.6	3	2.93	1.8
Sural CV (m/sec)	Week 0	3	16.10	27.9	3	15.73	27.3
	Week 10	3	45.63	7.6	3	43.43	1.0
Superficial peroneal SNAP amplitude (µV)	Week 0	4	0.00	0.0	4	0.00	0.0
	Week 10	4	0.58	1.2	4	0.80	1.6
Superficial peroneal CV (m/sec)	Week 0	3	0.00	0.0	4	0.00	0.0
	Week 10	3	17.73	30.7	4	12.58	25.2
Peroneal CMAP amplitude (mV)	Week 0	4	1.13	1.4	3	0.83	1.4
	Week 10	4	1.10	0.5	3	0.77	1.3
Peroneal CV (m/Sec)	Week 0	3	33.93	29.4	3	41.63	0.5
	Week 10	3	44.10	3.2	3	44.13	2.8
Peroneal F Wave-Min (msec)	Week 0	3	0.00	0.0	4	0.00	0.0
	Week 10	3	19.27	33.4	4	12.80	25.6
Tibial CMAP amplitude (mV)	Week 0	4	0.98	1.3	4	1.35	1.6
	Week 10	4	0.60	1.0	4	2.00	2.5
Tibial CV (m/sec)	Week 0	4	10.38	20.8	4	20.30	23.4
	Week 10	4	23.40	27.1	4	31.98	21.4
Tibial F Min (msec)	Week 0	4	0.00	0.0	4	0.00	0.0
	Week 10	4	0.00	0.0	4	28.08	32.5
Soleus H-Reflex latency (msec)	Week 0	4	8.85	17.7	4	17.50	20.4
	Week 10	4	9.40	18.8	4	26.10	17.6

N= number of subjects; SD= standard deviation; CV= conduction velocity; SNAP= sensory nerve action potential; CMAP= compound muscle action potential; F-Wave Min= F Wave Minimum latency

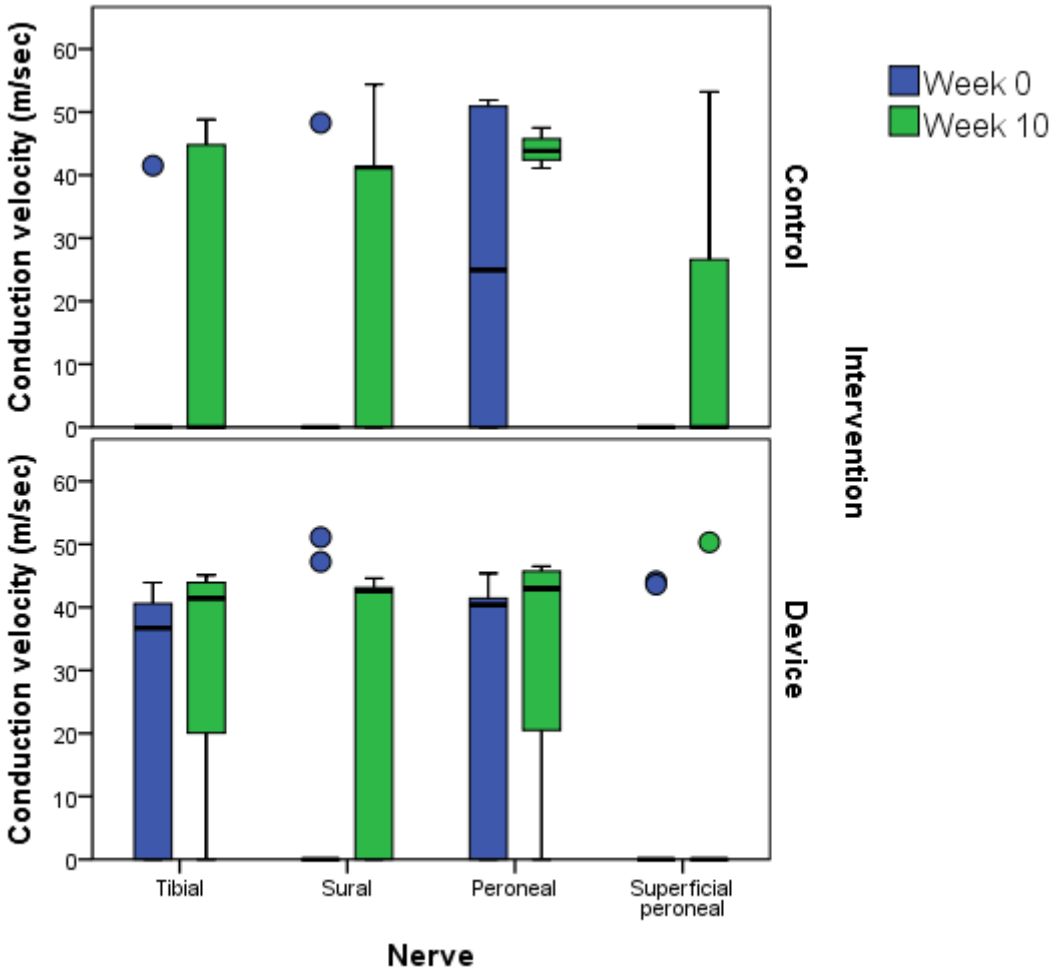


Figure 79 - Nerve conduction velocities for specific nerves during the NERVES protocol

Quality of life

Diabetes-specific quality of life assessment questionnaire scores are shown in Table 45. PAID questionnaire scores did not change in the control group, but decreased from mean 40.3 to 5 ($p<0.05$, Mann Whitney U). This indicates a statistically significant decrease in diabetic symptom severity. Changes in NTSS-6 and MNSI were not significant over time.

Generic quality of life questionnaires (EQ-5D, VAS and SF-12) did not change over time in either group.

Table 45 – Subject questionnaire scores at weeks 0 and 10 during NERVES trial

		Control			Device		
Questionnaire	Week	N	Mean	SD	N	Mean	SD
PAID	0	5	26.20	21.23	7	40.29	17.94
	10	5	27.40	21.93	2	5.00	4.24
NTSS-6	0	5	8.86	2.34	8	8.38	5.18
	10	5	8.86	2.43	2	2.83	2.59
MNSI	0	5	9.80	2.68	8	10.63	3.37
	10	5	10.10	2.97	2	10.25	6.72
EQ-5D	0	5	0.50	0.24	8	0.44	0.28
	10	5	0.58	0.21	2	0.38	0.65
VAS	0	5	56.00	18.17	8	39.38	17.61
	10	5	63.00	39.94	2	70.00	42.43
SF-12	0	5	72.02	14.70	9	58.08	27.07
	10	5	71.46	13.62	3	55.17	55.05

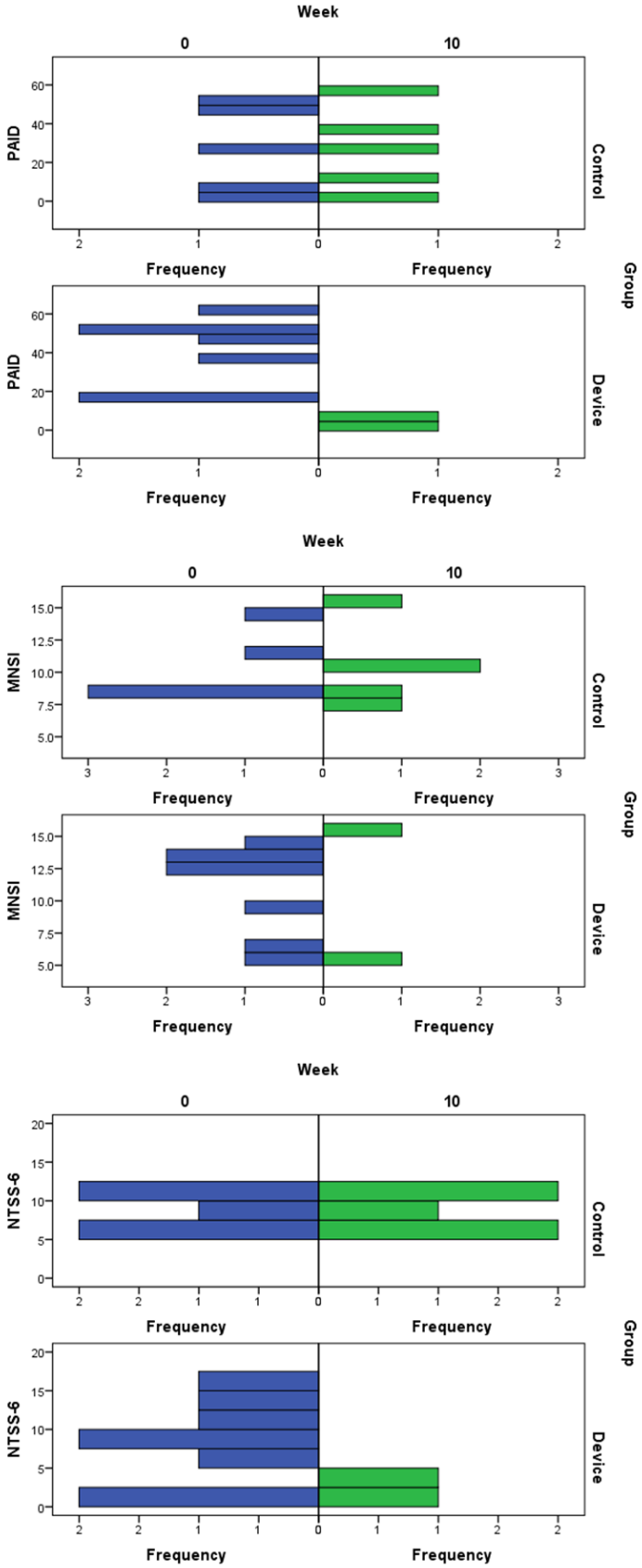


Figure 80 - Diabetes specific quality of life (morbidity) questionnaire results, with changes over 10 weeks

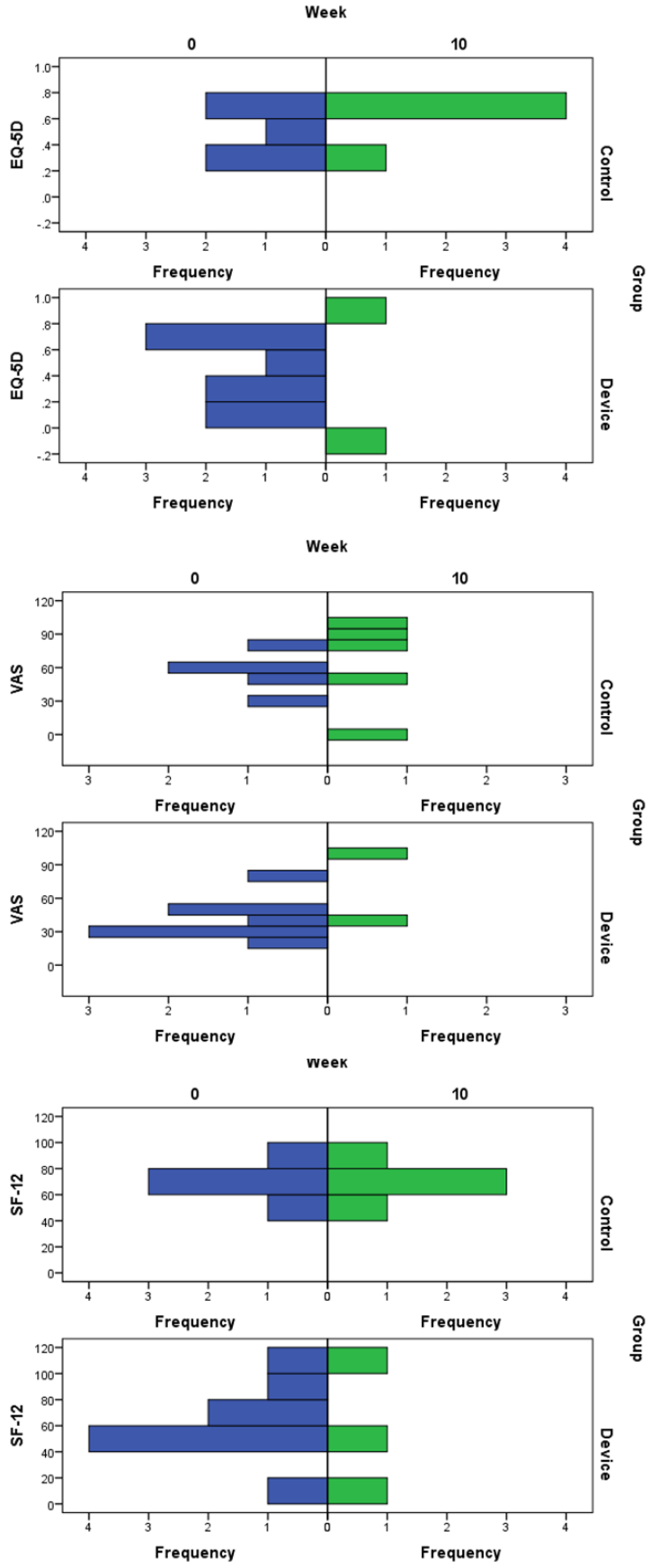


Figure 81 - Generic quality of life questionnaire results, with changes over 10 weeks

Haemodynamic measurements

Use of the geko in subjects with clinical diabetic neuropathy resulted in increases from baseline in arterial PV, TAMV and volume flow of 14%, 222% and 210% (n=5, P<0.05 for PV, Mann Whitney U; others not significant). Increases in venous parameters were 31%, 15%, and 33% (n=5, not significant). Results are tabulated in Table 46.

After activation of the device the surface temperature of the hand and foot did not change significantly (n=6). Laser Doppler fluximetry values in the foot and hand rose by 16% and 19% (not significant).

Table 46 - Absolute and percentage changes in haemodynamic variables from baseline with use of NMES

Parameter change from baseline	Mean	SD	% change from baseline
Arterial PV (cm/s)	11.53	9.2	14.15
Arterial TAMV (cm/s)	2.82	1.9	222.17
Arterial VF (ml/min)	92.14	74.0	209.60
Venous PV (cm/s)	4.09	9.8	31.19
Venous TAMV (cm/s)	0.63	2.8	15.41
Venous VF (ml/min)	36.89	67.4	32.82

N=5; PV – peak velocity; TAMV – time averaged maximum velocity; VF – volume flow; SD – standard deviation

7.4.3 Discussion

This pilot trial sought to evaluate the immediate and short-term effects of a neuromuscular device on diabetic neuropathy, neuropathic symptoms, and quality of life. It is significant when considering this data to bear in mind that recruitment was incomplete, and therefore there were different numbers of subjects allocated to control and device groups (5 versus 9). The incidence of female gender (80% versus 11%), insulin dependence (80% versus 56%), and ischaemic heart disease (20% versus 67%) could be said to be imbalanced between groups, and may have skewed data. Age, ABPI and BMI did not significantly vary between groups.

For both large mixed nerves and sensory nerves tested, there was no statistically significant differences in conduction velocities across the protocol in either the control or intervention group. The trial suffered from small numbers of both recruited subjects, and follow-up data. It is impossible to say whether increasing the statistical power would have allowed separation of the groups, but the data gathered here would go someway to allowing a power calculation. Whether this is worth pursuing is up for debate, as naked eye interrogation of the data seems to suggest that both the controls and the device groups parameters improved over time. This suggests that factors common to both groups may have a greater effect on primary outcomes. Behavioural studies have shown that recruitment into trials can lead to modification of behaviours; the “Hawthorne” effect (French 1953). A subject’s awareness of being observed may have changed factors not measured in the trial (e.g. medication adherence, or exercise regime etc) which may have had physiological and clinical effects confounding the measured outcomes.

Overall, no change in generic quality of life was seen in either the intervention or control group. There was a significant decrease in patient-reported disease severity, as reported by

the PAID questionnaire, but not the MNSI or NTSS-6. This is particularly interesting, and the PAID questionnaire specifically asks about how the subject feels about the impact of diabetic symptoms on their life, with heavy emphasis on psychological impact. The MNSI and NTSS-6 ask purely about presence or absence of neurological symptoms. This is suggestive that the psychological impact of using the device is greater than its biophysical effect.

Haemodynamic measurements of velocity and volume flow, taken at the level of the femoral vessels, were increased with bilateral device action. When compared to those figures achieved in healthy subjects, percentage increase from baseline in arterial parameters were comparable, whilst venous parameters were much reduced (e.g. venous TAMV increase of 15%/0.6cm/s versus 185%/2.4cm/s in healthy individuals). This may represent a vastly different baseline (e.g. resting venous tone), or a physiologically different response to stimulation (e.g. differences in arteriovenous sphincter reactivity, or restricted venous inflow through calcified microcirculatory channels). Laser doppler data from the foot support the lack of microcirculatory changes with the device, with fluximetry values in the foot resistant (16% increase, not statistically significant). Without further investigation it would be difficult to hypothesise further. Near-infrared spectroscopy of the lower limb skin and muscle, or toe nailfold capillaroscopy, may be useful in further studies.

The data presented here are far from conclusive, but do offer some interesting material with which to create hypotheses. It is not clear if ten weeks use of the device affects nerve conduction velocities, but seems unlikely, and further similar studies do not seem likely to produce positive results. There is evidence to suggest that in this group of people the psychological effects of using such a device are positive, and lead to a perceived improvement in disease severity. This has not translated into an overall increase in patient-reported generic

quality of life. The device is able to produce an increase in local blood flow in the ipsilateral femoral vessels, but its microcirculatory effects are attenuated compared to healthy subjects.

8 Conclusion and future work

Symptomatic vascular disease is common, with a spectrum of symptomatology ranging from cosmetic imperfection, to life-limiting morbidity, limb loss, and death. The cost to society of loss of productivity and consumption of health and social care resources are also significant.

Electrical stimulation of muscles and nerves is a promising therapy for disorders of the circulation, which has been incompletely explored in the context of evolving medical technologies.

8.1 Devices and anatomical application

There are many different electrical stimulation devices on the open market, with indications for use in both healthy people and patients with more specific vascular problems. Consensus in the literature on method of stimulation, anatomical stimulation site, and dosage regime has not been reached. The lack of controlled trials has been particularly noted.

8.1.1 Muscle target

It has been shown in the course of this project that intermittent electrical stimulation of the leg muscles over 5 minutes causes increased velocities in the femoral vessels, and increases fluximetry signal in the skin of both feet. Contraction of gastrocnemius causes high speed ejection of blood into the popliteal vein, making it an extremely powerful and efficient pumping unit. Vastus medialis and peroneus longus also appear to have a large enhancing effect on PV, with their respective actions on the thigh and foot pumps. For the purposes of

enhancing venous circulation, causing contraction of any of the muscles in this protocol may be useful in preventing venous stasis.

There is huge inter-subject variation in response to individual muscle stimulation, and it was not possible to conclusively identify the most effective muscle target. Differences between subjects are thought to be due to muscle mass and possibly metabolism, but would also rely on nerve function, and individual skin and subcutaneous tissue impedance.

Pain with stimulation was experienced mainly at electrode sites, with smaller electrical contact areas being more uncomfortable. This was the main complaint during the Muscle Pump trial, as opposed to fatigue from repetitive muscle contraction. The current magnitude also appeared to be roughly proportional to discomfort. Intensity of contraction can also be manipulated by varying other parameters such as frequency, pulse width, pulse interval, and voltage. Indirect muscle stimulation via a motor nerve allows usage of lower settings when compared to direct muscle stimulation.

Electrical stimulation by either the common peroneal nerve, or footplate device, produced comparative differences in blood flow measurements, causing a 50-100% increase in arterial TAMV and volume flow. The nerve stimulator appears to be a more efficient enhancer of venous clearance from the foot, however large differences in resting values may produce large artefact.

8.2 Adjuvant compression garments

In a healthy population, the addition of grade 2 graduated compression stockings to common peroneal nerve stimulation increased the percentage increase in arterial and venous TAMV

and flow rate in the femoral vessels. In fact it doubled volume flow changes with device activation compared to no compression. The addition of compression hosiery reduced recorded peak velocity values compared to baseline. The higher resting venous pressure in the compressed dependant limb is thought to decrease the venous reservoir capacity. It may also affect the microcirculation of the limb, but this has not been investigated.

There is the possibility that the use of NMES in conjunction with four-layer bandaging may enhance the blood supply to the skin of the foot.

8.3 Dosing regimen

6-10 weeks of repeated NMES stimulation appears to have beneficial effects on venous pain scores, claudication distances, and ulcer healing rates. It does not appear to alter symptomatic neuropathy. There is a lack of supportive evidence for a specific regime of stimulation, and it is likely to be both subject and disease dependant. Consensus is lacking.

8.4 Populations of interest

8.4.1 Prevention of venous thromboembolic events

It has been demonstrated that a significant increase in healthy subject venous peak velocity, TAMV and flow rate can be achieved with the use of NMES compared to baseline. It has been shown previously that an increase in venous flow volume and velocity causes venous emptying of the leg and facilitates clearance within the calf vessels and valve cusps which are the most common sites of DVT formation.

Literature meta-analysis has shown heparin to be effective in the prevention of DVT , however where heparin is contraindicated, NMES has a significant effect on risk reduction. In cases of very high risk, the use of NMES as an adjunct to heparin decreases incidence of DVT. The favourable side effect profile of NMES when compared to heparin has also been noted.

8.4.2 The treatment of venous disease

Circulatory parameters of speed and flow rate are increased by the use of NMES in subjects with clinically significant chronic venous insufficiency and/or obstruction. The benefits of repeated use include relief of leg oedema, and possible improvements in quality of life (AVVQ) of those with clinically severe venous symptoms. Evidence of improved perfusion of the skin of the foot may indicate its utility in the management of venous ulcers, especially where compression is not tolerated or contra-indicated. The mechanism for an enhanced effect with repeated use has not been elucidated.

8.4.3 Peripheral arterial disease

There is evidence both in the literature and in our experimental work presented here to support the use of supervised exercise and NMES in the management of intermittent claudication, with clinically important changes in treadmill distances seen with regular use. Trial subjects experienced improvements in both disease specific and generic quality of life scores, and it may be pragmatic to power future studies for this outcome. NMES use was associated with an increase in leg and foot in claudicants, as measured by ultrasound.

8.4.4 Diabetes (ulcers and neuropathy)

Literature search has shown many interventions that have been investigated for benefit in the management of diabetic neuropathy. These have mainly been exercise regimes, and some

belive benefit can be seen in as little as four weeks. Protocols of balance and stretching exercise are reported to improve function. The mechanism for this appears to be multifactorial, and it is unclear whether this is due to an improvement in function of damaged "hardware", or an increased ability of the residual muscle and nerves ability to compensate.

The ULCERS trial looked at a very small sample of subjects with diabetic foot ulcers, which would be very vulnerable to confounding factors such as activity, diet and environment. When looking at ulcer percentage volume changes, the group which used NMES healed faster overall than the group that did not use NMES. This was despite having larger ulcers at baseline. Generic quality of life scores increased much more in the device group than the control group, as measured by the VAS and SF-12. The EQ-5D seems to have changed by proportional amounts in both groups, which may point to the fact that functional tasks were not improved significantly by addition of the device. The effects of diabetes on activities of daily living are multi-modal. The changes in these scores may be used in the future to power future trial work, and aid future health care cost effectiveness analysis. Measurement of change in haemodynamic parameters in the femorals with use of the NMES device was attenuated compared to other patient groups.

The NERVES trial attempted to show that NMES use over 10 weeks could modify or reverse nerve conduction velocities in the leg, but could show no differences in either large mixed or sensory nerves. Improvement in both groups functional (particularly psychological) scores suggest a confounding Hawthorne effect. Overall, no change in generic quality of life was seen in either group. Haemodynamic measurements of velocity and volume flow, taken at the level of the femoral vessels, were increased with bilateral device action. When compared to those figures achieved in healthy subjects, percentage increase from baseline in arterial parameters

were comparable, whilst venous parameters were much reduced. This may represent a vastly different baseline (e.g. resting venous tone), or a physiologically different response to stimulation (e.g. differences in arteriovenous sphincter reactivity, or restricted venous inflow through calcified microcirculatory channels). Laser doppler data from the foot support the lack of microcirculatory changes with the device, with fluximetry values in the foot resistant to stimulation.

8.4.5 Oedema, and comparison of geko, Revitive, and graduated compression stockings

It has been shown that leg volumes change significantly over the course of a normal sedentaert working day. Compression stockings were more effective than NMES devices at reducing leg volume increase. The combination of increasing leg pump activity, and the application of external pressure may be beneficial in clearing pedal oedema. This also supports the use of combined compression and NMES in venous disease, where swelling is a symptomatic component. When comparing geko™ to Revitive™ to graduated compression stockings, differences in tolerability of the devices did not lead to poor compliance, and all subjects completed the whole protocol. A preference for compression stockings in healthy subjects over NMES is noted, although there was large inter-subject variability.

The application of any of these medical devices has been shown to have some or limited benefit in healthy subjects (e.g. footplate stimulation in very oedematous limbs, geko to maintain ambulation and reduce inactivity). Historically GCS reported compliance in chronic disease has been poor, often being described as uncomfortable and restrictive.

8.4.6 Other possible applications

Given more time and resources, it would have been very exciting to conduct a study looking at prevention of VTE in populations at high risk, particularly those with high bleeding risks, where heparin is contraindicated. Populations where increased blood flow and speed would be beneficial are those patients having surgical bypass operations, or perhaps those undergoing thrombolysis for clot.

The large increases in arterial inflow with the use of geko and compression may find utility in athletic recovery after training. The extra units of oxygen and carbohydrate per unit time delivered to the muscles and tissues of the leg may reduce the time needed to recover after exertion. This hypothesis could be tested by a protocol using endpoints such as blood sampling for anaerobic metabolic products (e.g. lactic acid), objective performance measures (e.g. time to complete set of aerobic manoeuvres), and subjective measures of muscle pain.

8.5 Method of effect

Previous studies have shown that the muscle-pumps of the leg play a crucial role in optimising circulation, and dysfunction may be associated with the onset of clinical symptoms of vascular disease. Repeated or regular NMES of the leg muscle-pumps might have a training and bulking effect on the stimulated muscles, in a similar action to physiotherapy or exercise training.

8.5.1 Systemic effect

Physiological parameters such as heart rate and diastolic blood pressure were not affected by the use of NMES, and other studies have shown NMES to be safe in even very sick populations such as cardiac intensive care. The Autonomic Activation trial showed some effect on diastolic blood pressure, but the clinical significance of this is not known. Repetitious stimulation with electricity via NMES produces a repeated sympathetic response that then starts to reduce in amplitude. This is thought to be due to system saturation and depletion of neurotransmitters or stress hormones.

In both healthy and diseased subjects, laser doppler changes in the arm either lag behind that of the leg, or rise as a result of device cessation. This is suggestive of the release of endogenous cytokines (such as nitric oxide, vascular endothelial growth factor, or some other metabolite) that affect downstream vascular tone or sphincter behaviour, over a local pressure effect on recruitment of arterioles and capillaries. It is striking that even brief stimulation periods of under 5 minutes have caused large increases in fluximetry, a surrogate measurement of red blood cell velocity in the skin. This may be due to several factors, both endocrine, neuronal and pressure related. The fact that subjects with clinically severe neuropathy failed to stimulate anywhere near as effectively as those without goes against a nervous-mediated response. Increased vessel shearing forces with increased velocities may have a fibrinolytic effect with the use of NMES. The beneficial effects seen in claudicants suggests that in addition to the increased hydrostatic pressures, vessel collateralisation may be affected, but I can provide no evidence for this with the work done so far.

8.5.2 NMES compared to intermittent pneumatic compression

NMES via the common peroneal nerve not only increases circulatory speeds and flow rates, it also compares favourably to approved devices (such as IPC) used in the treatment of vascular disorders. Tolerability scores for each device were also similar. The use of devices, such as NMES and IPC, have been shown to enhance microcirculation of the foot either during or immediately after usage. One of the benefits of electrical stimulation over IPC is that the action increases the activity of the users own muscles, as opposed to a passive compression system. Reports in the literature of improvement in muscle strength with electrical stimulation has been demonstrated. The cardiovascular benefits from NMES are unknown.

8.6 Limitations of trials

8.6.1 Measurements

Ultrasound measurements of venous flow are prone to variation, both due to the second-to-second changes in venous vascular tone, external compression, and cardiac output and respiratory intra-abdominal and thoracic pressures. Due to the high variability of venous measurements, and differing baseline values between subjects, this may spread values over a larger distribution, making statistical differences more difficult to elucidate. The use of air plethysmography to measure venous ejection fraction would be suited to measure the efficacy of the NMES device with relation to resting blood volume of the leg.

Measurement of leg ulcers sizes were limited by debridement, and when subjects were having multidisciplinary care, differences in wound care were noted. This could be heavily protocoled in future trials, perhaps with named wound care professionals per subject.

Technical problems were experienced with the autonomic recording equipment, which unfortunately nullified the protocols of three of our subjects. Metered auditory stimulation would probably perform better, and would more clearly demonstrate the effect of the NMES device. It would be helpful to physically correlate this with microneurography.

8.6.2 Populations

The small studies reported here were not stratified for age, BMI, sex, smoking, or disease severity. This would have been advantageous in order to draw more firm conclusions from the data. Baseline characteristics between device and controls in both the PAD and ULCERS trials were particularly different.

The relatively small sample sizes limit the statistical representation of findings within studies, which is therefore prone to type one error.

In all trials, efforts were taken not to restrict normal subject activity, in an effort to produce results that were most generally applicable to the general population. This does however introduce more variability between subjects, and makes comparison difficult.

Fluid intake, activity levels, hormonal profiles, diet and medication were not controlled, and this could have skewed results.

The lack of a true control to electrical stimulation does make trial design difficult, as it is fairly obvious to a sensible person whether they are receiving electrical shocks or not. In this thesis I have therefore tried to conduct trials using stimulation as an adjunct to normal clinical care.

Derived benefit is subject to a large placebo effect. The sensation of electrical stimulation may have beneficial effects on experience of pain, whether by a gating mechanism (now out of favour with neurophysiologists) or by a more psychological moderating influence.

The physical incapacity incumbent with diabetic foot ulcers meant that most trial weeks needed several home visits, to be present at the time of district nursing dressing changes for wound photography to be performed. Funding for a weekly clinic and patient transport would have been helpful, and needs to be factored in should this trial be considered for repetition. The results of this trial therefore are very much just a snapshot of a small group of patients, and not necessarily representative of the diabetic foot ulcer population.

8.6.3 Device failure

The non-invasive nature of transcutaneous stimulation means that some subjects remain recalcitrant to its effects. In the authors' experience, inability to elicit meaningful contraction renders treatment impossible. If oedema is a contributing factor, prior limb elevation or the use of graduated compression may help. Severe peripheral sensory loss and anoxia of tissues also seems to limit stimulation ability. The precise threshold nerve function necessary for successful device use is not yet known.

8.6.4 Skin reaction

Skin irritation at electrode adhesive sites occurred in five subjects who used the geko^{TM} device during the VeINS trial, which was treated with simple emollients. We have raised this issue with the manufacturer of the device, and other device adhesives are currently under investigation.

8.6.5 Adherence

We did not covertly monitor adherence to trial protocols which required home use by subjects over time, so are evaluating the effects of the devices on an intention-to-treat basis. The correlation between reported and actual compliance is not known, therefore the effect of the device on all parameters, including side-effects, may have been underestimated as a result.

8.6.6 Long term effects

The medium and long-term effects of muscle stimulation were not observed in this study. It would be clinically useful to understand the effects of electrical stimulation over hours, days and weeks, especially if stimulation induces a systemic response.

The long term tolerability of NMES device such as the geko™ and Revitive™ has yet to be evaluated. From the small pilot trials reported here, the maximum tolerated level is efficacious for producing maximum positive results whilst limiting discomfort, whilst repetitive stimulation may lead to improved tolerance of higher stimulation settings and experience of lower levels of discomfort.

8.6.7 The “ideal” device

When thinking about suitable devices it is important to consider the population targeted. It has been shown that different groups of people find different devices more tolerable, and factors such as tissue impedance and neuropathy will affect how the stimulation should best be applied anatomically. In elderly populations, impaired mobility, vision and dexterity will decrease ability to use devices unless they are simple to apply to the limb, easy to activate, deactivate, and adjust.

A device with modifiable stimulation settings and anatomical interfaces would be most cost-effective to build and market, and would allow subjects to adjust for comfort. The more tenable stimulation is, the greater the chances of good subject compliance with protocols.

The main source of discomfort reported with NMES usage originates at the electrode-skin interface, with larger surface contact of electrodes being more comfortable.

The combination of stimulation with graduated compression stockings may enhance the beneficial effects.

A mains operated footplate device seems ideal for those who have the time to sit and undergo a routine NMES protocol. A rechargeable portable device may be more suited for more ambulant patients, but would require thought as to anatomical application. This also requires an adhesive surface with low biological activity but high conduction values, preferably that can be changed and replaced at low unit cost.

9 References

Abraham, P., G. Leftheriotis, B. Desvaux, M. Saumet and J. L. Saumet (1994). "Diameter and blood velocity changes in the saphenous vein during thermal stress." *Eur J Appl Physiol Occup Physiol* **69**(4): 305-308.

Abraham, P., V. Mateus, F. Bieuzen, N. Ouedraogo, F. Cisse and G. Leftheriotis (2013). "Calf muscle stimulation with the Veinoplus device results in a significant increase in lower limb inflow without generating limb ischemia or pain in patients with peripheral artery disease." *Journal of Vascular Surgery* **57**(3): 714-719.

Abraira, C., J. A. Colwell, F. Q. Nuttall, C. T. Sawin, N. J. Nagel, J. P. Comstock, N. V. Emanuele, S. R. Levin, W. Henderson and H. S. Lee (1995). "Veterans Affairs Cooperative Study on glycemic control and complications in type II diabetes (VA CSDM). Results of the feasibility trial. Veterans Affairs Cooperative Study in Type II Diabetes." *Diabetes Care* **18**(8): 1113-1123.

Actegy. Retrieved 30th September 2015, from http://www.revitive.com/revitive-ix/.

Ahn, H., J. Lindhagen, G. E. Nilsson, E. G. Salerud, M. Jodal and O. Lundgren (1984). "Evaluation of Laser Doppler Flowmetry in the Assessment of Blood-Flow in the Feline Small-Intestine." *International Journal of Microcirculation-Clinical and Experimental* **3**(3-4): 478-478.

Ahroni, J. H. and E. J. Boyko (2000). "Responsiveness of the SF-36 among veterans with diabetes mellitus." *J Diabetes Complications* **14**(1): 31-39.

Alimi, Y. S., P. Barthelemy and C. Juhan (1994). "Venous pump of the calf: a study of venous and muscular pressures." *Journal of Vascular Surgery* **20**(5): 728-735.

Allen, J. D., T. Stabler, A. A. Kenjale, K. L. Ham, J. L. Robbins, B. D. Duscha, W. E. Kraus and B. H. Annex (2014). "Diabetes status differentiates endothelial function and plasma nitrite response to exercise stress in peripheral arterial disease following supervised training." *J Diabetes Complications* **28**(2): 219-225.

Allen, M., D. Stashuk, T. Doherty, K. Kimpinski and C. Rice (2014). "Neuromuscular remodeling and neuromuscular junction instability in human diabetic neuropathy (1168.1)." *The FASEB Journal* **28**(1 Supplement).

Allet, L., S. Armand, K. Aminian, Z. Pataky, A. Golay, R. A. de Bie and E. D. de Bruin (2010). "An exercise intervention to improve diabetic patients' gait in a real-life environment." *Gait & Posture* **32**(2): 185-190.

Allwood, M. J. (1957). "The effect of an increased local pressure gradient on blood flow in the foot." *Clin Sci (Lond)* **16**(2): 231-239.

Altman, D. G. and D. Moher (2013). "Declaration of transparency for each research article." *BMJ* **347**.

Alzamora, M. T., R. Forés, J. M. Baena-Díez, G. Pera, P. Toran, M. Sorribes, M. Vicheto, M. D. Reina, A. Sancho, C. Albaladejo and J. Llussà (2010). "The peripheral arterial disease study (PERART/ARTPER): prevalence and risk factors in the general population." *BMC Public Health* **10**: 38-38.

Amery, A., H. Bossaert and M. Verstraete (1969). "Muscle blood flow in normal and hypertensive subjects. Influence of age, exercise, and body position." *Am Heart J* **78**(2): 211-216.

Amos, A. F., D. J. McCarty and P. Zimmet (1997). "The rising global burden of diabetes and its complications: estimates and projections to the year 2010." *Diabet Med* **14 Suppl 5**: S1-85.

Anderson, C., S. Laubscher and R. Burns (1996). "Validation of the Short Form 36 (SF-36) health survey questionnaire among stroke patients." *Stroke* **27**(10): 1812-1816.

Anderson, P. A., T. J. Puschak and R. C. Sasso (2009). "Comparison of short-term SF-36 results between total joint arthroplasty and cervical spine decompression and fusion or arthroplasty." *Spine (Phila Pa 1976)* **34**(2): 176-183.

Anderson, S. I., P. Whatling, O. Hudlicka, P. Gosling, M. Simms and M. D. Brown (2004). "Chronic transcutaneous electrical stimulation of calf muscles improves functional capacity without inducing systemic inflammation in claudicants." *European Journal of Vascular and Endovascular Surgery* **27**(2): 201-209.

Andrianova GG, Kots YM, Marmyanov VA and X. VA. (1971). "Primenenie elektrostimuliatsii dlia trenirovki mishechnoj sili." *Novosti Meditsinskogo Priborostroeniia* **3**: 40-47.

Anthonysamy, D., Z. A. Azizi and H. M. Tajri (2012). "The effect of sequential intermittent pneumatic compression of foot and calf on popliteal artery mean systolic blood flow in patients with intermittent claudication." *Asian Journal of Surgery* **35**(4): 131-135.

Antonescu, I., F. Carli, N. E. Mayo and L. S. Feldman (2014). "Validation of the SF-36 as a measure of postoperative recovery after colorectal surgery." *Surgical endoscopy* **28**(11): 3168-3178.

Aragao, J. A., F. P. Reis, G. B. Pitta, F. Miranda, Jr. and L. F. Poli de Figueiredo (2006). "Anatomical study of the gastrocnemius venous network and proposal for a classification of the veins." *European Journal of Vascular and Endovascular Surgery* **31**(4): 439-442.

Araki, C. T., T. L. Back, F. T. Padberg, P. N. Thompson, Z. Jamil, B. C. Lee, W. N. Duran and R. W. Hobson (1994). "The significance of calf muscle pump function in venous ulceration." *Journal of Vascular Surgery* **20**(6): 872-877; discussion 878-879.

Armstrong, D. G., A. J. Boulton, G. Andros, C. Attinger, D. Eisenbud, L. A. Lavery, B. A. Lipsky, J. L. Mills, G. Sibbald, A. P. Smith, D. Wukich and D. J. Margolis (2009). "Defining success in clinical trials of diabetic foot wounds: the Los Angeles DFCon consensus." *Int Wound J* **6**(3): 211-213.

Arpinelli, F., G. Visona, R. Bruno, G. De Carli and G. Apolone (2000). "Health-related quality of life in asymptomatic patients with HIV. Evaluation of the SF-36 health survey in Italian patients." *Pharmacoeconomics* **18**(1): 63-72.

Arridge, S. R. (1999). "Optical tomography in medical imaging." *Inverse Problems* **15**(2): R41-R93.

Association, W. M. (2013). WMA Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects. http://www.wma.net/en/30publications/10policies/b3/.

Awdeh, H., K. Kassak, P. Sfeir, H. Hatoum, H. Bitar and A. Husari (2015). "The SF-36 and 6-Minute Walk Test are Significant Predictors of Complications After Major Surgery." *World Journal of Surgery*.

Azad, N., N. V. Emanuele, C. Abraira, W. G. Henderson, J. Colwell, S. R. Levin, F. Q. Nuttall, J. P. Comstock, C. T. Sawin, C. Silbert and F. A. Rubino (1999). "The effects of intensive glycemic control on neuropathy in the VA cooperative study on type II diabetes mellitus (VA CSDM)." *J Diabetes Complications* **13**(5-6): 307-313.

Babber, A., K. Williams and A. Davies (2014). Availability of Supervised Exercise Programmes for Patients with Peripheral Arterial Disease (National Audit), Imperial College London.

Back, T. L., F. T. Padberg, C. T. Araki, P. N. Thompson and R. W. Hobson (1995). "Limited range of motion is a significant factor in venous ulceration." *Journal of Vascular Surgery* **22**(5): 519-523.

Baker, L. L., B. R. Bowman and D. R. McNeal (1988). "Effects of waveform on comfort during neuromuscular electrical stimulation." *Clinical orthopaedics and related research*(233): 75-85.

Baker, S. R., M. C. Stacey, A. G. Jopp-McKay, S. E. Hoskin and P. J. Thompson (1991). "Epidemiology of chronic venous ulcers." *The British Journal of Surgery* **78**(7): 864-867.

Balducci, S., G. Iacobellis, L. Parisi, N. Di Biase, E. Calandriello, F. Leonetti and F. Fallucca (2006). "Exercise training can modify the natural history of diabetic peripheral neuropathy." *Journal of Diabetes and its Complications* **20**(4): 216-223.

Balducci, S., F. Leonetti, U. Di Mario and F. Fallucca (2004). "Is a long-term aerobic plus resistance training program feasible for and effective on metabolic profiles in type 2 diabetic patients?" *Diabetes Care* **27**(3): 841-842.

Baldwin, E. R., P. M. Klakowicz and D. F. Collins (2006). "Wide-pulse-width, high-frequency neuromuscular stimulation: implications for functional electrical stimulation." *J Appl Physiol (1985)* **101**(1): 228-240.

Baldwin, M. J., H. M. Moore, N. Rudarakanchana, M. Gohel and A. H. Davies (2013). "Post-thrombotic syndrome: a clinical review." *J Thromb Haemost* **11**(5): 795-805.

Balice-Gordon, R. J. (1997). "Age-related changes in neuromuscular innervation." *Muscle Nerve Suppl* **5**: S83-87.

Balice-Gordon, R. J., S. M. Breedlove, S. Bernstein and J. W. Lichtman (1990). "Neuromuscular junctions shrink and expand as muscle fiber size is manipulated: in vivo observations in the androgen-sensitive bulbocavernosus muscle of mice." *J Neurosci* **10**(8): 2660-2671.

Balsano, F., S. Coccheri, A. Libretti, G. G. Nenci, M. Catalano, G. Fortunato, S. Grasselli, F. Violi, H. Hellemans and P. Vanhove (1989). "Ticlopidine in the treatment of intermittent claudication: a 21-month double-blind trial." *J Lab Clin Med* **114**(1): 84-91.

Barendsen, G. J. and J. W. van den Berg (1984). "Venous capacity, venous refill time and the effectiveness of the calf muscle pump in normal subjects." *Angiology* **35**(3): 163-172.

Barros, M. V., N. Labropoulos, A. L. Ribeiro, R. Y. Okawa and F. S. Machado (2006). "Clinical significance of ostial great saphenous vein reflux." *European Journal of Vascular and Endovascular Surgery* **31**(3): 320-324.

Becker, J. and B. Schampi (1973). "The incidence of postoperative venous thrombosis of the legs. A comparative study on the prophylactic effect of dextran 70 and electrical calf-muscle stimulation." *Acta Chir Scand* **139**(4): 357-367.

Beebe-Dimmer, J. L., J. R. Pfeifer, J. S. Engle and D. Schottenfeld (2005). "The epidemiology of chronic venous insufficiency and varicose veins." *Ann Epidemiol* **15**(3): 175-184.

Beirne C, S. S. (2009). "Improving clinical and hemodynamic outcome in critical lower limb ischaemia with a sequential compression biomechanical device." *Vascular* **17**: S2.

Belkin, M., J. Knox, M. C. Donaldson, J. A. Mannick and A. D. Whittemore (1996). "Infrainguinal arterial reconstruction with nonreversed greater saphenous vein." *Journal of Vascular Surgery* **24**(6): 957-962.

Berg, D. K. and Z. W. Hall (1975). "Increased extrajunctional acetylcholine sensitivity produced by chronic acetylcholine sensitivity produced by chronic post-synaptic neuromuscular blockade." *The Journal of Physiology* **244**(3): 659-676.

Berg, W., C. Traneroth, A. Gunnarsson and C. Lossing (1990). "A method for measuring pressure sores." *Lancet* **335**(8703): 1445-1446.

Bergquist, A. J., J. M. Clair and D. F. Collins (2011). "Motor unit recruitment when neuromuscular electrical stimulation is applied over a nerve trunk compared with a muscle belly: triceps surae." *J Appl Physiol (1985)* **110**(3): 627-637.

Bickel, C. S., C. M. Gregory and J. C. Dean (2011). "Motor unit recruitment during neuromuscular electrical stimulation: a critical appraisal." *European Journal of Applied Physiology* **111**(10): 2399-2407.

Bigland-Ritchie, B., D. A. Jones and J. J. Woods (1979). "Excitation frequency and muscle fatigue: Electrical responses during human voluntary and stimulated contractions." *Exp Neurol* **64**(2): 414-427.

Bigland-Ritchie, B., I. Zijdewind and C. K. Thomas (2000). "Muscle fatigue induced by stimulation with and without doublets." *Muscle Nerve* **23**(9): 1348-1355.

Binder-Macleod, S. A. and C. B. Barker (1991). "Use of a catchlike property of human skeletal muscle to reduce fatigue." *Muscle Nerve*(September): 850-857.

Bircher, A., E. M. Deboer, T. Agner, J. E. Wahlberg and J. Serup (1994). "Guidelines for Measurement of Cutaneous Blood-Flow by Laser-Doppler Flowmetry - a Report from the Standardization Group of the European-Society-of-Contact-Dermatitis." *Contact Dermatitis* **30**(2): 65-72.

Blättler, W., N. Kreis, B. Lun, J. Winiger and F. Amsler (2008). "Leg symptoms of healthy people and their treatment with compression hosiery." *Phlebology* **23**(5): 214-221.

Bloor, K. (1961). "Natural History of Arteriosclerosis of the Lower Extremities: Hunterian Lecture delivered at the Royal College of Surgeons of England on 22nd April 1960." *Ann R Coll Surg Engl* **28**(1): 36-52.

Blumberg, H. and B. G. Wallin (1987). "Direct evidence of neurally mediated vasodilatation in hairy skin of the human foot." *The Journal of Physiology* **382**: 105-121.

Bogachev, V. Y., O. V. Golovanova, A. N. Kuznetsov, A. O. Shekoyan and N. V. Bogacheva (2011). "Electromuscular stimulation with VEINOPLUS for the treatment of chronic venous edema." *International Angiology* **30**(6): 567-570.

Bonner, R. and R. Nossal (1981). "Model for Laser Doppler Measurements of Blood-Flow in Tissue." *Applied Optics* **20**(12): 2097-2107.

Bosch, J. L. and M. G. Hunink (1997). "Meta-analysis of the results of percutaneous transluminal angioplasty and stent placement for aortoiliac occlusive disease." *Radiology* **204**(1): 87-96.

Bosi, E., G. Bax, L. Scionti, V. Spallone, S. Tesfaye, P. Valensi and D. Ziegler (2013). "Frequency-modulated electromagnetic neural stimulation (FREMS) as a treatment for symptomatic diabetic neuropathy: results from a double-blind, randomised, multicentre, long-term, placebo-controlled clinical trial." *Diabetologia* **56**(3): 467-475.

Bostrom, S., E. Holmgren, O. Jonsson, S. Lindberg, B. Lindstrom, I. Winso and B. Zachrisson (1986). "Post-operative thromboembolism in neurosurgery. A study on the prophylactic effect of calf muscle stimulation plus dextran compared to low-dose heparin." *Acta Neurochir (Wien)* **80**(3-4): 83-89.

Botter, A., G. Oprandi, F. Lanfranco, S. Allasia, N. A. Maffiuletti and M. A. Minetto (2011). "Atlas of the muscle motor points for the lower limb: implications for electrical stimulation procedures and electrode positioning." *European Journal of Applied Physiology* **111**(10): 2461-2471.

Boulton, A. J., L. Vileikyte, G. Ragnarson-Tennvall and J. Apelqvist (2005). "The global burden of diabetic foot disease." *Lancet* **366**(9498): 1719-1724.

Bradbury, A., C. Evans, P. Allan, A. Lee, C. V. Ruckley and F. G. R. Fowkes (1999). "What are the symptoms of varicose veins? Edinburgh vein study cross sectional population survey." *British Medical Journal* **318**(7180): 353-356.

Bradbury, A., C. J. Evans, P. Allan, A. J. Lee, C. V. Ruckley and F. G. R. Fowkes (2000). "The relationship between lower limb symptoms and superficial and deep venous reflux on duplex ultrasonography: The Edinburgh Vein Study." *Journal of Vascular Surgery* **32**(5): 921-930.

Bradley, E. L., 3rd and J. Sacerio (1972). "The velocity of ultrasound in human blood under varying physiologic parameters." *J Surg Res* **12**(4): 290-297.

Brandjes, D. P., H. R. Buller, H. Heijboer, M. V. Huisman, M. de Rijk, H. Jagt and J. W. ten Cate (1997). "Randomised trial of effect of compression stockings in patients with symptomatic proximal-vein thrombosis." *Lancet* **349**(9054): 759-762.

Brem, H. and M. Tomic-Canic (2007). "Cellular and molecular basis of wound healing in diabetes." *J Clin Invest* **117**(5): 1219-1222.

Brockes, J. P. and Z. W. Hall (1975). "Synthesis of acetylcholine receptor by denervated rat diaphragm muscle." *Proc Natl Acad Sci U S A* **72**(4): 1368-1372.

Broderick, B. J., O. Breathnach, F. Condon, E. Masterson and G. Olaighin (2013). "Haemodynamic performance of neuromuscular electrical stimulation (NMES) during recovery from total hip arthroplasty." *J Orthop Surg Res* **8**: 3.

Broderick, B. J., O. Breathnach, E. Masterson, P. P. Breen and O. L. G (2011). "Hemodynamic performance of NMES in the early post operative period following orthopaedic surgery."

Conference proceedings : .. **Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Conference. 2011**: 7630-7633.

Broderick, B. J., G. J. Corley, F. Quondamatteo, P. A. Grace, P. P. Breen and G. Olaighin (2008). "A haemodynamic study of the physiological mechanisms of the venous pump in the healthy human foot." *Conference proceedings : ..* **Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Conference. 2008**: 1411-1414.

Broderick, B. J., C. Kennedy, P. P. Breen, S. R. Kearns and G. Olaighin (2010). "The influence of orthopaedic implants on patient tolerance of neuromuscular electrical stimulation (NMES)." *Conf Proc IEEE Eng Med Biol Soc* **2010**: 5823-5826.

Broderick, B. J., C. Kennedy, P. P. Breen, S. R. Kearns and G. Olaighin (2011). "Patient tolerance of neuromuscular electrical stimulation (NMES) in the presence of orthopaedic implants." *Medical Engineering and Physics* **33**(1): 56-61.

Broderick, B. J., D. E. O'Briain, P. P. Breen, S. R. Kearns and G. Olaighin (2009). "A hemodynamic study of popliteal vein blood flow: the effect of bed rest and electrically elicited calf muscle contractions." *Conf Proc IEEE Eng Med Biol Soc* **2009**: 2149-2152.

Broderick, B. J., D. E. O'Briain, P. P. Breen, S. R. Kearns and G. Olaighin (2010). "A pilot evaluation of a neuromuscular electrical stimulation (NMES) based methodology for the prevention of venous stasis during bed rest." *Med Eng Phys* **32**(4): 349-355.

Brody, W. R. and J. D. Meindl (1974). "Theoretical Analysis of Cw Doppler Ultrasonic Flowmeter." *IEEE Transactions on Biomedical Engineering* **Bm21**(3): 183-192.

Brown, K. (2003). "A review to examine the use of SF-36 in cardiac rehabilitation." *British Journal of Nursing* **12**(15): 904-909.

Brown, M. D., C. J. Kelsall, M. Milkiewicz, S. Anderson and O. Hudlicka (2005). "A new model of peripheral arterial disease: sustained impairment of nutritive microcirculation and its recovery by chronic electrical stimulation." *Microcirculation* **12**(4): 373-381.

Brown, M. J. and S. J. Bird (2004). "Natural progression of diabetic peripheral neuropathy in the Zenarestat study population." *Diabetes Care* **27**(5): 1153-1159.

Browse, N. L. and D. Negus (1970). "The effect of intra-operative calf muscle stimulation on the prevention of postoperative deep-vein thrombosis." *The British Journal of Surgery* **57**(11): 861.

Bruce, R. A., F. Kusumi and D. Hosmer (1973). "Maximal oxygen intake and nomographic assessment of functional aerobic impairment in cardiovascular disease." *Am Heart J* **85**(4): 546-562.

Buller, B. Y. A. J., J. C. Eccles and R. M. Eccles (1960). "Interactions between motorneurones and muscles in respect of the characteristic speeds of their responses." *The Journal of Physiology* **150**: 417-439.

Bundens, W. P. (1995). "Use of the air plethysmograph in the evaluation and treatment of patients with venous stasis disease." *Dermatologic Surgery* **21**(1): 67-69.

Burnstock, G. and V. Ralevic (1994). "New insights into the local regulation of blood flow by perivascular nerves and endothelium." *British Journal of Plastic Surgery* **47**(8): 527-543.

Burroughs, T. E., R. Desikan, B. M. Waterman, D. Gilin and J. McGill (2004). "Development and Validation of the Diabetes Quality of Life Brief Clinical Inventory." *Diabetes Spectrum* **17**(1): 41-49.

Callam, M. J. (1994). "Epidemiology of varicose veins." *The British Journal of Surgery* **81**(2): 167-173.

Callam, M. J., D. R. Harper, J. J. Dale and C. V. Ruckley (1987). "Chronic ulcer of the leg: clinical history." *Br Med J (Clin Res Ed)* **294**(6584): 1389-1391.

Cambridge, N. A. (1977). "Electrical Apparatus used in Medicine before 1900." *Proc Roy Soc Med* **70**: 635-641.

Caravaggi, C., A. Sganzaroli, P. Galenda, M. Bassetti, R. Ferraresi and L. Gabrielli (2013). "The management of the infected diabetic foot." *Curr Diabetes Rev* **9**(1): 7-24.

Carlino, E., E. Frisaldi and F. Benedetti (2014). "Pain and the context." *Nat Rev Rheumatol* **10**(6): 348-355.
Carmeli, E., M. Moas, A. Z. Reznick and R. Coleman (2004). "Matrix metalloproteinases and skeletal muscle: A brief review." *Muscle Nerve* **29**(2): 191-197.
Carter, S. A. (1972). "Response of ankle systolic pressure to leg exercise in mild or questionable arterial disease." *The New England Journal of Medicine* **287**(12): 578-582.
Castaneda, C., J. E. Layne, L. Munoz-Orians, P. L. Gordon, J. Walsmith, M. Foldvari, R. Roubenoff, K. L. Tucker and M. E. Nelson (2002). "A randomized controlled trial of resistance exercise training to improve glycemic control in older adults with type 2 diabetes." *Diabetes Care* **25**(12): 2335-2341.
Charles, H. (1998). "Wound assessment: measuring the area of a leg ulcer." *British Journal of Nursing* **7**(13): 765-768.
Cheetham, D. R., L. Burgess, M. Ellis, A. Williams, R. M. Greenhalgh and A. H. Davies (2004). "Does supervised exercise offer adjuvant benefit over exercise advice alone for the treatment of intermittent claudication? A randomised trial." *European Journal of Vascular and Endovascular Surgery* **27**(1): 17-23.
Cheetham, D. R., R. M. Greenhalgh and A. H. Davies (2002). "A randomized study of once weekly supervised exercise motivation class versus exercise advice alone for the treatment of intermittent claudication (3-month results)." *British Journal of Surgery* **89**: 99-100.
Cheung, N. W., N. Cinnadaio, M. Russo and S. Marek (2009). "A pilot randomised controlled trial of resistance exercise bands in the management of sedentary subjects with type 2 diabetes." *Diabetes Res Clin Pract* **83**(3): e68-e71.
Chiu, K. W. H., R. S. M. Davies, P. G. Nightingale, A. W. Bradbury and D. J. Adam (2010). "Review of Direct Anatomical Open Surgical Management of Atherosclerotic Aorto-Iliac Occlusive Disease." *European Journal of Vascular and Endovascular Surgery* **39**(4): 460-471.
Chong, P. F., A. M. Garratt, J. Golledge, R. M. Greenhalgh and A. H. Davies (2002). "The intermittent claudication questionnaire: a patient-assessed condition-specific health outcome measure." *Journal of Vascular Surgery* **36**(4): 764-771; discussion 863-764.
Christopoulos, D., A. N. Nicolaides, G. Belcaro and P. Duffy (1990). "The Effect of Elastic Compression on Calf Muscle Pump Function." *Phlebology* **5**(1): 13-19.
Christopoulos, D., A. N. Nicolaides and G. Szendro (1988). "Venous reflux: quantification and correlation with the clinical severity of chronic venous disease." *The British Journal of Surgery* **75**(4): 352-356.
Clagett, G. P. and J. S. Reisch (1988). "Prevention of venous thromboembolism in general surgical patients. Results of meta-analysis." *Annals of surgery* **208**(2): 227-240.
Clarke Moloney, M., G. M. Lyons, P. Breen, P. E. Burke and P. A. Grace (2006). "Haemodynamic study examining the response of venous blood flow to electrical stimulation of the gastrocnemius muscle in patients with chronic venous disease." *European Journal of Vascular & Endovascular Surgery* **31**(3): 300-305.
Comerota, A. (2009). "Intermittent pneumatic compression improves healing of venous ulcers." *Vascular* **17**: S82-S82.
Comerota, A. J. (2011). "Intermittent pneumatic compression: Physiologic and clinical basis to improve management of venous leg ulcers." *Journal of Vascular Surgery* **53**(4): 1121-1129.
Comerota, A. J., M. L. Katz and J. V. White (1992). "Why does prophylaxis with external pneumatic compression for deep vein thrombosis fail?" *American Journal of Surgery* **164**(3): 265-268.
Conwit, R. A., D. Stashuk, B. Tracy, M. McHugh, W. F. Brown and E. J. Metter (1999). "The relationship of motor unit size, firing rate and force." *Clin Neurophysiol* **110**(7): 1270-1275.
Cordts, P. R., C. Hartono, W. W. LaMorte and J. O. Menzoian (1992). "Physiologic similarities between extremities with varicose veins and with chronic venous insufficiency utilizing air plethysmography." *American Journal of Surgery* **164**(3): 260-264.

Corley, G. J., S. I. Birlea, P. Breen and G. Olaighin (2009). Popliteal blood flow and plantar flexion force due to neuromuscular electrical stimulation (NMES) of the calf muscle pump are strongly associated with NMES intensity. Annual International Conference of the IEEE Engineering in Medicine and Biology Society.

Corley, G. J., P. P. Breen, S. I. Bîrlea, J. M. Serrador, P. A. Grace and G. ÓLaighin (2012). "Hemodynamic effects of habituation to a week-long program of neuromuscular electrical stimulation." *Medical Engineering & Physics* **34**(4): 459-465.

Corley, G. J., P. P. Breen, P. A. Grace and O. L. G (2008). "The effect of surface neuromuscular electrical stimulation and compression hosiery applied to the lower limb, on the comfort and blood flow of healthy subjects." *Conference proceedings : ..* **Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Conference. 2008**: 703-706.

Covington, J. S., J. W. Griffin, R. K. Mendius, R. E. Tooms and J. K. Clifft (1989). "Measurement of pressure ulcer volume using dental impression materials: suggestion from the field." *Physical Therapy* **69**(8): 690-694.

Crevenna, R., W. Mayr, M. Keilani, J. Pleiner, M. Nuhr, M. Quittan, R. Pacher, V. Fialka-Moser and M. Wolzt (2003). "Safety of a combined strength and endurance training using neuromuscular electrical stimulation of thighs muscles in patients with heart failure and bipolar sensing cardiac pacemakers." *Wien Klin Wochenschr* **115**(19-20): 710-714.

Criqui, M. H. and R. D. Langer (1992). "Mortality over a period of 10 years in patients with peripheral arterial disease." *… England Journal of …* **326**: 381-386.

Crisostomo, R. S., M. S. Candeias and P. A. Armada-da-Siva (2014). "The use of ultrasound in the evaluation of the efficacy of calf muscle pump function in primary chronic venous disease." *Phlebology* **29**(4): 247-256.

Cummins, H. Z., N. Knable and Y. Yeh (1964). "Observation of Diffusion Broadening of Rayleigh Scattered Light." *Phys Rev Lett* **12**(6): 150-153.

Currie, C. J., C. L. Morgan and J. R. Peters (1998). "The epidemiology and cost of inpatient care for peripheral vascular disease, infection, neuropathy, and ulceration in diabetes." *Diabetes Care* **21**(1): 42-48.

Curry, J. H. L. (1993). "The effect of chronic low frequency stimulation on performance and blood flow in human muscles." *The Journal of Physiology* **459**(1): 184P-243P.

Czyrny, J. J., R. E. Kaplan, G. E. Wilding, C. H. Purdy and J. Hirsh (2010). "Electrical foot stimulation: a potential new method of deep venous thrombosis prophylaxis." *Vascular* **18**(1): 20-27.

D'Hemecort P A, S. J. M., Karim M R. (1998). "Sodium carboxymethyl cellulose aqueous-based gel vs becaplermin gel in patients with nonhealing lower extremity diabetic ulcers." *Wounds* **10**(3): 69-75.

Da Silva, A., L. K. Widmer, H. W. Ziegler, C. Nissen and W. Schweizer (1979). "The Basle longitudinal study: report on the relation of initial glucose level to baseline ECG abnormalities, peripheral artery disease, and subsequent mortality." *J Chronic Dis* **32**(11-12): 797-803.

Dahl-Jorgensen, K., O. Brinchmann-Hansen, K. F. Hanssen, T. Ganes, P. Kierulf, E. Smeland, L. Sandvik and O. Aagenaes (1986). "Effect of near normoglycaemia for two years on progression of early diabetic retinopathy, nephropathy, and neuropathy: the Oslo study." *Br Med J (Clin Res Ed)* **293**(6556): 1195-1199.

Dai, G. H., O. Tsukurov, R. W. Orkin, W. M. Abbott, R. D. Kamm and J. P. Gertler (2000). "An in vitro cell culture system to study the influence of external pneumatic compression on endothelial function." *Journal of Vascular Surgery* **32**(5): 977-987.

Dalziel, C. F. (1972). "Electric shock hazard." *Spectrum, IEEE* **9**(2): 41-50.

Davies J, B. R., Farrelly I, Wakelin M (2008). "Improving the calf pump using home-based exercises for patients with chronic venous disease." *Wound* **3**(3): 48-58.

de Haro, J., F. Acin, A. Florez, S. Bleda and J. L. Fernandez (2010). "A prospective randomized controlled study with intermittent mechanical compression of the calf in patients with claudication." *Journal of Vascular Surgery* **51**(4): 857-862.

de Souza, R. J., A. de Souza and M. D. Nagvekar (2015). "Nerve conduction studies in diabetics presymptomatic and symptomatic for diabetic polyneuropathy." *Journal of Diabetes and its Complications* **29**(6): 811-817.

Dejode, L., M. Khurshid and W. Walther (1973). "The influence of electrical stimulation of the leg during surgical operations on the subsequent development of deep-vein thrombosis." *The British Journal of Surgery* **60**(1): 31-32.

Delis, K. (2005). "The case for intermittent pneumatic compression of the lower extremity as a novel treatment in arterial claudication." *Perspectives in Vascular Surgery & Endovascular Therapy* **17**(1): 29-42.

Delis, K. and A. Knaggs (2005). "Duration and amplitude decay of acute arterial leg inflow enhancement with intermittent pneumatic leg compression: An insight into the implicated physiologic mechanisms." *Journal of Vascular Surgery* **42**(4): 717-725.

Delis, K. and A. Nicolaides (2005). "Effect of intermittent pneumatic compression of foot and calf on walking distance, hemodynamics, and quality of life in patients with arterial claudication: A prospective randomized controlled study with 1-year follow-up." *Annals of surgery* **241**(3): 431-441.

Delis, K. T., M. Husmann, E. Kalodiki, J. H. Wolfe and A. N. Nicolaides "In situ hemodynamics of perforating veins in chronic venous insufficiency." *Journal of Vascular Surgery* **33**(4): 773-782.

Delis, K. T., M. J. Husmann, A. N. Nicolaides, J. H. Wolfe and N. J. Cheshire (2002). "Enhancing foot skin blood flux in peripheral vascular disease using intermittent pneumatic compression: controlled study on claudicants and grafted arteriopaths." *World Journal of Surgery* **26**(7): 861-866.

Delis, K. T., N. Labropoulos, A. N. Nicolaides, B. Glenville and G. Stansby (2000). "Effect of intermittent pneumatic foot compression on popliteal artery haemodynamics." *European Journal of Vascular and Endovascular Surgery* **19**(3): 270-277.

Delis, K. T., A. N. Nicolaides, N. Labropoulos and G. Stansby (2000). "The acute effects of intermittent pneumatic foot versus calf versus simultaneous foot and calf compression on popliteal artery hemodynamics: A comparative study." *Journal of Vascular Surgery* **32**(2): 284-292.

Delis, K. T., A. N. Nicolaides, J. H. Wolfe and G. Stansby (2000). "Improving walking ability and ankle brachial pressure indices in symptomatic peripheral vascular disease with intermittent pneumatic foot compression: a prospective controlled study with one-year follow-up." *Journal of Vascular Surgery* **31**(4): 650-661.

Delitto, A., M. J. Strube, A. D. Shulman and S. D. Minor (1992). "A study of discomfort with electrical stimulation." *Physical Therapy* **72**(6): 410-421; discussion on 421-414.

Dellantonio, R., D. Paladini, A. Cinti, P. Fumelli, I. Testa and F. Angeleri (1992). "Autonomic diabetic neuropathy: skin sympathetic response and neurovegetative cardiovascular functionality tests." *Funct Neurol* **7**(2): 115-120.

Deltombe, T., J. Jamart, S. Recloux, C. Legrand, N. Vandenbroeck, S. Theys and P. Hanson (2007). "Reliability and limits of agreement of circumferential, water displacement, and optoelectronic volumetry in the measurement of upper limb lymphedema." *Lymphology* **40**(1): 26-34.

Deschenes, M. R., M. A. Roby, M. K. Eason and M. B. Harris (2010). "Remodeling of the neuromuscular junction precedes sarcopenia related alterations in myofibers." *Exp Gerontol* **45**(5): 389-393.

Devlin, N. J. and P. F. Krabbe (2013). "The development of new research methods for the valuation of EQ-5D-5L." *Eur J Health Econ* **14 Suppl 1**: S1-3.

Dharmarajah, B., T. R. Lane, H. M. Moore, H. M. Neumann, E. Rabe, C. H. Wittens and A. H. Davies (2014). "The Future of Phlebology in Europe." *Phlebology* **29**(1 suppl): 181-185.

Dillon, R. S. (1980). "Effect of Therapy with the Pneumatic End-Diastolic Leg Compression Boot on Peripheral Vascular Tests and on the Clinical Course of Peripheral Vascular-Disease." *Angiology* **31**(9): 614-638.

Dillon, R. S. (1986). "Treatment of resistant venous stasis ulcers and dermatitis with the end-diastolic pneumatic compression boot." *Angiology* **37**(1): 47-56.

Dillon, R. S. (1997). "Fifteen years of experience in treating 2177 episodes of foot and leg lesions with the circulator boot. Results of treatments with the circulator boot." *Angiology* **48**(5 Pt 2): S17-34.

Dillon, R. S. (1997). "Patient assessment and examples of a method of treatment. Use of the circulator boot in peripheral vascular disease." *Angiology* **48**(5 Pt 2): S35-58.

Dix, F. P., R. Brooke and C. N. McCollum (2003). "Venous disease is associated with an impaired range of ankle movement." *European Journal of Vascular & Endovascular Surgery* **25**(6): 556-561.

Donnelly, R., A. Emslie-Smith, I. Gardner and A. Morris (2000). "ABC of arterial and venous disease: vascular complications of diabetes." *BMJ* **320**: 1062-1066.

Doran, F. S., M. Drury and A. Sivyer (1964). "A Simple Way to Combat the Venous Stasis Which Occurs in the Lower Limbs during Surgical Operations." *The British Journal of Surgery* **51**: 486-492.

Doran, F. S. and H. M. White (1967). "A demonstration that the risk of postoperative deep venous thrombosis is reduced by stimulating the calf muscles electrically during the operation." *The British Journal of Surgery* **54**(8): 686-689.

Doran, F. S., M. White and M. Drury (1970). "A clinical trial designed to test the relative value of two simple methods of reducing the risk of venous stasis in the lower limbs during surgical operations, the danger of thrombosis, and a subsequent pulmonary embolus, with a survey of the problem." *The British Journal of Surgery* **57**(1): 20-30.

Dormandy, J. A. and R. B. Rutherford (2000). "Management of peripheral arterial disease (PAD). TASC Working Group. TransAtlantic Inter-Society Consensus (TASC)." *Journal of Vascular Surgery* **31**(1 Pt 2): S1-S296.

Doucet, B. M., A. Lam and L. Griffin (2012). "Neuromuscular electrical stimulation for skeletal muscle function." *Yale J Biol Med* **85**(2): 201-215.

Downie, S. P., D. N. Firmin, N. B. Wood, S. A. Thom, A. D. Hughes, J. N. H. Wolfe and X. Y. Xu (2007). "Role of MRI in investigating the effects of elastic compression stockings on the deformation of the superficial and deep veins in the lower leg." *Journal of Magnetic Resonance Imaging* **26**(1): 80-85.

Drory, V. E. and A. D. Korczyn (1993). "Sympathetic skin response: age effect." *Neurology* **43**(9): 1818-1820.

Duchenne, G. B. (1871). A treatise on localized electrization, and its applications to pathology and therapeutics.

Dumville, J. C., A. J. Lee, F. B. Smith and F. G. R. Fowkes (2004). "The health-related quality of life of people with peripheral arterial disease in the community: the Edinburgh Artery Study." *The British Journal of General Practice* **54**(508): 826-831.

Eaton, W. and L. Kessler (1981). "Rates of symptoms of depression in a national sample." *American Journal of Epidemiology* **114**(4): 528-538.

Eaton WW, M. C., Smith C, Tien A, Ybarra M (2004). Center for Epidemiologic Studies Depression Scale: Review and revision (CESD and CESD-R). The Use of Psychological Testing for Treatment Planning and Outcomes Assessment. M. Maruish, Lawrence Erlbaum**:** 363-377.

Eberhardt, R. T. and J. D. Raffetto (2005). "Chronic venous insufficiency." *Circulation* **111**(18): 2398-2409.

Eftimie, R., H. R. Brenner and A. Buonanno (1991). "Myogenin and MyoD join a family of skeletal muscle genes regulated by electrical activity." *Proc Natl Acad Sci U S A* **88**(4): 1349-1353.

Eklöf, B., R. B. Rutherford, J. J. Bergan, P. H. Carpentier, P. Gloviczki, R. L. Kistner, M. H. Meissner, G. L. Moneta, K. Myers, F. T. Padberg, M. Perrin, C. V. Ruckley, P. C. Smith and T. W. Wakefield (2004). "Revision of the CEAP classification for chronic venous disorders: consensus statement." *Journal of Vascular Surgery* **40**(6): 1248-1252.

Elie, B. and P. Guiheneuc (1990). "Sympathetic Skin-Response - Normal Results in Different Experimental Conditions." *Electroencephalography and Clinical Neurophysiology* **76**(3): 258-267.

Elie, B. and P. Guiheneuc (1990). "Sympathetic skin response: normal results in different experimental conditions." *Electroencephalography and Clinical Neurophysiology* **76**(3): 258-267.

Enden, T. R., Y. Haig, N. E. Klow, C. E. Slagsvold, L. Sandvik, W. Ghanima, G. Hafsahl, P. A. Holme, L. O. Holmen, A. M. Njaastad, G. Sandbaek and P. M. Sandset (2011). "Improved Functional Outcome after Additional Catheter-Directed Thrombolysis for Acute Iliofemoral Deep Vein Thrombosis: Results of a Randomized Controlled Clinical Trial (The CaVenT Study)." *Blood* **118 (21)**.

Engel, G. L. (1981). "The Clinical Application of the Biopsychosocial Model." *Journal of Medicine and Philosophy* **6**(2): 101-124.

Ertekin, C., N. Ertekin, S. Mutlu, S. Almis and A. Akcam (1987). "Skin potentials (SP) recorded from the extremities and genital regions in normal and impotent subjects." *Acta Neurol Scand* **76**(1): 28-36.

Escribano, J. M., J. Juan, R. Bofill, J. Maeso, A. Rodriguez-Mori and M. Matas (2003). "Durability of reflux-elimination by a minimal invasive CHIVA procedure on patients with varicose veins. A 3-year prospective case study." *European Journal of Vascular and Endovascular Surgery* **25**(2): 159-163.

Evander, E., A. Evander and E. Scigala (1984). "Use of photoplethysmography to evaluate the effectiveness of elastic support stockings." *Bruit* **8**: 75-78.

Eze, A. R., A. J. Comerota, P. L. Cisek, B. S. Holland, R. P. Kerr and R. Veeramasuneni (1996). "Intermittent calf and foot compression increases lower extremity blood flow." *American Journal of Surgery* **172**(2): 130-134; discussion 135.

Faghri, P. D., H. F. Pompe Van Meerdervort, R. M. Glaser and S. F. Figoni (1997). "Electrical stimulation-induced contraction to reduce blood stasis during arthroplasty." *IEEE Transactions on Rehabilitation Engineering* **5**(1): 62-69.

Faghri, P. D., J. J. Votto and C. F. Hovorka (1998). "Venous hemodynamics of the lower extremities in response to electrical stimulation." *Archives of Physical Medicine and Rehabilitation* **79**(7): 842-848.

Fagius, J. and B. G. Wallin (1980). "Sympathetic reflex latencies and conduction velocities in normal man." *J Neurol Sci* **47**(3): 433-448.

Fahim, M. A. (1989). "Rapid neuromuscular remodeling following limb immobilization." *Anat Rec* **224**(1): 102-109.

Fahim, M. A., M. Y. Hasan and W. B. Alshuaib (2000). Early morphological remodeling of neuromuscular junction in a murine model of diabetes.

Fahim, M. A. and N. Robbins (1986). "Remodelling of the neuromuscular junction after subtotal disuse." *Brain Res* **383**(1–2): 353-356.

Falanga, V. "Wound healing and its impairment in the diabetic foot." *The Lancet* **366**(9498): 1736-1743.

Fanelli, G., M. Zasa, M. Baciarello, R. Mazzani, S. Di Cianni, M. Rossi and A. Casati (2008). "Systemic hemodynamic effects of sequential pneumatic compression of the lower limbs: a prospective study in healthy volunteers." *Journal of Clinical Anaesthesia* **20**(5): 338-342.

Ferris, L., B. King, P. Spence and H. Williams (1936). "Effect of electric shock on the heart." *BSTJ: Technical Digests* **15**(3).

Finch C, Z. P. (1988). Mortality from diabetes. The Diabetes Annual. K. L. Alberti K. Amsterdam, Elsevier**:** 1-16.

Flanc, C., V. V. Kakkar and M. B. Clarke (1968). "The detection of venous thrombosis of the legs using 125I-labelled fibrinogen." *British Journal of Surgery* **55**(10): 742-747.

Flynn, M. D. and J. E. Tooke (1995). "Diabetic Neuropathy and the Microcirculation." *Diabetic Medicine* **12**(4): 298-301.

Fokkenrood, H. J., B. L. Bendermacher, G. J. Lauret, E. M. Willigendael, M. H. Prins and J. A. Teijink (2013). "Supervised exercise therapy versus non-supervised exercise therapy for intermittent claudication." *Cochrane Database of Systematic Reviews* **8**: Cd005263.

Forst, T., a. Pfützner, R. Bauersachs, M. Arin, B. Bach, H. Biehlmaier, E. Küstner and J. Beyer "Comparison of the microvascular response to transcutaneous electrical nerve stimulation and postocclusive ischemia in the diabetic foot." *Journal of Diabetes and its Complications* **11**(5): 291-297.

Franceschi, C. (1997). "[Measures and interpretation of venous flow in stress tests. Manual compression and Parana manoeuver. Dynamic reflux index and Pstakis index]." *J Mal Vasc* **22**(2): 91-95.

French, J. (1953). Experiments in Field Settings. Research Methods in the Behavioural Sciences. L. Festinger and D. Katz. New York, Holt, Rinehardt, Winston**:** 98-135.

Fuglevand, A. J., D. A. Winter, A. E. Patla and D. Stashuk (1992). "Detection of motor unit action potentials with surface electrodes: influence of electrode size and spacing." *Biol Cybern* **67**(2): 143-153.

Ganot (1859). Introductory course of natural philosophy for the use of schools and academies. Popular Physics. W. G. Peck**:** 479.

Gardner, A. W., P. S. Montgomery and A. Afaq (2007). "Exercise performance in patients with peripheral arterial disease who have different types of exertional leg pain." *Journal of Vascular Surgery* **46**(1): 79-86.

Gardner, A. W., J. S. Skinner, B. W. Cantwell and L. K. Smith (1991). "Progressive vs single-stage treadmill tests for evaluation of claudication." *Medicine & Science in Sports & Exercise* **23**(4): 402-408.

Gardner, S. E., R. A. Frantz and B. N. Doebbeling (2001). "The validity of the clinical signs and symptoms used to identify localized chronic wound infection." *Wound Repair and Regeneration* **9**(3): 178-186.

Gardner, S. E., S. L. Hillis, K. Heilmann, J. A. Segre and E. A. Grice (2013). "The neuropathic diabetic foot ulcer microbiome is associated with clinical factors." *Diabetes* **62**(3): 923-930.

Garratt, A. M., L. M. Macdonald, D. A. Ruta, I. T. Russell, J. K. Buckingham and Z. H. Krukowski (1993). "Towards measurement of outcome for patients with varicose veins." *Qual Health Care* **2**(1): 5-10.

Garratt, A. M., D. A. Ruta, M. I. Abdalla and I. T. Russell (1996). "Responsiveness of the SF-36 and a condition-specific measure of health for patients with varicose veins." *Quality of Life Research* **5**(2): 223-234.

Gatchel, R. J. (2004). "Comorbidity of chronic pain and mental health disorders: the biopsychosocial perspective." *Am Psychol* **59**(8): 795-805.

Gibson, A. P., J. C. Hebden and S. R. Arridge (2005). "Recent advances in diffuse optical imaging." *Phys Med Biol* **50**(4): R1-43.

Gibson, K., M. Meissner and D. Wright (2012). "Great saphenous vein diameter does not correlate with worsening quality of life scores in patients with great saphenous vein incompetence." *Journal of Vascular Surgery* **56**(6): 1634-1641.

Gill, R. W. (1985). "Measurement of blood flow by ultrasound: Accuracy and sources of error." *Ultrasound in Medicine & Biology* **11**(4): 625-641.

Gjores, J. E. and O. Thulesius (1977). "Compression treatment in venous insufficiency evaluated with foot volumetry." *Vasa* **6**(4): 364-368.
Gobbo, M., P. Gaffurini, L. Bissolotti, F. Esposito and C. Orizio (2011). "Transcutaneous neuromuscular electrical stimulation: influence of electrode positioning and stimulus amplitude settings on muscle response." *European Journal of Applied Physiology* **111**(10): 2451-2459.
Goddard, A. A., C. S. Pierce and K. J. McLeod (2008). "Reversal of lower limb edema by calf muscle pump stimulation." *J Cardiopulm Rehabil Prev* **28**(3): 174-179.
Goldhaber-Fiebert, J. D., S. N. Goldhaber-Fiebert, M. L. Tristan and D. M. Nathan (2003). "Randomized controlled community-based nutrition and exercise intervention improves glycemia and cardiovascular risk factors in type 2 diabetic patients in rural Costa Rica." *Diabetes Care* **26**(1): 24-29.
Gonzalez-Consuegra, R. V. and J. Verdu (2011). "Quality of life in people with venous leg ulcers: an integrative review." *J Adv Nurs* **67**(5): 926-944.
Gouin-Thibault, I., E. Pautas and V. Siguret (2005). "Safety profile of different low-molecular weight heparins used at therapeutic dose." *Drug Saf* **28**(4): 333-349.
Graven-Nielsen, T., H. Lund, L. Arendt-Nielsen, B. Danneskiold-Samsøe and H. Bliddal (2002). "Inhibition of maximal voluntary contraction force by experimental muscle pain: A centrally mediated mechanism." *Muscle Nerve* **26**(5): 708-712.
Green DA, S. P., Hunter SP (2008). "Effect of percutaneous electrical stimulation of the sole upon lower limb blood pooling induced by protracted sitting in man." *HIgh Tech Health Ltd*.
Gregg, E. W., P. Sorlie, R. Paulose-Ram, Q. Gu, M. S. Eberhardt, M. Wolz, V. Burt, L. Curtin, M. Engelgau, L. Geiss, h. national and s. nutrition examination (2004). "Prevalence of lower-extremity disease in the US adult population >=40 years of age with and without diabetes: 1999-2000 national health and nutrition examination survey." *Diabetes Care* **27**(7): 1591-1597.
Griffin, J. W., E. A. Tolley, R. E. Tooms, R. A. Reyes and J. K. Clifft (1993). "A comparison of photographic and transparency-based methods for measuring wound surface area." *Physical Therapy* **73**(2): 117-122.
Griffin, M., A. N. Nicolaides, D. Bond, G. Geroulakos and E. Kalodiki (2010). "The efficacy of a new stimulation technology to increase venous flow and prevent venous stasis." *European Journal of Vascular & Endovascular Surgery* **40**(6): 766-771.
Group, T. D. C. a. C. T. R. (1995). "The effect of intensive diabetes therapy on the development and progression of neuropathy. The Diabetes Control and Complications Trial Research Group." *Annals of Internal Medicine* **122**(8): 561-568.
Group, T. D. R. (1988). "Reliability and Validity of a Diabetes Quality-of-Life Measure for the Diabetes Control and Complications Trial (DCCT)." *Diabetes Care* **11**(9): 725-732.
Grunau, R. V. E., M. F. Whitfield, J. H. Petrie and E. L. Fryer (1994). "Early pain experience, child and family factors, as precursors of somatization: a prospective study of extremely premature and fullterm children." *Pain* **56**(3): 353-359.
Gutrecht, J. A. (1994). "Sympathetic skin response." *J Clin Neurophysiol* **11**(5): 519-524.
Guyatt, G. H., D. H. Feeny and D. L. Patrick (1993). "Measuring Health-Related Quality-of-Life." *Annals of Internal Medicine* **118**(8): 622-629.
Guyenet, P. G. (2006). "The sympathetic control of blood pressure." *Nat Rev Neurosci* **7**(5): 335-346.
Hackman, W. (1978). Early electrical investigations. Electricity from glass: The history of the frictional electrical machine 1600-1850. G. Turner. The Netherlands, Sijthoff and Noordhoff.
Haenen, J. H., M. C. Janssen, a. J. Brakkee, H. Van Langen, H. Wollersheim, T. M. De Boo, S. H. Skotnicki and T. Thien (2000). "Venous reflux has a limited effect on calf muscle pump dysfunction in post-thrombotic patients." *Clinical Science* **98**: 449-454.
Hall, Z. W. and J. R. Sanes (1993). "Synaptic structure and development: The neuromuscular junction." *Cell* **72, Supplement**(0): 99-121.

Hamburg, N. M. and G. J. Balady (2011). "Exercise Rehabilitation in Peripheral Artery Disease: Functional Impact and Mechanisms of Benefits." *Circulation* **123**(1): 87-97.

Hann, D., K. Winter and P. Jacobsen (1999). "Measurement of depressive symptoms in cancer patients: Evaluation of the center for epidemiological studies depression scale (Ces-d)." *Journal of Psychosomatic Research* **46**(5): 437-443.

Harvey, W. (1628). Exercitio Anatomica de Motu Cordis et Sanguinis Animalibus, Baltimore.

Heath, M. E. and S. B. Gibbs (1992). "High-voltage pulsed galvanic stimulation: effects of frequency of current on blood flow in the human calf muscle." *Clin Sci (Lond)* **82**(6): 607-613.

Herrmann, L. G. and M. R. Reid (1934). "Passive vascular exercises: Treatment of peripheral obliterative arterial diseases by rhythmic alternation of environmental pressure." *Archives of surgery* **29**(5): 697-704.

HES (2010/11). Hospital Episode Statistics.

Hey-Mogensen, M., K. Højlund, B. F. Vind, L. Wang, F. Dela, H. Beck-Nielsen, M. Fernström and K. Sahlin (2010). "Effect of physical training on mitochondrial respiration and reactive oxygen species release in skeletal muscle in patients with obesity and type 2 diabetes." *Diabetologia* **53**(9): 1976-1985.

Hiatt, W. R., A. T. Hirsch, J. G. Regensteiner and E. P. Brass (1995). "Clinical trials for claudication. Assessment of exercise performance, functional status, and clinical end points. Vascular Clinical Trialists." *Circulation* **92**(3): 614-621.

Hiatt, W. R., A. T. Hirsch, J. G. Regensteiner, E. P. Brass and a. t. V. C. Trialists (1995). "Clinical Trials for Claudication: Assessment of Exercise Performance, Functional Status, and Clinical End Points." *Circulation* **92**(3): 614-621.

Hiatt, W. R., D. Nawaz, J. G. Regensteiner and K. F. Hossack (1988). "The Evaluation of Exercise Performance in Patients with Peripheral Vascular Disease." *Journal of Cardiopulmonary Rehabilitation and Prevention* **8**(12): 525-532.

Hiatt, W. R., J. G. Regensteiner, M. E. Hargarten, E. E. Wolfel and E. P. Brass (1990). "Benefit of exercise conditioning for patients with peripheral arterial disease." *Circulation* **81**(2): 602-609.

Hill, J. A., J. T. O'Brien, E. Scott, C. R. Conti and C. J. Pepine (1984). "Effects of bepridil on exercise tolerance in chronic stable angina: a double-blind, randomized, placebo-controlled, crossover trial." *American Journal of Cardiology* **53**(6): 679-683.

Hirai, M., H. Iwata, K. Miyazaki, A. Koyama, K. Ikeda, M. Hukiage and H. Kitamura (2013). "Effect of gaiters on muscle pump activity in healthy volunteers." *Phlebology* **28**(6): 293-298.

Hjermstad, M. J., P. M. Fayers, D. F. Haugen, A. Caraceni, G. W. Hanks, J. H. Loge, R. Fainsinger, N. Aass and S. Kaasa (2011). "Studies Comparing Numerical Rating Scales, Verbal Rating Scales, and Visual Analogue Scales for Assessment of Pain Intensity in Adults: A Systematic Literature Review." *J Pain Symptom Manage* **41**(6): 1073-1093.

Hopman, W. M., M. B. Harrison, H. Coo, E. Friedberg, M. Buchanan and E. G. VanDenKerkhof (2009). "Associations between chronic disease, age and physical and mental health status." *Chronic Dis Can* **29**(3): 108-116.

Hopman, W. M., E. G. VanDenKerkhof, M. E. Carley, J. L. Kuhnke and M. B. Harrison (2014). "Factors associated with health-related quality of life in chronic leg ulceration." *Qual Life Res* **23**(6): 1833-1840.

Hordern, M. D., J. S. Coombes, L. M. Cooney, L. Jeffriess, J. B. Prins and T. H. Marwick (2009). "Effects of exercise intervention on myocardial function in type 2 diabetes." *Heart* **95**(16): 1343-1349.

Hordern, Matthew D., Louise M. Cooney, Elaine M. Beller, Johannes B. Prins, Thomas H. Marwick and Jeff S. Coombes (2008). Determinants of changes in blood glucose response to short-term exercise training in patients with Type 2 diabetes.

Howard, L. S. and R. J. Hughes (2013). "NICE guideline: management of venous thromboembolic diseases and role of thrombophilia testing." *Thorax* **68**(4): 391-393.

Howell-Jones, R. S., M. J. Wilson, K. E. Hill, A. J. Howard, P. E. Price and D. W. Thomas (2005). "A review of the microbiology, antibiotic usage and resistance in chronic skin wounds." *J Antimicrob Chemother* **55**(2): 143-149.

Hudlicka, O., S. Beck, A. Crow, C. Shearman and G. Tsang (1994). "The Effect of Chronic Electrical-Stimulation on Muscle Performance in Patients with Peripheral Vascular Diseases." *Journal of Physiology-London* **477P**: P38-P38.

Hultman, E., H. Sjöholm, I. Jäderholm-Ek and J. Krynicki (1983). "Evaluation of methods for electrical stimulation of human skeletal muscle in situ." *Pflügers Archiv* **398**: 139-141.

Humbert, P., S. Meaune and T. Gharbi (2004). "Wound healing assessment." *Phlebolymphology* **47**: 312-319.

Hunter, J. (1773). "Anatomical observations on the torpedo." *Phil Trans Roy Soc* **63**: 481-489.

Huskisson, E. C. (1974). "Measurement of pain." *Lancet* **2**(7889): 1127-1131.

Hussain, S. T., R. E. Smith, R. F. M. Wood and M. Bland (1996). "Observer variability in volumetric blood flow measurements in leg arteries using duplex ultrasound." *Ultrasound in Medicine & Biology* **22**(3): 287-291.

Huxley, A. F. and R. Niedergerke (1954). "Structural changes in muscle during contraction; interference microscopy of living muscle fibres." *Nature* **173**(4412): 971-973.

Huxley, H. and J. Hanson (1954). "Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation." *Nature* **173**(4412): 973-976.

IARC (2002). WHO Classification of Soft Tissue Tumours. Lyons, IARC Press.

ICAI (1997). "Long-term mortality and its predictors in patients with critical leg ischaemia. The Study Group of Criticial Chronic Ischemia of the Lower Exremities." *European Journal of Vascular and Endovascular Surgery* **14**(2): 91-95.

Iwatsu, K., S. Yamada, Y. Iida, H. Sampei, K. Kobayashi, M. Kainuma and A. Usui (2015). "Feasibility of Neuromuscular Electrical Stimulation Immediately After Cardiovascular Surgery." *Archives of Physical Medicine and Rehabilitation* **96**(1): 63-68.

Izumi, M., M. Ikeuchi, K. Aso, N. Sugimura, Y. Kamimoto, T. Mitani, T. Ueta, T. Sato, M. Yokoyama, T. Sugiura and T. Tani (2014). "Less deep vein thrombosis due to transcutaneous fibular nerve stimulation in total knee arthroplasty: a randomized controlled trial." *Knee Surgery, Sports Traumatology, Arthroscopy*: 1-7.

Izumi, M., M. Ikeuchi, T. Mitani, S. Taniguchi and T. Tani "Prevention of venous stasis in the lower limb by transcutaneous electrical nerve stimulation." *European Journal of Vascular & Endovascular Surgery* **39**(5): 642-645.

Izumi, M., M. Ikeuchi, T. Mitani, S. Taniguchi and T. Tani (2010). "Prevention of venous stasis in the lower limb by transcutaneous electrical nerve stimulation." *European Journal of Vascular & Endovascular Surgery* **39**(5): 642-645.

Jabre, J. F. and N. T. Spellman (1996). "The demonstration of the size principle in humans using macro electromyography and precision decomposition." *Muscle Nerve* **19**(3): 338-341.

Jang, Y. C. and H. Van Remmen (2011). "Age-associated alterations of the neuromuscular junction." *Experimental Gerontology* **46**(2–3): 193-198.

Jenkinson, C., L. Wright and A. Coulter (1994). "Criterion validity and reliability of the SF-36 in a population sample." *Quality of Life Research* **3**(1): 7-12.

Jensen, J. L., J. Seeley and B. Gillin (1998). "Diabetic foot ulcerations. A controlled, randomized comparison of two moist wound healing protocols: Carrasyn Hydrogel Wound dressing and wet-to-moist saline gauze." *Adv Wound Care* **11**(7 Suppl): 1-4.

Jezernik, S., R. G. Wassink and T. Keller (2004). "Sliding mode closed-loop control of FES: controlling the shank movement." *IEEE Transactions on Biomedical Engineering* **51**(2): 263-272.

Jones, M., I. Edwards and L. Gifford (2002). "Conceptual models for implementing biopsychosocial theory in clinical practice." *Manual Therapy* **7**(1): 2-9.

Jorgensen, J. O., N. J. Lalak, L. North, K. Hanel, D. R. Hunt and D. L. Morris (1994). "Venous stasis during laparoscopic cholecystectomy." *Surg Laparosc Endosc* **4**(2): 128-133.

Joyce, C. R., D. W. Zutshi, V. Hrubes and R. M. Mason (1975). "Comparison of fixed interval and visual analogue scales for rating chronic pain." *Eur J Clin Pharmacol* **8**(6): 415-420.

Jull, A., N. Walker, M. Hackett, M. Jones, A. Rodgers, N. Birchall, R. Norton and S. MacMahon (2004). "Leg ulceration and perceived health: a population based case-control study." *Age Ageing* **33**(3): 236-241.

Jungbeck, C., K. Peterson, G. Danielsson and L. Norgren (2002). "Effects of Compression Hosiery in Female Workers with a Standing Profession." *Phlebology* **16**(3): 117-120.

Kahn, S. R., T. Ducruet, D. L. Lamping, L. Arsenault, M. J. Miron, A. Roussin, S. Desmarais, F. Joyal, J. Kassis, S. Solymoss, L. Desjardins, M. Johri and I. Shrier (2005). "Prospective evaluation of health-related quality of life in patients with deep venous thrombosis." *Arch Intern Med* **165**(10): 1173-1178.

Kahn, S. R., H. Shbaklo, D. L. Lamping, C. A. Holcroft, I. Shrier, M. J. Miron, A. Roussin, S. Desmarais, F. Joyal, J. Kassis, S. Solymoss, L. Desjardins, M. Johri and J. S. Ginsberg (2008). "Determinants of health-related quality of life during the 2 years following deep vein thrombosis." *J Thromb Haemost* **6**(7): 1105-1112.

Kakrani, A. L., V. S. Gokhale, K. V. Vohra and N. Chaudhary (2014). "Clinical and nerve conduction study correlation in patients of diabetic neuropathy." *J Assoc Physicians India* **62**(1): 24-27.

Kan, Y. M. and K. T. Delis (2001). "Hemodynamic effects of supervised calf muscle exercise in patients with venous leg ulceration: a prospective controlled study." *Archives of surgery* **136**(12): 1364-1369.

Kaplan, R. E., J. J. Czyrny, T. S. Fung, J. D. Unsworth and J. Hirsh (2002). "Electrical foot stimulation and implications for the prevention of venous thromboembolic disease." *Thrombosis and Haemostasis* **88**(2): 200-204.

Karatzanos, E., V. Gerovasili, D. Zervakis, E. S. Tripodaki, K. Apostolou, I. Vasileiadis, E. Papadopoulos, G. Mitsiou, D. Tsimpouki, C. Routsi and S. Nanas (2012). "Electrical muscle stimulation: an effective form of exercise and early mobilization to preserve muscle strength in critically ill patients." *Crit Care Res Pract* **2012**: 432752.

Karjalainen, K., A. Malmivaara, M. van Tulder, R. Roine, M. Jauhiainen, H. Hurri and B. Koes (2003). "Multidisciplinary biopsychosocial rehabilitation for subacute low back pain among working age adults." *Cochrane Database of Systematic Reviews*(2): CD002193.

Katz, R. T., D. Green, T. Sullivan and G. Yarkony (1987). "Functional electric stimulation to enhance systemic fibrinolytic activity in spinal cord injury patients." *Archives of Physical Medicine and Rehabilitation* **68**(7): 423-426.

Kavros, S. J., K. T. Delis, N. S. Turner, A. E. Voll, D. A. Liedl, P. Gloviczki and T. W. Rooke (2008). "Improving limb salvage in critical ischemia with intermittent pneumatic compression: a controlled study with 18-month follow-up." *Journal of Vascular Surgery* **47**(3): 543-549.

Kecelj-Leskovec, N., M. Jezersek, J. Mozina, M. D. Pavlovic and T. Lunder (2007). "Measurement of venous leg ulcers with a laser-based three-dimensional method: comparison to computer planimetry with photography." *Wound Repair and Regeneration* **15**(5): 767-771.

Khalil, Z., R. Ogrin and P. Darzins (2007). "The effect of sensory nerve stimulation on sensory nerve function in people with peripheral neuropathy associated with diabetes." *Neurological Research* **29**(7): 743-748.

Kherif, Dehaupas, Lafuma, Fardeau and Alameddine (1998). "Matrix metalloproteinases MMP-2 and MMP-9 in denervated muscle and injured nerve." *Neuropathology and Applied Neurobiology* **24**(4): 309-319.

Kluding, P. M., M. Pasnoor, R. Singh, S. Jernigan, K. Farmer, J. Rucker, N. K. Sharma and D. E. Wright (2012). "The effect of exercise on neuropathic symptoms, nerve function, and cutaneous innervation in people with diabetic peripheral neuropathy." *Journal of Diabetes and its Complications* **26**(5): 424-429.

Knezevic, W. and S. Bajada (1985). "Peripheral autonomic surface potential: a quantitative technique for recording autonomic neural function in man." *Clin Exp Neurol* **21**: 201-210.

Knutson, J. S., M. Y. Harley, T. Z. Hisel and J. Chae (2007). "Improving hand function in stroke survivors: a pilot study of contralaterally controlled functional electric stimulation in chronic hemiplegia." *Archives of Physical Medicine* **88**(April): 513-520.

Kohara, N., J. Kimura, R. Kaji, Y. Goto, J. Ishii, M. Takiguchi and M. Nakai (2000). "F-wave latency serves as the most reproducible measure in nerve conduction studies of diabetic polyneuropathy: multicentre analysis in healthy subjects and patients with diabetic polyneuropathy." *Diabetologia* **43**(7): 915-921.

Kolari, P. J. and K. Pekanmaki (1986). "Intermittent pneumatic compression in healing of venous ulcers." *Lancet* **2**(8515): 1108-1108.

Kolari, P. J., K. Pekanmaki and R. T. Pohjola (1988). "Transcutaneous oxygen tension in patients with post-thrombotic leg ulcers: Treatment with intermittent pneumatic compression." *Cardiovascular Research* **22**(2): 138-141.

Komiyama, T., A. Onozuka, T. Miyata and H. Shigematsu (2002). "Oxygen saturation measurement of calf muscle during exercise in intermittent claudication." *European Journal of Vascular and Endovascular Surgery* **23**(5): 388-392.

Komiyama, T., H. Shigematsu, H. Yasuhara, Y. Hosoi and T. Muto (1996). "An objective evaluation of muscle oxygen content in claudicants receiving drug therapy." *International Angiology* **15**(3): 215-218.

Kots YM and X. VA (1971). "Trenirovka mishechnoj sili metodom elektro-stimuliatsii: soobschenie 2, trenirovka metodom elektricheskogo razdrazenii mishechi." *Teor Pract Fis Cult.* **4**: 66-72.

Koyuncu, E., G. F. Nakipoglu-Yuzer, A. Dogan and N. Ozgirgin (2010). "The effectiveness of functional electrical stimulation for the treatment of shoulder subluxation and shoulder pain in hemiplegic patients: A randomized controlled trial." *Disabil Rehabil* **32**(7): 560-566.

Kröger, K., A. Stang, J. Kondratieva, S. Moebus, E. Beck, A. Schmermund, S. Möhlenkamp, N. Dragano, J. Siegrist, K.-H. Jöckel and R. Erbel (2006). "Prevalence of Peripheral Arterial Disease – Results of the Heinz Nixdorf Recall Study." *European Journal of Epidemiology* **21**(4): 279-285.

Kucera, P., Z. Goldenberg and E. Kurca (2004). "Sympathetic skin response: review of the method and its clinical use." *Bratisl Lek Listy* **105**(3): 108-116.

Kumru, H., J. Vidal, M. Perez, P. Schestatsky and J. Valls-Sole (2009). "Sympathetic skin responses evoked by different stimuli modalities in spinal cord injury patients." *Neurorehabil Neural Repair* **23**(6): 553-558.

Labropoulos, N., L. R. Leon, Jr., A. Bhatti, S. Melton, S. S. Kang, A. M. Mansour and M. Borge (2005). "Hemodynamic effects of intermittent pneumatic compression in patients with critical limb ischemia." *Journal of Vascular Surgery* **42**(4): 710-716.

Lachmann, E. A., J. L. Rook, R. Tunkel and W. Nagler (1992). "Complications associated with intermittent pneumatic compression." *Arch Phys Med Rehabil* **73**(5): 482-485.

Lagan, K., A. Dusoir, S. McDonough and G. Baxter (2000). "Wound measurement: The comparative reliability of direct versus photographic tracings analyzed by planimetry versus digitizing techniques." *Archives of Physical Medicine and Rehabilitation* **81**(8): 1110-1116.

Laing, S. and R. M. Greenhalgh (1986). "Treadmill testing in the assessment of peripheral arterial disease." *International Angiology* **5**(4): 249-252.

Landis, E. and J. Gibbon (1934). "The effect of of alternate suction and pressure on blood flow to the lower extremities." *Annals of Internal Medicine* **8**: 282-295.

Lane, T. R. A., A. C. Shepherd, M. Gohel, I. J. Franklin and A. H. Davies (2013). "Big Veins, Big Deal - Vein Diameter Affects Disease Severity, not Quality of Life." *Journal of Vascular Surgery: Venous and Lymphatic Disorders* **1**(1): 101.

Largus, S. "Compositiones Medicae."

Lattimer, C. R., E. Kalodiki, G. Geroulakos, D. Hoppensteadt and J. Fareed (2015). "Endogenous pro-thrombotic biomarkers from the arm and leg may not have the same value." *Phlebology*.

Lauritzen, T., K. Frost-Larsen, H. W. Larsen and T. Deckert (1985). "Two-year experience with continuous subcutaneous insulin infusion in relation to retinopathy and neuropathy." *Diabetes* **34 Suppl 3**: 74-79.

Laverick, M., R. McGivern, M. Crone and R. Mollan (1990). "A comparison of the effects of electrical calf muscle stimulation and the venous foot pump on venous blood flow in the lower leg." *Phlebology* **5**: 285-290.

Lavery, L. A., S. A. Vela, D. C. Lavery and T. L. Quebedeaux (1996). "Reducing dynamic foot pressures in high-risk diabetic subjects with foot ulcerations: A comparison of treatments." *Diabetes Care* **19**(8): 818-821.

Layden, J., J. Michaels, S. Bermingham, B. Higgins and G. Guideline Development (2012). "Diagnosis and management of lower limb peripheral arterial disease: summary of NICE guidance." *BMJ* **345**: e4947.

Le Tohic, A., H. Bastian, M. Pujo, P. Beslot, R. Mollard and P. Madelenat (2009). "[Effects of electrostimulation (Veinoplus) on lower limbs venous insufficiency-related symptoms during pregnancy. Preliminary study]." *Gynecol Obstet Fertil* **37**(1): 18-24.

Leahy, M. J., F. F. M. de Mul, G. E. Nilsson and R. Maniewski (1999). "Principles and practice of the laser-Doppler perfusion technique." *Technology and Health Care* **7**(2): 143-162.

Lee, A. P., R. Ice, R. Blessey and M. E. Sanmarco (1979). "Long-term effects of physical training on coronary patients with impaired ventricular function." *Circulation* **60**(7): 1519-1526.

Lee, H. L., T. Mehta, B. Ray, M. S. Heng, P. T. McCollum and I. C. Chetter (2007). "A non-randomised controlled trial of the clinical and cost effectiveness of a Supervised Exercise Programme for claudication." *European Journal of Vascular and Endovascular Surgery* **33**(2): 202-207.

Lehman, W., R. Craig and P. Vibert (1994). "Ca2+-Induced Tropomyosin Movement in Limulus Thin-Filaments Revealed by 3-Dimensional Reconstruction." *Nature* **368**(6466): 65-67.

Leknes, S., C. Berna, M. C. Lee, G. D. Snyder, G. Biele and I. Tracey (2013). "The importance of context: when relative relief renders pain pleasant." *Pain* **154**(3): 402-410.

Levin, M. E. (1995). "Preventing amputation in the patient with diabetes." *Diabetes Care* **18**(10): 1383-1394.

Lewis, P., J. V. Psaila, W. T. Davies, K. McCarty and J. P. Woodcock (1986). "Measurement of volume flow in the human common femoral artery using a duplex ultrasound system." *Ultrasound in Medicine & Biology* **12**(10): 777-784.

Lindgarde, F., R. Jelnes, H. Bjorkman, G. Adielsson, T. Kjellstrom, I. Palmquist and L. Stavenow (1989). "Conservative drug treatment in patients with moderately severe chronic occlusive peripheral arterial disease. Scandinavian Study Group." *Circulation* **80**(6): 1549-1556.

Lindhagen, J., H. Ahn, E. G. Salerud, M. Jodal and O. Lundgren (1984). "Laser Doppler Flowmetry in the Assessment of Intestinal Blood-Flow in Man." *International Journal of Microcirculation-Clinical and Experimental* **3**(3-4): 478-478.

Lindstrom, B., C. Holmdahl and O. Jonsson (1982). "Prediction and prophylaxis of postoperative thromboembolism: A comparison between peroperative calf muscle stimulation with groups of impulses and dextran 40." *British Journal of Surgery* **69**(11): 633-637.

Lipton, J. A. and J. J. Marbach (1984). "Special Issue: Chronic PainEthnicity and the pain experience." *Social Science & Medicine* **19**(12): 1279-1298.

Liu, H. I., D. P. Currier and A. J. Threlkeld (1987). "Circulatory response of digital arteries associated with electrical stimulation of calf muscle in healthy subjects." *Physical Therapy* **67**(3): 340-345.

Lobastov, K., V. Barinov, L. Laberko, V. Obolensky, V. Boyarintsev and G. Rodoman (2014). "Electrical calf muscle stimulation with Veinoplus device in postoperative venous thromboembolism prevention." *International Angiology* **33**(1): 42-49.

Lomo, T. and J. Rosenthal (1972). "Control of ACh sensitivity by muscle activity in the rat." *The Journal of Physiology* **221**(2): 493-513.

Lomo, T. and R. H. Westgaard (1975). "Further studies on the control of ACh sensitivity by muscle activity in the rat." *The Journal of Physiology* **252**(3): 603-626.
Loubser, P. G., D. Cardus, L. R. Pickard and W. G. McTaggart (1988). "Effects of unilateral, low-frequency, neuromuscular stimulation on superficial circulation in lower extremities of patients with peripheral vascular disease." *Medical Instrumentation* **22**(2): 82-87.
Louridas, G., R. Saadia, J. Spelay, A. Abdoh, W. Weighell, A. S. Arneja, J. Tanner and R. Guzman (2002). "The ArtAssist Device in chronic lower limb ischemia. A pilot study." *International Angiology* **21**(1): 28-35.
Loven, C. (1866). "Uber die Erweiterung von Arterien in Folge einer Nervenerregung." *Ber Verh konigl-sachs Ges Wiss:Math-phys Classe* **18**: 85-110.
Lucas, C. C., Jody; Harrison, Deannine; De Haan, Rob J (2002). "Pressure ulcer surface area measurement using instant full-scale photography and transparency tracings." *Advances in Skin & Wound Care* **15**(1): 17-23.
Luff, A. R. (1998). "Age-associated changes in the innervation of muscle fibers and changes in the mechanical properties of motor units." *Ann N Y Acad Sci* **854**: 92-101.
Lundeberg, T. C., S. V. Eriksson and M. Malm (1992). "Electrical nerve stimulation improves healing of diabetic ulcers." *Annals of Plastic Surgery* **29**(4): 328-331.
Lurie, F., D. J. Awaya, R. L. Kistner and B. Eklof (2003). "Hemodynamic effect of intermittent pneumatic compression and the position of the body." *Journal of Vascular Surgery* **37**(1): 137-142.
Lustman, P. J., R. J. Anderson, K. E. Freedland, M. de Groot, R. M. Carney and R. E. Clouse (2000). "Depression and poor glycemic control: a meta-analytic review of the literature." *Diabetes Care* **23**(7): 934-942.
Lyons, G. M., G. E. Leane and P. A. Grace (2002). "The effect of electrical stimulation of the calf muscle and compression stocking on venous blood flow velocity." *European Journal of Vascular & Endovascular Surgery* **23**(6): 564-566.
MacDougall, D. A., A. L. Feliu, S. J. Boccuzzi and J. Lin (2006). "Economic burden of deep-vein thrombosis, pulmonary embolism, and post-thrombotic syndrome." *Am J Health Syst Pharm* **63**(20 Suppl 6): S5-15.
Macefield, V. G. and B. G. Wallin (1999). "Firing properties of single vasoconstrictor neurones in human subjects with high levels of muscle sympathetic activity." *The Journal of Physiology* **516 (Pt 1)**: 293-301.
Macefield, V. G. and B. G. Wallin (1999). "Respiratory and cardiac modulation of single sympathetic vasoconstrictor and sudomotor neurones to human skin." *The Journal of Physiology* **516 (Pt 1)**: 303-314.
Mackman, N. (2012). "New insights into the mechanisms of venous thrombosis." *J Clin Invest* **122**(7): 2331-2336.
Madden, K. M., C. Lockhart, T. F. Potter and D. Cuff (2010). "Aerobic training restores arterial baroreflex sensitivity in older adults with type 2 diabetes, hypertension, and hypercholesterolemia." *Clin J Sport Med* **20**(4): 312-317.
Maddox, D. (2012). "Effects of venous leg ulceration on patients' quality of life." *Nurs Stand* **26**(38): 42-49.
Maffiuletti, N. A. (2010). "Physiological and methodological considerations for the use of neuromuscular electrical stimulation." *European Journal of Applied Physiology* **110**(2): 223-234.
Maiman, T. H. (1960). "Stimulated Optical Radiation in Ruby." *Nature* **187**(4736): 493-494.
Maiorana, A., G. O'Driscoll, C. Goodman, R. Taylor and D. Green (2002). "Combined aerobic and resistance exercise improves glycemic control and fitness in type 2 diabetes." *Diabetes Res Clin Pract* **56**(2): 115-123.
Majeske, C. (1992). "Reliability of wound surface area measurements." *Physical Therapy* **72**(2): 138-141.

Malhotra, V., S. Singh, O. P. Tandon, S. V. Madhu, A. Prasad and S. B. Sharma (2002). "Effect of Yoga asanas on nerve conduction in type 2 diabetes." *Indian J Physiol Pharmacol* **46**(3): 298-306.
Mallik, A. and A. I. Weir (2005). "Nerve conduction studies: essentials and pitfalls in practice." *Journal of Neurology, Neurosurgery & Psychiatry* **76**(suppl 2): ii23-ii31.
Man, I., G. Lepar, M. Morrissey and J. Cywinski (2003). "Effect of neuromuscular electrical stimulation on foot/ankle volume during standing." *Medicine & Science in Sports & Exercise* **35**(4): 630-634.
Marsden, G., M. Perry, K. Kelley, A. H. Davies and G. Guideline Development (2013). "Diagnosis and management of varicose veins in the legs: summary of NICE guidance." *BMJ* **347**: f4279.
Maselli, R. A., J. B. Jaspan, B. C. Soliven, A. J. Green, J. P. Spire and B. G. Arnason (1989). "Comparison of sympathetic skin response with quantitative sudomotor axon reflex test in diabetic neuropathy." *Muscle Nerve* **12**(5): 420-423.
Maurins, U., B. H. Hoffmann, C. Losch, K. H. Jockel, E. Rabe and F. Pannier (2008). "Distribution and prevalence of reflux in the superficial and deep venous system in the general population--results from the Bonn Vein Study, Germany." *Journal of Vascular Surgery* **48**(3): 680-687.
Mayrovitz, H. N. and L. B. Soontupe (2009). "Wound areas by computerized planimetry of digital images: accuracy and reliability." *Advances in Skin & Wound Care* **22**(5): 222-229.
McCully, K. K., S. Iotti, K. Kendrick, Z. Wang, J. D. Posner, J. Leigh, Jr. and B. Chance (1994). "Simultaneous in vivo measurements of HbO2 saturation and PCr kinetics after exercise in normal humans." *J Appl Physiol (1985)* **77**(1): 5-10.
McDermott, M. M., K. Liu, P. Greenland, J. M. Guralnik, M. H. Criqui, C. Chan, W. H. Pearce, J. R. Schneider, L. Ferrucci, L. Celic, L. M. Taylor, E. Vonesh, G. J. Martin and E. Clark (2004). "Functional decline in peripheral arterial disease: associations with the ankle brachial index and leg symptoms." *Journal of the American Medical Association* **292**(4): 453-461.
MDRTC MNSI tool. Michigan Diabetes Research and Training Center, University of Michigan.
Meijer, W. T., A. W. Hoes, D. Rutgers, M. L. Bots, A. Hofman and D. E. Grobbee (1998). "Peripheral arterial disease in the elderly: The Rotterdam Study." *Arterioscler Thromb Vasc Biol* **18**(2): 185-192.
Meissner, M. H. (2005). "Lower extremity venous anatomy." *Seminars in interventional radiology* **22**(3): 147-156.
Meissner, M. H., G. Moneta, K. Burnand, P. Gloviczki, J. M. Lohr, F. Lurie, M. A. Mattos, R. B. McLafferty, G. Mozes, R. B. Rutherford, F. Padberg and D. S. Sumner (2007). "The hemodynamics and diagnosis of venous disease." *Journal of Vascular Surgery* **46**: 4S-24S.
Mendoza, E. and H. A. Berger (2001). "Provokationsmanöver für die duplex-sonographische Diagnostik der Varikosis." *Gefässchirurgie* **6**(1): 43-46.
Merli, G. J., G. J. Herbison, J. F. Ditunno, H. H. Weitz, J. H. Henzes, C. H. Park, M. Jaweed and J. Heltzel (1988). "Deep vein thrombosis: Prophylaxis in acute spinal cord injured patients." *Archives of Physical Medicine and Rehabilitation* **69**(9): 661-664.
Merli, G. J., G. J. Herbison, J. F. Ditunno, H. H. Weitz, J. H. Henzes, C. H. Park and M. M. Jaweed (1988). "Deep vein thrombosis: prophylaxis in acute spinal cord injured patients." *Arch Phys Med Rehabil* **69**(9): 661-664.
MHRA. Retrieved 04/10/2015, from www.MHRA.gov.uk.
Middlebrooke, A. R., L. M. Elston, K. M. MacLeod, D. M. Mawson, C. I. Ball, A. C. Shore and J. E. Tooke (2006). "Six months of aerobic exercise does not improve microvascular function in type 2 diabetes mellitus." *Diabetologia* **49**(10): 2263-2271.
Miller, B. F., K. G. Gruben and B. J. Morgan (2000). "Circulatory responses to voluntary and electrically induced muscle contractions in humans." *Physical Therapy* **80**(1): 53-60.
Moffatt, C. J., P. J. Franks, D. C. Doherty, R. Smithdale and A. Steptoe (2009). "Psychological factors in leg ulceration: a case-control study." *Br J Dermatol* **161**(4): 750-756.
Moghtaderi, A., A. Bakhshipour and H. Rashidi (2006). "Validation of Michigan neuropathy screening instrument for diabetic peripheral neuropathy." *Clin Neurol Neurosurg* **108**(5): 477-481.

Moharic, M. and H. Burger (2010). "Effect of transcutaneous electrical nerve stimulation on sensation thresholds in patients with painful diabetic neuropathy: an observational study." *Int J Rehabil Res* **33**(3): 211-217.

Montori, V. M., S. J. Kavros, E. E. Walsh and T. W. Rooke (2002). "Intermittent compression pump for nonhealing wounds in patients with limb ischemia. The Mayo Clinic experience (1998-2000)." *International Angiology* **21**(4): 360-366.

Moore, H. M., T. R. Lane, A. Thapar, I. J. Franklin and A. H. Davies (2013). "The European burden of primary varicose veins." *Phlebology* **28 Suppl 1**: 141-147.

Morgan, R. H., G. Carolan, J. V. Psaila, A. M. N. Gardner, R. H. Fox and J. P. Woodcock (1991). "Arterial Flow Enhancement by Impulse Compression." *Vascular Surgery* **25**(1): 8-16.

Morgan, R. H., J. V. Psaila, J. Stone, G. Carolan and J. P. Woodcock (1991). "Effect of postural change on common femoral artery volume flow, measured by duplex ultrasound, in normal subjects and patients with peripheral vascular disease." *Journal of Biomedical Engineering* **13**(3): 244-248.

Morris, R. J. (2008). "Intermittent pneumatic compression - systems and applications." *Journal of Medical Engineering & Technology* **32**(3): 179-188.

Morris, R. J. and J. P. Woodcock (2002). "Effects of supine intermittent compression on arterial inflow to the lower limb." *Archives of surgery* **137**(11): 1269-1273.

Morris, R. J. and J. P. Woodcock (2004). "Evidence-based compression: prevention of stasis and deep vein thrombosis." *Annals of surgery* **239**(2): 162-171.

Morris, R. J. and J. P. Woodcock (2004). "Intermittent venous compression, and the duration of hyperaemia in the common femoral artery." *Clinical Physiology and Functional Imaging* **24**(4): 237-242.

Mortimer, J. T., R. Magnusson and I. Petersen (1970). "Conduction velocity in ischemic muscle: effect on EMG frequency spectrum." *Am J Physiol* **219**(5): 1324-1329.

Morton, R. D., D. J. West, J. W. Stephens, S. C. Bain and R. M. Bracken (2010). "Heart rate prescribed walking training improves cardiorespiratory fitness but not glycaemic control in people with type 2 diabetes." *J Sports Sci* **28**(1): 93-99.

Mosti, G. and H. Partsch (2013). "Occupational leg oedema is more reduced by antigraduated than by graduated stockings." *European Journal of Vascular and Endovascular Surgery* **45**(5): 523-527.

Moxey, P. W., J. Brownrigg, S. S. Kumar, G. Crate, P. J. Holt, M. M. Thompson, K. G. Jones and R. J. Hinchliffe (2013). "The BASIL survival prediction model in patients with peripheral arterial disease undergoing revascularization in a university hospital setting and comparison with the FINNVASC and modified PREVENT scores." *Journal of Vascular Surgery* **57**(1): 1-7.

Murabito, J. M., J. C. Evans, M. G. Larson, K. Nieto, D. Levy, P. W. Wilson and S. Framingham (2003). "The ankle-brachial index in the elderly and risk of stroke, coronary disease, and death: the Framingham Study." *Arch Intern Med* **163**(16): 1939-1942.

Murray, J. "Success of Dr Murray's exhausted air-bath in cholera." *London Medical and Surgical Journal* **1**: 806.

Musselman, D. L., E. Betan, H. Larsen and L. S. Phillips (2003). "Relationship of depression to diabetes types 1 and 2: epidemiology, biology, and treatment." *Biol Psychiatry* **54**(3): 317-329.

Musselman, D. L., D. L. Evans and C. B. Nemeroff (1998). "The relationship of depression to cardiovascular disease: Epidemiology, biology, and treatment." *Archives of General Psychiatry* **55**(7): 580-592.

Nadland, I. H., L. Walloe and K. Toska (2009). "Effect of the leg muscle pump on the rise in muscle perfusion during muscle work in humans." *European Journal of Applied Physiology* **105**(6): 829-841.

Nakayama, K. and H. Furuhata (1974). "Necessary Requirements for Blood Flowmetering by Ultrasonic Doppler Method." *Electronics & Communications in Japan* **57**(12): 85-92.

Narayana, P. A., J. Ophir and N. F. Maklad (1984). "The attenuation of ultrasound in biological fluids." *J Acoust Soc Am* **76**(1): 1-4.

Navarro, T. P., K. T. Delis and A. P. Ribeiro (2002). "Clinical and hemodynamic significance of the greater saphenous vein diameter in chronic venous insufficiency." *Archives of surgery* **137**(11): 1233-1237.

Neglen, P., I. I. o. t. P. V. S. Writing Group, B. Eklof, A. Kulwicki, A. Davies, T. Deschamps, M. Garcia, P. Gloviczki, N. Labropoulos, A. Nicolaides, H. Partsch, M. Perrin, E. Rabe and C. Wittens (2010). "Prevention and treatment of venous ulcers in primary chronic venous insufficiency." *Journal of Vascular Surgery* **52**(5 Suppl): 15S-20S.

Nelson, E. A., R. Mani, K. Thomas and K. Vowden (2011). "Intermittent pneumatic compression for treating venous leg ulcers." *Cochrane Database of Systematic Reviews*(2): CD001899-CD001899.

Neville, C. M., M. Schmidt and J. Schmidt (1992). "Response of myogenic determination factors to cessation and resumption of electrical activity in skeletal muscle: a possible role for myogenin in denervation supersensitivity." *Cell Mol Neurobiol* **12**(6): 511-527.

Newson, T. P., A. Obeid, R. S. Wolton, D. Boggett and P. Rolfe (1987). "Laser Doppler Velocimetry - the Problem of Fiber Movement Artifact." *Journal of Biomedical Engineering* **9**(2): 169-172.

Niakan, E. and Y. Harati (1988). "Sympathetic skin response in diabetic peripheral neuropathy." *Muscle Nerve* **11**(3): 261-264.

NICE (2013). Varicose veins: diagnosis and management. London. **CG168**.

NICE (2014). NICE quality standard QS52. Lower limb peripheral artery disease (NICE clinical guideline 147).

Nicolaides, A. N., E. Cardiovascular Disease, T. Research, S. European Society of Vascular, T. I. A. S. A. C. Organization, A. International Union of and C. Union Internationale de Phlebologie at the Abbaye des Vaux de (2000). "Investigation of chronic venous insufficiency: A consensus statement (France, March 5-9, 1997)." *Circulation* **102**(20): E126-163.

Nicolaides, A. N., V. V. Kakkar, E. S. Field and P. Fish (1972). "Optimal electrical stimulus for prevention of deep vein thrombosis." *Br Med J* **3**(5829): 756-758.

Nicolaides, A. N., V. V. Kakkar, E. S. Field and J. T. Renney (1971). "The origin of deep vein thrombosis: a venographic study." *The British Journal of Radiology* **44**(525): 653-663.

Nicolaides, A. N., C. Miles, M. Hoare, P. Jury, E. Helmis and R. Venniker (1983). "Intermittent sequential pneumatic compression of the legs and thromboembolism-deterrent stockings in the prevention of postoperative deep venous thrombosis." *Surgery* **94**(1): 21-25.

Nilsson, G. E., T. Tenland and P. A. Oberg (1980). "New Instrument for Continuous Measurement of Tissue Blood-Flow by Light Beating Spectroscopy." *IEEE Transactions on Biomedical Engineering* **27**(1): 12-19.

Nordin, M. and J. Fagius (1995). "Effect of noxious stimulation on sympathetic vasoconstrictor outflow to human muscles." *The Journal of Physiology* **489 (Pt 3)**: 885-894.

O'Donnell, T. F., Jr., D. A. Rosenthal, A. D. Callow and B. L. Ledig (1979). "Effect of elastic compression on venous hemodynamics in postphlebitic limbs." *Journal of the American Medical Association* **242**(25): 2766-2768.

O'Meara, S., N. Cullum, E. A. Nelson and J. C. Dumville (2012). "Compression for venous leg ulcers." *Cochrane Database of Systematic Reviews* **11**: CD000265.

Oates, C. (2001). Cardiovascular Haemodynamics and Doppler Waveforms Explained.

Ochi, M., K. Kohara, Y. Tabara, T. Kido, E. Uetani, N. Ochi, M. Igase and T. Miki (2010). "Arterial stiffness is associated with low thigh muscle mass in middle-aged to elderly men." *Atherosclerosis* **212**(1): 327-332.

Ohnhaus, E. E. and R. Adler (1975). "Methodological problems in the measurement of pain: A comparison between the verbal rating scale and the visual analogue scale." *Pain* **1**(4): 379-384.

Oldfield, M. (2005). "Microvascular filtration capacity is modified by chronic muscle stimulation in ischaemic human limbs without changes in local vascular control (abstract)." *Microcirculation* **12**: 645-689.
ONS (2007). Office of National Statistics. http://www.ons.gov.uk/.
Orme, J. G., J. Reis and E. J. Herz (1986). "Factorial and discriminant validity of the center for epidemiological studies depression (CES-D) scale." *Journal of Clinical Psychology* **42**(1): 28-33.
Ozer, K., S. P. Chesher and L. R. Scheker (2006). "Neuromuscular electrical stimulation and dynamic bracing for the management of upper-extremity spasticity in children with cerebral palsy." *Developmental Medicine and Child Neurology* **48**(7): 559-563.
Padberg Jr, F. T., M. V. Johnston and S. A. Sisto (2004). "Structured exercise improves calf muscle pump function in chronic venous insufficiency: a randomized trial." *Journal of Vascular Surgery* **39**(1): 79-87.
Pambianco, G., T. Orchard and P. Landau (1995). "Deep vein thrombosis: prevention in stroke patients during rehabilitation." *Arch Phys Med Rehabil* **76**(4): 324-330.
Panzram, G. (1987). "Mortality and survival in type 2 (non-insulin-dependent) diabetes mellitus." *Diabetologia* **30**(3): 123-131.
Parisi, A. F., E. D. Folland and P. Hartigan (1992). "A comparison of angioplasty with medical therapy in the treatment of single-vessel coronary artery disease. Veterans Affairs ACME Investigators." *The New England Journal of Medicine* **326**(1): 10-16.
Park, Y. C., A. Esa, T. Sugiyama, S. Kaneko and T. Kurita (1988). "Sympathetic Skin-Response - a New Test to Diagnose Ejaculatory Dysfunction." *Journal of Urology* **139**(3): 539-541.
Partsch, H. (1984). "Do we need firm compression stockings exerting high pressure?" *Vasa* **13**(1): 52-57.
Partsch, H., J. Winiger and B. Lun (2004). "Compression Stockings Reduce Occupational Leg Swelling." *Dermatologic Surgery* **30**(5): 737-743.
Peach, G., M. Griffin, K. G. Jones, M. M. Thompson and R. J. Hinchliffe (2012). "Diagnosis and management of peripheral arterial disease." *BMJ* **345**: e5208.
Peters, E. J., L. A. Lavery, D. G. Armstrong and J. G. Fleischli (2001). "Electric stimulation as an adjunct to heal diabetic foot ulcers: a randomized clinical trial." *Archives of Physical Medicine and Rehabilitation* **82**(6): 721-725.
Peters, E. J. and B. A. Lipsky (2013). "Diagnosis and management of infection in the diabetic foot." *Med Clin North Am* **97**(5): 911-946.
Peters, J. G., D. G. Armstrong, R. P. Wunderlich, J. Bosma, S. Stacpoole-Shea and L. Lavery (1998). "The benefit of electrical stimulation to enhance perfusion in persons with diabetes mellitus." *The Journal of Foot and Ankle Surgery* **37**(5): 396-400.
Petrofsky, J. S., D. Lawson, L. Berk and H. Suh (2010). "Enhanced healing of diabetic foot ulcers using local heat and electrical stimulation for 30 min three times per week." *Journal Of Diabetes* **2**(1): 41-46.
Pette, D. and G. Vrbová (1999). "What does chronic electrical stimulation teach us about muscle plasticity?" *Muscle and Nerve* **22**(6): 666-677.
Phan, T. G. and A. Bullen (2010). "Practical intravital two-photon microscopy for immunological research: faster, brighter, deeper." *Immunol Cell Biol* **88**(4): 438-444.
Piette, J., M. Huchet, A. Duclert, A. Fujisawa-Sehara and J. P. Changeux (1992). "Localization of mRNAs coding for CMD1, myogenin and the alpha-subunit of the acetylcholine receptor during skeletal muscle development in the chicken." *Mech Dev* **37**(1-2): 95-106.
Plassmann, P. and T. D. Jones (1998). "MAVIS: a non-invasive instrument to measure area and volume of wounds. Measurement of Area and Volume Instrument System." *Med Eng Phys* **20**(5): 332-338.
Pollock, A. V. "Calf-muscle stimulation as a prophylactic method against deep vein thrombosis." *Triangle* **16**(1): 41-45.

Pollock, A. V. (1977). "Calf-muscle stimulation as a prophylactic method against deep vein thrombosis." *Triangle* **16**(1): 41-45.
Price, D. D., P. A. McGrath, A. Rafii and B. Buckingham (1983). "The validation of visual analogue scales as ratio scale measures for chronic and experimental pain." *Pain* **17**(1): 45-56.
Radloff, L. S. (1977). "The CES-D Scale: A Self-Report Depression Scale for Research in the General Population." *Applied Psychological Measurement* **1**(3): 385-401.
Raju, S., K. Hollis and P. Neglen (2007). "Use of compression stockings in chronic venous disease: patient compliance and efficacy." *Ann Vasc Surg* **21**(6): 790-795.
Ramaswami, G., M. D'Ayala, L. Hollier, R. Deutsch and A. McElhinney (2005). "Rapid foot and calf compression increases walking distance in patients with intermittent claudication: Results of a randomized study." *Journal of Vascular Surgery* **41**(5): 794-801.
Rasmussen, L. H., M. Lawaetz, L. Bjoern, B. Vennits, A. Blemings and B. Eklof (2011). "Randomized clinical trial comparing endovenous laser ablation, radiofrequency ablation, foam sclerotherapy and surgical stripping for great saphenous varicose veins." *The British Journal of Surgery* **98**(8): 1079-1087.
Reddy, J., K. Wilhelm and L. Campbell (2013). "Putting PAID to Diabetes-Related Distress: The Potential Utility of the Problem Areas in Diabetes (PAID) Scale in Patients with Diabetes." *Psychosomatics* **54**(1): 44-51.
Regensteiner, J. G., M. E. Hargarten, R. B. Rutherford and W. R. Hiatt (1993). "Functional benefits of peripheral vascular bypass surgery for patients with intermittent claudication." *Angiology* **44**(1): 1-10.
Reiber, G. (2001). The Diabetic Foot. St. Louis, Mosby.
Reiber, G., Boyko, E, Smith, D (1995). Lower extremity foot ulcers and amputations in diabetes.
Reichard, P., B. Y. Nilsson and U. Rosenqvist (1993). "The effect of long-term intensified insulin treatment on the development of microvascular complications of diabetes mellitus." *The New England Journal of Medicine* **329**(5): 304-309.
Revitive. Retrieved 02/12/2013, from http://www.revitive.com/does-revitive-work/.
Richardson, J. K., D. Sandman and S. Vela (2001). "A focused exercise regimen improves clinical measures of balance in patients with peripheral neuropathy." *Arch Phys Med Rehabil* **82**(2): 205-209.
Riva, C., B. Ross and G. B. Benedek (1972). "Laser Doppler measurements of blood flow in capillary tubes and retinal arteries." *Invest Ophthalmol* **11**(11): 936-944.
Rizzoli, R., J.-Y. Reginster, J.-F. Arnal, I. Bautmans, C. Beaudart, H. Bischoff-Ferrari, E. Biver, S. Boonen, M.-L. Brandi, A. Chines, C. Cooper, S. Epstein, R. Fielding, B. Goodpaster, J. Kanis, J.-M. Kaufman, A. Laslop, V. Malafarina, L. Mañas, B. Mitlak, R. Oreffo, J. Petermans, K. Reid, Y. Rolland, A. Sayer, Y. Tsouderos, M. Visser and O. Bruyère (2013). "Quality of Life in Sarcopenia and Frailty." *Calcified Tissue International* **93**(2): 101-120.
RM Fish, L. G. (2009). "Conduction of electrical current to and through the human body: a review." *Eplasty* **9**: e44.
Rogers, L. C., N. J. Bevilacqua, D. G. Armstrong and G. Andros (2010). "Digital planimetry results in more accurate wound measurements: a comparison to standard ruler measurements." *J Diabetes Sci Technol* **4**(4): 799-802.
Rosenberg, I. L., M. Evans and A. V. Pollock (1975). "Prophylaxis of postoperative leg vein thrombosis by low dose subcutaneous heparin or peroperative calf muscle stimulation: a controlled clinical trial." *Br Med J* **1**(5959): 649-651.
Rosenberg, I. L., M. Evans and A. V. Pollock (1975). "Prophylaxis of postoperative leg vine thrombosis by low dose subcutaneous heparin or peroperative calf muscle stimulation: a controlled clinical trial." *Br Med J* **1**(5959): 649-651.
Rutherford, R. B., F. T. Padberg, Jr., A. J. Comerota, R. L. Kistner, M. H. Meissner and G. L. Moneta (2000). "Venous severity scoring: An adjunct to venous outcome assessment." *Journal of Vascular Surgery* **31**(6): 1307-1312.

Sachdeva, A., M. Dalton, S. V. Amaragiri and T. Lees (2014). "Graduated compression stockings for prevention of deep vein thrombosis." *Cochrane Database of Systematic Reviews* **12**: CD001484.

Scholkmann, F., S. Kleiser, A. J. Metz, R. Zimmermann, J. M. Pavia, U. Wolf and M. Wolf (2014). "A review on continuous wave functional near-infrared spectroscopy and imaging instrumentation and methodology." *NeuroImage* **85**: 6-27.

Seenan, C., P. A. Roche, C. W. Tan and M. T (2012). "Modification of experimental, lower limb ischemic pain with transcutaneous electrical nerve stimulation." *Clin J Pain* **28**(8): 693-699.

SF-36.org. "SF-36 Tool." from http://www.sf-36.org/tools/sf12.shtml.

Shalhoub, J., M. Hamish and A. H. Davies (2009). "Supervised exercise for intermittent claudication - an under-utilised tool." *Ann R Coll Surg Engl* **91**(6): 473-476.

Shammas, N. W. (2007). "Epidemiology, classification, and modifiable risk factors of peripheral arterial disease." *Vasc Health Risk Manag* **3**(2): 229-234.

Shaw, J., C. M. Hughes, K. M. Lagan, P. M. Bell and M. R. Stevenson (2007). "An Evaluation of Three Wound Measurement Techniques in Diabetic Foot Wounds." *Diabetes Care* **30**(10): 2641-2642.

Shetty, R., H. Sreekar, S. Lamba and A. K. Gupta (2012). "A novel and accurate technique of photographic wound measurement." *Indian J Plast Surg* **45**(2): 425-429.

Shiman, M. I., B. Pieper, T. N. Templin, T. J. Birk, A. R. Patel and R. S. Kirsner (2009). "Venous ulcers: A reappraisal analyzing the effects of neuropathy, muscle involvement, and range of motion upon gait and calf muscle function." *Wound Repair and Regeneration* **17**(2): 147-152.

Shimbo, D., K. W. Davidson, D. C. Haas, V. Fuster and J. J. Badimon (2005). "Negative impact of depression on outcomes in patients with coronary artery disease: mechanisms, treatment considerations, and future directions." *Journal of Thrombosis and Haemostasis* **3**(5): 897-908.

Shung, K. K. (1983). "Blood visualization by ultrasonic scanners." *J Clin Ultrasound* **11**(3): A17-18.

Sigvant, B., K. Wiberg-Hedman, D. Bergqvist, O. Rolandsson, B. Andersson, E. Persson and E. Wahlberg (2007). "A population-based study of peripheral arterial disease prevalence with special focus on critical limb ischemia and sex differences." *Journal of Vascular Surgery* **45**(6): 1185-1191.

Simka, M. (2004). "Calf muscle pump dysfunction in the patients with severe chronic venous insufficiency." *Phlebolymphology* **47**: 299-303.

Simka, M. (2007). "Calf muscle pump impairment and delayed healing of venous leg ulcers: Air plethysmographic findings." *Journal of Dermatology* **34**(8): 537-544.

Smith, D. O. and M. R. Chapman (1987). "Acetylcholine receptor binding properties at the rat neuromuscular junction during aging." *J Neurochem* **48**(6): 1834-1841.

Smith, P. C., S. Sarin, J. Hasty and J. H. Scurr (1990). "Sequential gradient pneumatic compression enhances venous ulcer healing: A randomized trial." *Surgery* **108**(5): 871-875.

Snyder, R. J. and K. K. Lanier (2002). "Offloading difficult wounds and conditions in diabetic patient." *Ostomy Wound Management* **48**(1): 22-28, 30, 32-25.

Soliven, B., R. Maselli, J. Jaspan, A. Green, H. Graziano, M. Petersen and J. P. Spire (1987). "Sympathetic skin response in diabetic neuropathy." *Muscle Nerve* **10**(8): 711-716.

Somerville, J. J., G. O. Brow, P. J. Byrne, R. D. Quill and W. G. Fegan (1974). "The effect of elastic stockings on superficial venous pressures in patients with venous insufficiency." *The British Journal of Surgery* **61**(12): 979-981.

Son, Y.-J., J. T. Trachtenberg and W. J. Thompson (1996). "Schwann cells induce and guide sprouting and reinnervation of neuromuscular junctions." *Trends in Neurosciences* **19**(7): 280-285.

Soosainathan, A., H. M. Moore, M. S. Gohel and A. H. Davies (2013). "Scoring systems for the post-thrombotic syndrome." *Journal of Vascular Surgery* **57**(1): 254-261.

Spincemaille, G. H. J. J., H. C. W. D. Vet, D. T. Ubbink and M. J. H. M. Jacobs (2001). "The Results of Spinal Cord Stimulation in Critical Limb Ischaemia : a Review." *European Journal of Vascular and Endovascular Surgery* **20**: 99-105.

Sprigle, S., M. Nemeth and A. Gajjala (2012). "Iterative design and testing of a hand-held, non-contact wound measurement device." *Journal of Tissue Viability* **21**(1): 17-26.

Sritharan, K., T. R. Lane and A. H. Davies (2012). "The burden of depression in patients with symptomatic varicose veins." *European Journal of Vascular and Endovascular Surgery* **43**(4): 480-484.

Stanton, A. W., J. W. Northfield, B. Holroyd, P. S. Mortimer and J. R. Levick (1997). "Validation of an optoelectronic limb volumeter (Perometer)." *Lymphology* **30**(2): 77-97.

Stefanovska, A. and L. Vodovnik (1985). "Change in muscle force following electrical stimulation. Dependence on stimulation waveform and frequency." *Scand J Rehabil Med* **17**(3): 141-146.

Stern, M. D. (1975). "In vivo evaluation of microcirculation by coherent light scattering." *Nature* **254**(5495): 56-58.

Stonebridge, P. A., N. Chalmers, I. Beggs, A. W. Bradbury and C. V. Ruckley (1995). "Recurrent varicose veins: a varicographic analysis leading to a new practical classification." *The British Journal of Surgery* **82**(1): 60-62.

Stratton, I. M., A. I. Adler and H. A. W. Neil (2000). "Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study." *BMJ*(Ukpds 35): 405-412.

Sultan, S., N. Hamada, E. Soylu, A. Fahy, N. Hynes and W. Tawfick (2011). "Sequential compression biomechanical device in patients with critical limb ischemia and nonreconstructible peripheral vascular disease." *Journal of Vascular Surgery* **54**(2): 440-446; discussion 446-447.

Syrjala, K. L. and M. E. Chapko (1995). "Evidence for a biopsychosocial model of cancer treatment-related pain." *Pain* **61**(1): 69-79.

Tan, C.-W., F. Coutts and C. Bulley (2013). "Measurement of lower limb volume: agreement between the vertically oriented perometer and a tape measure method." *Physiotherapy* **99**(3): 247-251.

TASC2 (2007). Diagnosis and treatment of acute limb ischemia. M. Nehler.

Taveggia, G., J. H. Villafañe, F. Vavassori, C. Lecchi, A. Borboni and S. Negrini (2014). "Multimodal Treatment of Distal Sensorimotor Polyneuropathy in Diabetic Patients: A Randomized Clinical Trial." *Journal of Manipulative and Physiological Therapeutics* **37**(4): 242-252.

Taylor, L. M., Jr., J. M. Edwards and J. M. Porter (1990). "Present status of reversed vein bypass grafting: five-year results of a modern series." *Journal of Vascular Surgery* **11**(2): 193-205; discussion 205-196.

Tesfaye, S., N. D. Harris, R. M. Wilson and J. D. Ward (1992). "Exercise-induced conduction velocity increment: a marker of impaired peripheral nerve blood flow in diabetic neuropathy." *Diabetologia*: 155-159.

Tesfaye, S. and D. Selvarajah (2012). "Advances in the epidemiology, pathogenesis and management of diabetic peripheral neuropathy." *Diabetes/Metabolism Research Reviews* **28 Suppl 1**: 8-14.

Thawer, H. A., P. E. Houghton, M. G. Woodbury, D. Keast and K. Campbell (2002). "A comparison of computer-assisted and manual wound size measurement." *Ostomy Wound Management* **48**(10): 46-53.

Thomas, J. L., G. N. Jones, I. C. Scarinci, D. J. Mehan and P. J. Brantley (2001). "The Utility of the Ces-D as a Depression Screening Measure among Low-Income Women Attending Primary Care Clinics." *The International Journal of Psychiatry in Medicine* **31**(1): 25-40.

Toyokura, M. (2012). "Within-subject consistency of sympathetic-skin-response waveform across different modalities of stimulation." *Autonomic Neuroscience* **169**(2): 135-138.

Treat-Jacobson, D., U. G. Bronas and A. S. Leon (2009). "Efficacy of arm-ergometry versus treadmill exercise training to improve walking distance in patients with claudication." *Vascular Medicine* **14**(3): 203-213.

Truchon, M. (2001). "Determinants of chronic disability related to low back pain: towards an integrative biopsychosocial model." *Disabil Rehabil* **23**(17): 758-767.

Tsang, G. M., M. a. Green, a. J. Crow, F. C. Smith, S. Beck, O. Hudlicka and C. P. Shearman (1994). "Chronic muscle stimulation improves ischaemic muscle performance in patients with peripheral vascular disease." *European Journal of Vascular Surgery* **8**(4): 419-422.

Tucker, A. T., A. Maass, D. S. Bain, L. H. H. Chen, M. Azzam, H. Dawson and A. Johnston (2010). "Augmentation of venous, arterial and microvascular blood supply in the leg by isometric neuromuscular stimulation via the peroneal nerve." *The International Journal of Angiology* **19**(1): e31-37.

Tuomilehto, J., Rastenyte, D (1997). Epidemioloy of microvascular disease and hypertension in diabetes mellitus. International textbook of diabetes mellitus. K. Alberti, Zimmet, P, DeFronzo, R, Keen, H.

Turns, M. (2012). "The diabetic foot: an overview for community nurses." *Br J Community Nurs* **17**(9): 422, 424-427, 430-423.

Uhl, J. F., J. P. Benigni, A. Cornu-Thenard, J. Fournier and E. Blin (2015). "Relationship between medical compression and intramuscular pressure as an explanation of a compression paradox." *Phlebology* **30**(5): 331-338.

Uhl, J. F. and C. Gillot (2015). "Anatomy of the veno-muscular pumps of the lower limb." *Phlebology* **30**(3): 180-193.

Unruh, A. M. (1996). "Gender variations in clinical pain experience." *Pain* **65**(2–3): 123-167.

Urbankova, J. (2005). "Intermittent pneumatic compression and deep vein thrombosis prevention." *Thrombosis and Haemostasis* **94**: 1181.

Vaizey, C. J., M. A. Kamm and I. C. Turner (1999). "Effects of short term sacral nerve stimulation on anal and rectal function in patients with anal incontinence." *Gut*: 407-412.

van Beekvelt, M. C. P., W. N. J. C. van Asten and M. T. E. Hopman (2000). "The effect of electrical stimulation on leg muscle pump activity in spinal cord-injured and able-bodied individuals." *European Journal of Applied Physiology* **82**(5-6): 510-516.

van Bemmelen, P. S., M. a. Mattos, K. J. Hodgson, L. D. Barkmeier, D. E. Ramsey, W. E. Faught and D. S. Sumner (1993). "Does air plethysmography correlate with duplex scanning in patients with chronic venous insufficiency?" *Journal of Vascular Surgery* **18**(5): 796-807.

van den Bos, R., L. Arends, M. Kockaert, M. Neumann and T. Nijsten (2009). "Endovenous therapies of lower extremity varicosities: a meta-analysis." *Journal of Vascular Surgery* **49**(1): 230-239.

Varatharajan, L., K. Williams, H. Moore and A. H. Davies (2014). "The effect of footplate neuromuscular electrical stimulation on venous and arterial haemodynamics." *Phlebology*.

Vasquez, M. A. and C. E. Munschauer (2012). "Revised venous clinical severity score: a facile measurement of outcomes in venous disease." *Phlebology* **27 Suppl 1**: 119-129.

Vasquez, M. A., E. Rabe, R. B. McLafferty, C. K. Shortell, W. A. Marston, D. Gillespie, M. H. Meissner and R. B. Rutherford (2010). "Revision of the venous clinical severity score: Venous outcomes consensus statement: Special communication of the American Venous Forum Ad Hoc Outcomes Working Group." *Journal of Vascular Surgery* **52**(5): 1387-1396.

Vella, A., L. A. Carlson, B. Blier, C. Felty, J. D. Kuiper and T. W. Rooke (2000). "Circulator boot therapy alters the natural history of ischemic limb ulceration." *Vascular Medicine* **5**(2): 128.

Velmahos, G. C., P. Petrone, L. S. Chan, S. E. Hanks, C. V. Brown and D. Demetriades "Electrostimulation for the prevention of deep venous thrombosis in patients with major trauma: a prospective randomized study." *Surgery* **137**(5): 493-498.

Velmahos, G. C., P. Petrone, L. S. Chan, S. E. Hanks, C. V. Brown and D. Demetriades (2005). "Electrostimulation for the prevention of deep venous thrombosis in patients with major trauma: a prospective randomized study." *Surgery* **137**(5): 493-498.

Venkataraman, K., H. L. Wee, M. K. Leow, E. S. Tai, J. Lee, S. C. Lim, S. Tavintharan, T. Y. Wong, S. Ma, D. Heng and J. Thumboo (2013). "Associations between complications and health-related quality of life in individuals with diabetes." *Clin Endocrinol (Oxf)* **78**(6): 865-873.

Vetrugno, R., R. Liguori, P. Cortelli and P. Montagna (2003). "Sympathetic skin response: basic mechanisms and clinical applications." *Clin Auton Res* **13**(4): 256-270.

Veves, A., H. J. Murray, M. J. Young and A. J. M. Boulton (1992). "The risk of foot ulceration in diabetic patients with high foot pressure: a prospective study." *Diabetologia* **35**(7): 660-663.

Vinik, A. I. (1999). "Diabetic neuropathy: pathogenesis and therapy." *Am J Med* **107**(2B): 17S-26S.

Vinik, A. I., T. S. Park, K. B. Stansberry and G. L. Pittenger (2000). "Diabetic neuropathies." *Diabetologia* **43**(8): 957-973.

von Elm, E., D. G. Altman, M. Egger, S. J. Pocock, P. C. Gotzsche, J. P. Vandenbroucke and S. Initiative (2007). "The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies." *Bull World Health Organ* **85**(11): 867-872.

Vowden, K. (2001). "The use of intermittent pneumatic compression in venous ulceration." *British Journal of Nursing* **10**(8): 491-509.

Wall, P. D. (1978). "The gate control theory of pain mechanisms. A re-examination and re-statement." *Brain* **101**(1): 1-18.

Ware, J., Jr., M. Kosinski and S. D. Keller (1996). "A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity." *Med Care* **34**(3): 220-233.

Ware, J. E., Jr. and C. D. Sherbourne (1992). "The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection." *Med Care* **30**(6): 473-483.

Warwick, D., K. Dewbury and A. Forrester (2013). "Intermittent pneumatic compression. A comparison of femoral vein velocity with five different devices." *International Angiology* **32**(4): 404-409.

Warwick, D. J. and A. Shaikh (2013). "Neuromuscular electrostimulation via the common peroneal nerve promotes lower limb blood flow in a below-knee cast. A potential for thromboprophylaxis." *Bone and Joint …* **2**(9): 179-185.

Watahiki, Y., M. Baba, M. Matsunaga, K. Takebe and T. Onuma (1989). "Sympathetic skin response in diabetic neuropathy." *Electromyogr Clin Neurophysiol* **29**(3): 155-159.

Welch, G., K. Weinger, B. Anderson and W. H. Polonsky (2003). "Responsiveness of the Problem Areas In Diabetes (PAID) questionnaire." *Diabetic Medicine* **20**(1): 69-72.

Welch, G. W., A. M. Jacobson and W. H. Polonsky (1997). "The Problem Areas in Diabetes Scale: An evaluation of its clinical utility." *Diabetes Care* **20**(5): 760-766.

Welkie, J. F., A. J. Comerota, M. L. Katz, S. C. Aldridge, R. P. Kerr and J. V. White (1992). "Hemodynamic deterioration in chronic venous disease." *Journal of Vascular Surgery* **16**(5): 733-740.

Wernig, A. and A. A. Herrera (1986). "Sprouting and remodelling at the nerve-muscle junction." *Prog Neurobiol* **27**(3): 251-291.

White, J. V. and J. I. Zarge (1996). "The plantar venous plexus and applications of A-V impulse system technology." *International Angiology* **15**(3): 42-50.

White, R. H. (2003). "The epidemiology of venous thromboembolism." *Circulation* **107**(23 Suppl 1): I4-8.

WHO (2013). Diabetes fact sheet No.312.

Wiesinger, G. F., R. Crevenna, M. J. Nuhr, M. Huelsmann, V. Fialka-Moser and M. Quittan (2001). "Neuromuscular electric stimulation in heart transplantation candidates with cardiac pacemakers." *Archives of Physical Medicine and Rehabilitation* **82**(10): 1476-1477.

Wilcott, R. (1962). "Palmar skin sweating vs. palmar skin resistance and skin potential. ." *Journal of Comparative and Physiological Psychology* **55**(3): 327-331.

Williams, K., H. Moore and A. Davies (2015). "Haemodynamic changes with the use of neuromuscular electrical stimulation compared to intermittent pneumatic compression." *Phlebology* **30**(5): 365-372.

Williams, K. J., O. Ayekoloye, H. M. Moore and A. H. Davies (2014). "The calf muscle pump revisited." *Journal of Vascular Surgery: Venous and Lymphatic Disorders*: 1-6.

Williams, K. J. and A. H. Davies (2015). "Comparative accuracy and reliability of 3D cameras in simulated wound measurement (abstract)." *British Journal of Surgery* **102**(S5): 47.

Witzemann, V. and B. Sakmann (1991). "Differential regulation of MyoD and myogenin mRNA levels by nerve induced muscle activity." *FEBS Lett* **282**(2): 259-264.

Wu, C. H. (1984). "Electric Fish and the Discovery of Animal Electricity." *American Scientist* **72**(6): 598-607.

Wycherley, T. P., G. D. Brinkworth, M. Noakes, J. D. Buckley and P. M. Clifton (2008). "Effect of caloric restriction with and without exercise training on oxidative stress and endothelial function in obese subjects with type 2 diabetes." *Diabetes, Obesity and Metabolism* **10**(11): 1062-1073.

Yang, D., Y. K. Vandongen and M. C. Stacey (1999). "Effect of exercise on calf muscle pump function in patients with chronic venous disease." *The British Journal of Surgery* **86**(3): 338-341.

Yoo, M., L. D'Silva, K. Martin, N. Sharma, M. Pasnoor, J. LeMaster and P. Kluding (2015). "Pilot Study of Exercise Therapy on Painful Diabetic Peripheral Neuropathy." *Pain Med* **16**(8): 1482-1489.

Zhang, Q., J. Styf, L. Ekstrom and A. K. Holm (2014). "Effects of electrical nerve stimulation on force generation, oxygenation and blood volume in muscles of the immobilized human leg." *Scandinavian Journal of Clinical and Laboratory Investigation*.

Ziaja, D., P. Kocełak, J. Chudek and K. Ziaja (2011). "Compliance with compression stockings in patients with chronic venous disorders." *Phlebology* **26**(8): 353-360.

Zwierska, I., R. D. Walker, S. A. Choksy, J. S. Male, A. G. Pockley and J. M. Saxton (2005). "Upper- vs lower-limb aerobic exercise rehabilitation in patients with symptomatic peripheral arterial disease: a randomized controlled trial." *Journal of Vascular Surgery* **42**(6): 1122-1130.

10 Appendices

Section	Title	Page
10.1	Tables relating to NMES in venous disease	361
10.2	Tables relating to NMES in peripheral arterial disease	383
10.3	Tables pertaining to Exercise and Electrical Stimulation in Diabetes	390
10.4	Results tables pertaining to small trials and bench testing of NMES devices	397
10.5	Trial subject information sheets	408
10.6	Associated academic activities	442
Error! Reference source not found.	**Error! Reference source not found.**	**Error! Bookmark not defined.**

10.1 Tables relating to NMES in venous disease

Table 47 - Effect of electrical stimulation on venous haemodynamics of healthy individuals

Author	Year	Trial design	Population	Device/ electrodes	Outcome measure	Results
Faghri(Faghri *et al.* 1998)	1998	Case series 3 Exercise protocols: a) Voluntary exercise (VOL) - 10x Tiptoe exercise (standing) b) Electrical stimulation (ES) - Calf muscle stimulation on non-weight bearing leg (standing). c) Combined (ES/VOL) - 10 x Voluntary toe raise + ES Before each protocol, participant relaxed in supine position (5 min) for calibration. Leg elevated to 45°to empty the leg veins (MinVV)	Healthy (n=10)	EMPI Respond Select Dual Channel Neuromuscular Electrical Stimulator Channel 1: Gastrocnemius-soleus stim Channel 2: Tibialis anterior stim 35 pulses per second balanced continuous symmetrical biphasic waveform Electrodes Self-adhesive reusable carbonized skin electrodes	<u>Air plethysmograph</u> Venous volume (VV) Ejected volume (EV) Ejected fraction (EF) Residual volume (RV) Residual volume fraction (RVF) Venous filling index (VFI)	- EV was significantly higher in ES/VOL group (ES/VOL 97.8±37.5ml vs VOL 80.6±31.7ml vs ES 51.7±25.9ml). ES produced up to 60% EV produced by voluntary contraction. No difference between EF after VOL and after ES/VOL. ES induced contraction produced a significantly lower EF than VOL and ES/VOL (60% of voluntary contraction). ES induced exercise produced highest RV and RVF followed by ES/VOL and VOL. There was no significant difference in venous filling index between the groups.
Miller(Miller *et al.* 2000)	2000	Case series Participants studied supine in a temperature controlled laboratory. 2 protocols: a) TENS b) Voluntary exercise Participants asked to mimic electrically evoked contractions to a metronome with a specified duty cycle and an oscilloscope.	Healthy (n=14)	Theratouch 7.7 point stimulator Sinusoidal waveform Carrier frequency 2500Hz Burst frequency 20Hz Duty cycle 4s "on" 4s “off" Electrode: Empi series 9000§ 20.3cm2 electrode - stimulating: over tibial nerve - dispersive: over medial belly of gastrocmenius muscle.	<u>a) Calf blood flow (venous occlusion plethysmography)</u>	a) Mean calf blood flow, calf vascular resistance, mean arterial pressure and heart rate were similar at baseline. b) Calf blood flow (ml/100ml/min): i) Both voluntary contractions and TENS increased calf blood flow (Voluntary: 10.3±0.3 post exercise vs 4.7±0.2 baseline; TENS: 11.1±0.3 post exercise vs 4.5±0.1 baseline;p<0.05). ii) Calf blood flow remained significantly increase at 15s post exercise compared to baseline with TENS protocol only (6.9±0.2 vs 4.5±0.1, p<0.05). d) Calf vascular resistance (arbitrary units) i) Both voluntary contractions and TENS reduced calf vascular resistance (Voluntary: 10.3±0.5 post exercise vs 22.7±1.1 baseline; TENS: 9.1±0.3 post exercise vs 22.8±0.8 baseline; p<0.05). ii)Calf vascular resistance remained significantly decreased at 15s post exercise compared to baseline with TENS protocol only (14.5±0.5 vs 22.8±0.8, p<0.05)

Lyons(IARC 2002)	2002	Case series Participants seated 4 protocols: Group 1: Resting Group 2: NMES with varying parameters: a) 200µs, 25Hz b) 200µs, 35Hz c) 300µs, 25Hz d) 300µs, 35Hz Group 3: GCS Group 4: NMES + GCS Graduated compression stocking (GCS): Medilastex calf length GCS. Ankle pressure 25.2- 32.3mmHg.	Healthy (n=10)	Device BMR NeuroTech NT2000 Pulse width 200-300µs Pulse frequency 25-35Hz Contraction time 2s, relaxation time 6s, ramp up tome 500ms, ramp down time 200ms. Pulse ampltude: 50% maximum amplitude. Electrodes PALS© round 50mm diameter surface electrodes on calf and above achilles tendon.	<u>Doppler ultrasound</u> Popliteal vein a)PVV (cm/s)	a)Resting PVV 12cm/s (range 9-15cm/s) b)NMES: Pulse duration 300µs and pulse frequency of 35Hz produced the greatest increase of 266% (43cm/s vs 12cm/s, $p<0.05$). c) NMES + GCS: All four varations in NMES parameters produced statistically significant higher velocities than with NMES alone ($p<0.001$). Increase in PVV at the highest setting (300µs, 35Hz) in NMES and compression group was 502% vs 266% with NMES alone.
Kaplan(Kaplan *et al.* 2002)	2002	Case series Participants seated in chair for 4hrs. 2 protocols: Group 1: Calf stimulation (n=30) Group 2: foot stimulation (n=19) Limited to 2 visits to toilet, 16 ounces of fluid and a normal lunch. One leg treated (randomly allocated). Contralateral as control.	Healthy (n=49)	FocusTM Neuromuscular Stimulation System Empi 50 pulses per second phase duration 300µs ramp up 2s, ramp down 2s, 12s "on" 48s "off". Intensity to cause muscle twitch. Electrodes Group 1: electrodes over lateral and medial gastrocnemius. Group 2: electrodes over sole of foot.	Femoral and popliteal venous blood flow (m/s) at 0,15,120 and 240 min.	a)Calf stimulation: Significant increase in femoral ($p<0.035$) and popliteal ($p<0.003$) blood flow compared to control. b) Foot stimulation: Significant increase in femoral ($p<0.0001$) and popliteal ($p<0.009$) blood flow compared to control. c) No significant difference in magnitude of blood flow effects between calf and foot stimulation for either femoral or popliteal blood flow.

Broderick(Broderick *et al*. 2008)	2008	Case series 6 Protocols: a) NMES1:Flexor digitorum brevis b) NMES2: Abductor hallucis c) WB: Weight bearing exercise (20s off, 5s on) d) FTC: Fast toe curls (10 toe curls, one per second, 10s relaxation) e) STC: Slow toe curls (toes stretched out fully before curled for 10s, with 10s relaxation) f) TS: Toe spreads (spread and close toes, 10s relaxation)	Healthy (n=4)	Duo-STIM Pulse width 350µs Interpulse interval 100µs Pulse frequency 36Hz Ramp up time 750ms Extension time 1000ms Rampdown time 750ms Electrodes - Two 3.8cm round PALS ® Ultraflex electrodes.	Doppler ultrasound Posterior tibial vein a) Peak venous velocity (PVV) b) Volume of blood expelled (Vol) Popliteal vein a) Peak venous velocity (PVV) b) Volume of blood expelled (Vol)	a) Posterior tibial vein PVV i) PVV for WB (57±6cm/s), FTC (40±14 cm/s) and STC (48±35 cm/s) were not significantly different ii) PVV for WB, FTC and STC were significantly higher than for toe spreads, NMES1 and NMES 2. b) Popliteal vein PVV i) No significant difference between WB (88±26 cm/s), FTC (63±13 cm/s), STC 62±21 cm/s and TS 47±60cm/s. c) Posterior tibial vein volume of blood expelled: i) was significnatly higher with FTC (2.11 ±0.8ml) vs other protocols ii) NMES 1 and 2 reported one and no reading respectively. d) Popliteal vein volume of blood expelled: i) FTC (59 ±25 ml) produced largest volumes followed by WB. ii) NMES1 and 2 reported one and no reading respectively.
Corley(Corley *et al*. 2008)	2008	Case series 7 day trial 3x 40 minute sessions daily for 6 days, 1 session on 7th day. Participants wore Grade 2 knee length compression stockings (Scholl consumer products) over electrodes and leads on right leg. Patients scanned in seated position.	healthy (n=2)	Duo-STIM trapezoidal symmetric biphasic burst Pulse width 350µs Pulse interval 100µs Frequency 36Hz Ramp up time 1s, ramp down time 1s. 25s rest between contractions. Electrodes Two 3.8cm round PALS® Ultraflex electrodes	Doppler ultrasound a) Popliteal vein: i) Peak venous velocity (PVV) ii) Venous volume flow	Venous hemodynamics a) PVV and venous volume decreased in first two days and increased over the second half of the week, likely due to decrease in tolerable stimulation intensity or poor electrode placement.
Green(Green DA 2008)	2008	Case series PRE: Resting measurements over 5min STASIS: Remain seated over 40 min STIM: Foot stimulation for 10 min	Healthy (n=18)	Device CIRCULATION BOOSTERTM Stimulator intensity set to 50% pain threshold.	Ultrasound Femoral peak venous velocity	a) Femoral peak venous velocity caused by 10% MVC (59.2±4.3;p=0.000) and Stim (32.0±5.0;p=0.000) were significantly greater than PRE 7.0±0.6cm/s. b) Peak venous velocity difference between PRE and 10%MVC was significantly greater than STIM (p=0.001).

Broderick(Broderick *et al.* 2009)	2009	Case series 2 Protocols: a) Resting 4 hour bed rest b) Electrical Stimulation (ES) Electrical stimulation whilst having bed rest	Healthy (n=10)	Device DuoSTIM 30s rest between contractions Pulse width 350µs Interpulse interval 100µs Frequency 36Hz Contraction time 1.2s Ramp up time 500ms ramp down time 300ms 5x5cm PALS electrodes over motor points of calf muscles.	Ultrasound Popliteal vein a) Volume flow b) Peak venous velocity	a) Volume flow: - reduced by 37% after 2 hours ($p<0.01$) and by 45% ($p<0.01$) at 4hr. - was ~211% greater in the stimulation group compared to resting ($p<0.001$). b) Peak Venous Velocity: - was greater by ~573% compared to resting ($p<0.001$)
Corley(Corley *et al.* 2009)	2009	Case series One week long trial 3x 30min stimulation daily Class 2 graduated compression hosiery (Scholl consumer products, UK) worn over electrodes and lead wires continuously for 7 days.	Healthy (n=14)	Device Duo-STIM Pulse width 350µs Interpulse interval 100µs Frequency 36 Hz Ramp up, contraction, ramp down 1s Relaxation time 12s Electrodes Two round 5cm diameter PALS® electrodes over calf muscles.	Ultaasound a) Popliteal vein peak velocity	a) Popliteal venous peak velocity: i) due to NMES (17.39±0.84cm/s) significantly higher than resting (7±0.16 cm/s), $p<0.001$) ii) induced by NMES correlated strongly with plantar flexion force ($r=0.67$; $p<0.001$)
Griffin(Griffin *et al.* 2010)	2010	Case series Participants positioned in semi-recumbent position. 12 Different rates of stimulation applied: 2, 3, 4, 5, 6, 8, 12, 15, 20, 30, 60, 120 stimulations per minute A 3-5 min interval was allowed between different stimulations. Each measurements repeated x3. Limb to be stimulated determined randomly.	Healthy (n=24)	Device VEINOPLUS® Bursts of square wave 50ml duration Adjustable voltage 0-5 VRMS Maximum stimulation energy per phase range 3-24 microCoulombs (µC). Electrodes - VeinoPack 5x13cm to posterior aspect of calf.	Ultrasound a) Popliteal vein i) peak systolic velocity (PSV) ii) vein diameter iii) Time averaged mean velocity (TAMV) b) Ejected volume during single stimulus (ml)= volume flow x ejection time	a) Mean popliteal vein peak systolic volume (PSV): - PSV increased by a factor of 10 (96-105cm/s) between stimulations of 2-8 per minute but decreased to 355cm/s at stimulation of 120 per minute. b) Ejected volume per stimulus decreased in a similar fashion: Ejected volume at 2 stimuli per minute was 19x higher than 120 stimuli per minute. c) Ejected volume per minute increased 12 times from 20ml/min (2 stimuli per minute) to 240ml/min (120 stimuli per minute)

Izumi(Izumi *et al.*)	2010	Case series Participant prone on table. 4 protocols: a) Thromboprophylactic transcutaneous electrical nerve stimulation (TpTENS) b) Electrical Muscle Stimulation (EMS) c) Intermittent Pneumatic Compression (IPC) - FLOWTRON DVT AC500 - Compression pressure 40mmHg - Inflation / deflation cyce of 1 min d) Active ankle dorsiflexion e) Muscle squeeze (30mmHg)	Healthy (n=10)	Device SEN-5201 NIHON KOHDEN, Tokyo, Japan Square wave pulse Pulse duration 500ms Intensity 100V Electrodes a) TpTENS Rate 10Hz Electrode placement: Left common peroneal nerve b) EMS Rate 50Hz Electrode placement: motor point tibialis anterior.	Ultrasound Popliteal vein a) Peak venous velocity (PVV) b) Mean venous velocity (MVV) c) Venous Flow Volume (VF)	a) Median Peak Venos Velocity: i) was at least 2.5x higher in all groups compared to baseline ($p<0.01$). ii) was significantly higher in TpTENS (102 cm/s) compared to IPC (65cm/s) ($p=0.022$) b) Median venous flow volume (Range): i) was greater than baseline by 2.1-2.7x in all groups ($p<0.01$). ii) was not significantly different between the 5 groups.
Czyny(Czyrny *et al.* 2010)	2010	Case series 2 Protocols, 48 hrs between sessions: a) Electrical stimulation b)Intermittent Pneumatic Compression (IPC) Knee high Tyco Healthcare Kendall Novamedix A-V Impulse system 130mmHg impulse pressure with 3s impulse duration. Participants seated for 4 hrs. Limited to 2 visits to toilet, 16 ounces of fluid and a normal lunch. One leg treated (randomly allocated).	Healthy (n=40)	Device Focus Neuromuscular Stimulation System Biphasic square wave Phase duration 300µs. Ramp up 2s, ramp down 2s. Frequency 50Hz Stimulation cycle 12s on 48s off per minute. Intensity increased to cause muscle twitch. Electrodes: Surface electrodes on plantar aspect of foot.	Ultrasound Popliteal and femoral peak venous velocities at 0, 15, 120 and 240 minutes. a) Primary analysis: paired t-test accounting for non-inferiority index of 5.0cm/s b) Secondary analysis: Accounts baseline differences using mixed model method.	a) Primary analysis: Electrical stimulation is non-inferior (<5cm/s) to IPC, achieving non-inferior flow values at 120 minutes for the femoral vein ($p=0.005$) and 120min ($p=0.004$) and 240min ($p=0.014$) for the popliteal vein. b) Secondary analysis: Electrical stimulation is non-inferior to IPC when measured at the femoral vein (time=120 minutes, $p=0.08$) and popliteal vein (time =120 min, $p=0.18$; time=240 min, $p=0.043$). c) Treatments were uncomfortable: 92.5% IPC and 82.5% of electrical foot stimulation. d) 62.5% found IPC more comfortable than ES.

Tucker(Tucker *et al*. 2010)	2010	case series 4 hour experiment in a temperature and humidity controlled environment Participants seated in economy airline seat position. One limb received electrical stimulation. Contralateral limb acts as control.	Healthy (n=30)	Device not specified Pulse width 200µs. Pulse amplitude varied between 1mA to 40mA. Frequency varied from 1-5 Hz. 15 programmes administered sequentially. Each programme lasts 5 minutes, followed by 5 minutes response recording and 5 minutes of recovery. Electrodes Over common peroneal nerve	Photoplethysmograph (PPG) a) venous emptying Strain gauge plethysmograph (SPG) b) amplitude of cyclic change in calf circumference Laser Doppler Fluxmetry c) Microcirculatory flux d) Tissue oxygen tension Ultrasound Femoral vein e) Venous volume flow f) Peak venous velocity	a) higher venous emptying at higher amplitude (40mA) and frequency(5Hz) (p=0.0004, r2=0.56) b) Stimulation frequency a significant positive effect on SPG amplitude (p<0.001, r2= 0.84). - Stimulation caused a 55-70% change in calf circumference produced by voluntary dorsiflexion. c) Both frequency and current significantly increased microcirculatory flux (p<0.01) with a strongly positive correlation (r2=0.86) with frequency. -There was a significant increase in temperature in the stimulated versus unstimulated leg. d) Decrease in tissue oxygen tension over 4 hours in both limbs, but tissue oxygenation was significantly higher in the stimulated leg (p=0.03) e) Venous volume flow: All stimulation programmes increased venous volume flow versus baseline (p<0.01), with a positive correlation with amplitude (r2=0.55) and frequency (r2=0.82). f) Peak Venous Velocity (PVV): Increase significantly with all programmes. Positive correlation seen with frequency (r2= 0.72) and amplitude (r2=0.74).
Broderick (Broderick *et al*. 2010)	2010	Case series Random sequence of 6 exercises 1) standing weight bearing 2) standing toe curls 3) NMES foot 4) Intermittent pneumatic compression on the foot. Novamedix AV Impulse system; Inflation every 20s, inflation time 3s, inflation pressure 200mmHg 5) Supine weight bearing 6) Supine toe curls 3 reps of each exercise, lasting 3s each	Healthy (n=10)	DuoSTIM Pulse width 350µs Interpulse interval 100µs Frequency 36 Hz, Delay time 20s (rest period) Ramp up tome 500ms, contraction time 1s, ramp down time 500ms. Two neurostimulation PALS electrodes; active electrode over posterior tibial nerve (5x5cm), reference electrode over metatarsal heads (5x10cm).	Ultrasound Popliteal, posterior tibial and peroneal veins Parameters: a) peak venous velocity (PVV) b) intensity weighted mean velocity (IWMV) c) blood vessel cross sectional area d) EVV= cross sectional area x mean velocity x duration	Upright position: a) EVV was highest during weight bearing and toe curls. b) Lowest EVV was in NMES, which was not significantly different from resting. Supine position: a) EVV was highest during weight bearing and toe curls. b) Lowest EVV was in NMES, which was not significantly different from resting . Ejected venous volume was ~78% higher when upright vs supine. Vessel diameter was 25% larger when standing vs supine.

Broderick (Broderick *et al.* 2010)	2010	Case series Two protocols: a) Resting: 4h bed rest (n=10) b) Electrical Stimulation Group 1 (S1, n=5) stimulation applied alternately to each leg with 30s rest between contractions Group 2 (S2, n=5) stimulation applied to one leg only with 60s rest between contractions.	Healthy (n=10)	DuoSTIM Pulse width 350µs Interpulse interval 100µs Frequency 36 Hz, Contraction time 1.2s, ramp up time 400ms, ramp down time 300ms. 5x5cm PALS self adhesive, skin surface electrodes over motor points of calf muscle.	Ultrasound Popliteal vein a) peak venous velocity (PVV) b) Volume flow (VF)	Effect of resting a) Peak venous velocity (PVV): - no difference in resting PVV between 0, 2 and 4 hours. b) Volume flow (VF): -Resting VF decreased by 43% and 47% at 2hr and 4hr, respectively. Effect of electrical stimulation: a) increased PVV by 650% vs resting ($p<0.001$). b) increased volume flow by 301% vs resting ($p<0.001$) Effect on contralateral limb: a) no change in volume flow ($p=0.39$) over time, but volume flow in contralateral limb of stimulation group significantly different from resting group ($p<0.05$)
Corley(Corley *et al.* 2012)	2012	Randomised control vs NMES. 7 day protocol. Intervention group: Self-administered 3x 30 minute sessions daily for 6 days. Minimum 2hr rest period between each session. One of three daily sessions was supervise in laboratory setting. Control group: Did not receive electrical stimulation. Class 2 Graduated Compression Stockings (Scholl Consumer Products) worn 24 hours a day over lead wires in both groups.	Healthy (n= 24) (10 control, 14 stim)	DuoSTIM balanced biphasic waveform pulse width 350µs interpulse interval 100µs frequency 36 Hz Ramp up 1s, on 1s, ramp down 1s, off time 12s Two round 5cm diameter neurostimulation electrodes (PALS®, Axelgaard Manufacturing) over soleus motor points on right calf.	Ultrasound Popliteal vein: a) PVV b) EVV= CSVA x TAMV pre, during and post ES. Measurements taken 3x with 1 min break in between.	a) Baseline: No difference in popliteal PVV and EVV between groups. b) Peak Venous velocity (PVV): -did not change in control and NMES groups at baseline vs 30 min -resting PVV did not change significantly from baseline in both groups over 7 days. -increased significantly in NMES group (92±106%) vs control (39±40%). c) Ejected venous volume (EVV): - did not change significantly in either group during resting and NMES. - resting EVV did not change significantly from baseline in either group over 7 days. - increased significantly due to NMES from baseline (100±122%) vs control (27±28%).

Table 48 - Venous Haemodynamics in perioperative patients

Author	Year	Trial design	Population	Device/ electrodes	Outcome measure	Results
Doran(Doran *et al.* 1964)	1964	Case series Contralateral leg as control	Peri-operative patients (n=50)	Device not specified 30 galvanic shocks per minute Pulse width 500ms	Radioactive Na24Cl a) venous return (VR)	a) 29 results reliable (19 technical difficulties, 2 blocked catheters intraoperatively). b) In the control group, intraoperative slowing of VR in 26/33 patients: - Time of venous return x2 (12/26), x3-4 (7/26), x5 (2/26) c) Inpatients receiving ES (n=29), a variable result was found: -VR increased (n=10), remained same (n=6), and decreased (n=13).
Nicolaides(Nicolaides *et al.* 1972)	1972	Two part trial Part A: Case series to identify optimum electrical stimulation (n=10) Part B: DVT prophylaxis	Peri-operative patients (n=116)	Thromboprophylactor (Stanley Cox medical equipment) 50ms square wave every 5s Every alternate wave reveresed polarity Intensity adjusted to produce brisk plantar flexion. Two 6x15cm pads on upper and lower part of posterior calf of randomly selected leg.	Doptone (doppler blood flow detector) a) Change in femoral venous velocity 125I-fibrinogen test (pre and post op day 1 and alternate days up to day 10 postop). b) DVT	Part A: a) Stimulation duration: i)< 50ms duration required higher intensity to achieve same increase in femoral vein velocity. ii) A stimulation of 100ms produced 2 muscle contractions. iii) The optimal stimulation duratoin was 50ms. b) Resting period <4s did not allow soleal veins to fill completely before stimulation and resulted in progressive reduction stroke volume.

Velmahos(Velmahos *et al*. 2005)	2005	Randomised control trial 2 groups: Group A: Electrical muscle stimulation (EMS) (n=30) 2x30 minutes treatment a day for minimum 7 days maximum 14 days. Group B: Control (n=30) Standard prophylaxis (heparin / sequential compression device) after contraindication for use was no longer present.	Major trauma patients (ISS>9) not suitable for heparin (n=60)	Lymphavision (Bexley trading, San Rafael Calif) Voltage 0-120V 3ms stimulus at frequency 1.75Hz (105/minute) Inversion polarity every 5secs. Electrodes Calf and medial thigh of both extremeties	Ultrasound (baseline) Venous velocity (cm/min) and max venous diameter a) popliteal vein b) superficial femoral vein c) common femoral vein d) external iliac vein	4 EMS and 9 control were excluded due to lack of outcome evaluation. b) Secondary outcome: i) venous velocity increased significantly compared to baseline in EMS and control groups. ii) There was no significant difference in venous velocity and diameter in EMS group and control group except for the measurements made at left superficial femoral and popliteal veins at 20 minutes.
Broderick (Broderick *et al*. 2011)	2010	Case serie	3/52 Post operative total hip arthroplasty (n=10) and total knee arthroplasty (n=10).	Device Duo-STIM Pulse width 350µs Frequency 36 Hz, Ramp up time 500ms, "on" time 1s, ramp down time 500ms Electrodes Four 5x5cm PALS UltraStim, two applied over each calf muscle.	Ultrasound Popliteal vein: a) peak venous velocity (PVV) b) mean venous velocity (MVV) c) Volume flow	Peak venous velocity(PVV) a) no significant difference between PVV of patients post THA and post TKA b) Almost 200% increase in PVV with stimulation compared to resting ($p<0.001$) Mean venous velocity (MVV) a) no significant difference in MVV between patients post THA and post KA b) Approximately 60% increase MVV with stimulation compared to resting (($p<0.001$) Volume flow a) increased by 60% compared to resting.

Broderick(Broderick *et al*. 2011)	2011	Case series Stimulation for 4 hours.	Day 1 postop total hip arthroplasty (n=5)	Duo-STIM Biphasic Square Wave Pulse width 350µs Interpulse inteval 100µs Frequency 36Hz Contraction time 1s, ramp up 500ms, ramp down 500ms. Stimulation applied to alternate leg every 30s. Electrodes Two 5x5cm PALS UltraStim applied to motor points of soleus muscles of both legs.	Ultrasound Popliteal vein a) Peak venous velocity b) vein diameter c) Volume flow	a) blood flow measurements were successfully measured in 4 out of 5 patients. b) At the end of treatment, stimulated peak venous velocities were significantly higher than resting ($p<0.05$). c) At the end of treatment, stimulated volume flow was not significantly different from resting ($p=0.19$).
Broderick (Broderick *et al*. 2013)	2013	Case series Stimulation for 4 hours.	Day 1 postop total hip arthroplasty (n=11)	Duo-STIM Biphasic Square Wave Pulse width 350µs Interpulse inteval 100µs Frequency 36Hz Contraction time 1s, ramp up time 500ms, ramp down time 500ms. Stimulation applied alternatively to each leg every 30s. Electrodes Two 5x5cm PALS UltraStim applied to each calf muscle.	Ultrasound Popliteal vein a) Peak venous velocity b) Vein diameter c) Time averaged mean velocity (TAMEAN) iv) Volume flow	a) Peak venous velocity was 99% (12±5.9 vs 22.5±16.8cm/s, $p=0.006$) and 288% (13.8±7.6 vs 43.9±13.7cm/s, $p=0.018$) higher than resting on operated and nonoperated limb respectively. b) Mean venous velocity was 178% (2.3±1.4 cm/s, vs 7±5.7 cm/s, $p=0.003$) and 354% (3.7±2 vs 12.9±4.3 cm/s, $p=0.018$) higher than resting on the operated and non-operated limb respectively. c) Volume flow was 159% (78.7±61.1 vs 230.4±215.2ml/min, $p=0.003$) and 614% (112.6±69.5 vs 457.6±215ml/min, $p=0.018$) higher than resting in the operated and non-operated limb, respectively. d) No significant difference in NMES voltage applied to operated and unoperated limb (39±12.3V versus 40.5±12.5V, $p=0.68$)

Table 49 - Venous haemodynamics in patients with chronic venous disease

Author	Year	Trial design	Participants	Device/ electrodes	Outcome measure	Results
Clarke-Moloney(Clarke Moloney *et al.* 2006)	2006	Case series 6 Groups: Test 1: Standing Test 2: Voluntary contraction Patient changed from standing to 'tip toe' position Test 3: Standing with NMES applied Test 4: Standing with* compression bandaging. Test 5: Voluntary contraction with *compression bandaging. Test 6: Standing with *compression bandaging and NMES * 4 layer compression bandaging (ProforeTM)	CEAP 6 (n=10)	BMR NeuroTech NT2000TM Pulse duration 300µs Pulse frequency 35Hz Contraction time 2s Relaxation time 6s Ramp up time 0.5s, ramp down time 0.2s Electrodes Two 5cm diameter neurostimulation hypoallergenic skin surface electrodes (PALS® Nidd Valley Medical) applied to calf muscles	Ultrasound Popliteal vein a) peak venous velocity	a) Median (range) of peak venous velocities (cm/s) i) Stationary : 7.3 (4.0 - 15) ii) Voluntary contraction : 70 (29-206) ($p<0.005$) iii) Stationary with NMES: 13 (9.4 - 27) ($p<0.005$ vs stationary) iv) Stationary with compression: 5.8 (3.0 - 14) v) Voluntary contraction with compression: 73 (27 - 213) ($p<0.005$) vi) NMES and compression : 15 (7.2 - 49) ($p<0.005$ vs stationary with compression)

Table 50 - The effect of electrical stimulation on oedema

Author	Year	Trial design	Population	Device/ electrodes	Outcome measure	Results
Bogachev(Bogachev *et al.* 2011)	2011	Case series Day 1-10: morning, afternoon and evening Day 11-20: morning and evening Day 21-30: evening only Patients not allowed to wear bandages or compression stockings or oedema curing medicines during trial. Measurements taken at baseline and day 35, to allow for a 5 day washout period.	30 patients with venous oedema 32 limbs 22 limbs C3sEpAspPr 10 limbs C3sEsAspdPr	Device VEINOPLUS® 20 minutes per treatment. Intensity 1-50	a) circumference supramalleolar segment b) refilling time (RT) c) CIVIQ d) Pain	22 patients fully observed schedule. a) Evening oedema : i) disappeared in 59.4% of cases (19 limbs) ii) reduced in 34.4% (11 limbs) iii) remained unchanged in 6.2% cases (2 limbs). iv) Group average circumference was 276.5±1.1mm at baseline, which reduced to 256.2±0.7mm (Δ circumference 20.3mm, p<0.001). b) The refill time increased from 17.3±0.9s to 21.5±1.1s (ΔRT = 4.2s, p<0.001) c) The average severity score reduced from 8.33±1.1 to 3.8±0.9 points. d) The presence of venous pain reduced from 87.5% to 34.5% of patients.
Man(Man *et al.* 2003)	2003	Case series Participants underwent 2 protocols a week apart. a) 30 minutes standing with NMES b) 30 minutes standing without NMES The order of treatment was decided by coin flip.	Healthy (n=20)	P4 Microstim "HEALTHFIT" Low voltage rectangular waveform. 30 minute stumulation pattern with 360 cycles. Each cycle lasts 5s. Interpulse interval 45-125 Hz Pulse duration 60-240µs Average frequency 80Hz (range 45-120 Hz) Stimulation delivered in bursts lasting 1.25s 10cm diameter disks Two electrodes on muscle belly gastrocnemius and tibialis anterior, each.	Foot and ankle volume (ankle volumeter)	Foot and ankle volume: i) No significant difference between mean pretest values between both groups (p=0.253). ii) Significant difference in standing still without NMES group (p<0.001) iii) No significant difference in standing still with NMES group (p=0.185) iv) Percentage change in volume from pretest to post test without NMES was 3.5% and with NMES 0.8%.

Green(Green DA 2008)	2008	Case series PRE: Resting measurements over 5min STASIS: Remain seated over 40 min STIM: Foot stimulation for 10 min	Healthy (n=18)	Device CIRCULATION BOOSTERTM Stimulator intensity set to 50% pain threshold.	a) Limb volume b) Subjective swelling and discomfort	a) Limb volume: i) PRE vs STASIS: Significant swelling was evoked in the foot (378±33 vs 407±40, p=0.006), ankle (323 ±38 vs 333±38; p=0.001) , subcalf (1690±197 vs 1752 ±201, p=0.030) and calf (1323±169 vs 1338±174; p=0.031). ii) STIM vs PRE: No significant difference in swelling in foot (378±33 vs 390±35; p=0.088), subcalf (1690±197 vs 1712±198, p=0.087) and calf (1323±169 vs 1330±172; p=0.142). b) Following STASIS, subjects reported significant swelling and discomfort greater than PRE. This was significantly reduced by STIM (p=0.013).
Broderick (Broderick *et al*. 2010)	2010	Case series Two protocols: a) Resting: 4h bed rest (n=10) b) Electrical Stimulation Group 1 (S1, n=5) stimulation applied alternately to each leg with 30s rest between contractions Group 2 (S2, n=5) Stimulation applied to one leg only with 60s rest between contractions.	Healthy (n=10)	Device DuoSTIM Pulse width 350µs Interpulse interval 100µs Frequency 36 Hz, Contraction time 1.2s, ramp up time 400ms, ramp down time 300ms Electrodes 5x5cm PALS electrodes over motor points of calf muscle.	Figure of 8 tape Ankle thickness at 0 and 4 hrs.	Ankle thickness No difference in ankle thickness observed in both protocols.
Goddard(Goddard *et al*. 2008)	2008	Case series 90 minute protocol 15 min thermal equiilbration 15 min venous assessment (supine) 30 min quiet sitting 30 min sitting with plantar stimulation	Healthy women (n=54)	Juvent Medical Sinusoidal waveform Pulse width 50µm Frequency 45Hz Electrodes Juvent Medical frontal aspect plantar surface of foot.	Air Plethysmography a) Venous filling index b) Calf ejection fraction	a) Of 54 volunteers, 7 demonstrated venous insufficiency. b) Participants reanalysed according to oedematous (increase calf volume during sitting) and non-oedematous group: i) In oedematous group (44% of group): - lower limb pooling increased relatively linearly over the 30 mins of quiet sitting. - commencement of stimulation resulted in a rapid ejection of 3ml of fluid followed by a sustaine decrease in volume at an average rate of 2.7±0.1ml/h. ii) In the nonoedematous group: - there wa a constant decrease in calf volume over 30 minutes of quiet sitting (-8.9±0.2ml/h) - Plantar stimulation accelerated fluid ejection in the first 5 minutes (-36ml/h) followed by an average ejection rate of 5.7±0.1ml/h.

Table 51 - Electrical stimulation as a method of thromboprophylaxis

Author	Year	Trial design	Participants	Device/ electrodes	Outcome measure	Results
Doran(Doran *et al.*	1967	Case series Contralateral leg as control (8andomised)	Intra operative patients (n=200)	Intermittent galvanic current (not described)	a) Clinical signs of DVT	a) DVT affected 8.5% of unstimulated versus 2.5% of stimulated limbs.
Doran(Doran *et al.*	1970	Case series: ES on one leg, 15° elevation of contralateral leg.	Intra-operative patients (n=200)	Not described	a) Clinical signs of DVT	a) DVT affected 3.5% stimulated limbs and 6% elevated limbs
Browse(Browse *et al.* 1970)	1970	Case series Contralateral leg as control (randomised)	Intra-operative patients (n=110)	a) SS Electronics Diagnostic Stimulator Type V MK III, or b) battery operated Medelec TS2 Square wave galvanic current Calf stimulation 2x contraction Pulse width 30ms Voltage: 15V (lightly anaesthetised) or 45V (with neuromuscular block). Two 12x9cm electrodes over posterior calf .	I125-fibrinogen uptake test on 1st, 3rd and 5th postop day. a) DVT	a) Number of limbs stimulated: 61 left legs, 49 right legs b) DVT was present: i) in a total of 25 (23%) patients. ii) bilaterally DVT in 7 (6.4%) patients iii) in 9 (8.2%) stimulated legs and 23 (20.9%) unstimulated leg. c) electrical stimulation does not significantly reduce the incidence of DVT in peri-operative patients.

Nicolaides(Nicolaides *et al*. 1972)	1972	Two part trial Part A: See venous hemodynamics Part B: Non randomised control trial (n=116) a) ES group: intraoperative one leg electrical stimulation (n=60) b) Control group: Routine physiotherapy (n=56)	Intra-operative patients (n=116)	Thromboprophylactor (Stanley Cox medical equipment) 50ms square wave every 5s Every alternate wave reverses polarity. Intensity adjusted to produce brisk plantar flexion Two 6x15cm electrode pads on upper and lower part of posterior calf of randomly selected leg.	125I-fibrinogen test (pre and post op day 1 and alternate days up to day 10 postop). b) DVT	Part B: a) Control group: DVT affected 13 (23%) right and 12 (21%) left legs. b) ES group: DVT affected only 1 (1.6%) stimulated limb, but 9 (15%) unstimulated limbs in this group. c) ARR 21.5% (9.8, 34.2) for stimulated leg versus control group.
de Jode (Dejode *et*	1973	Case series Contralateral leg as control	Intra-operative patients (n=64)	Device As per Browse and Negus and Doran.	I125-fibrinogen uptake test a) DVT	a) Incidence of DVT: 39% b) No significant difference in DVT between stimulated and unstimulated limbs (13% vs 15%)
Becker(Becker *et al*. 1973)	1973	Randomised control trial Three groups: Group A: no prophylaxis (n=35) Group B: ES calf muscle (n=39) Group C: Dextran 70 (n=42) 500ml on induction and 500ml next morning	Intra-operative patients (n=116)	Siemens Therapy Stimulator Galvanic stimulation Pulse width 10ms Frequency 30 per minute Electrodes: Tin plates 10x14cm covered by humid rubber foam sponges. Anodes to tibial nerve, cathodes over alluces tendon.	125I-fibrinogen uptake test Phlebography (not performed in all patients) a) DVT	a) In Group A (control), total incidence of DVT was 31.4% b) In Group B (ES), total incidence of DVT was 5.1% c) In group C (Dextran70), total incidence of DVT was 30.9% d) There was a significantly lower incidence of DVT in patients treated with ES compared to Dextran 70 and controls (p<0.01).

Rosenberg(Rosenberg *et al.* 1975)	1975	Randomised control trial 3 groups: a) Intraoperative bilateral EMS b) Subcutaneous heparin 5000iu 8 hourly for 6 days c) Control : early mobilisation	Intra-operative patients (n= 295)	Thrombophylactor Direct current 50ms duration every 5s Each pulse automatically reversed in polarity One Electrode on upper calf, other above ankle.	125I-fibrinogen uptake test (FUT) a) DVT	a) Twenty-two withdrawals from trial (14 failed to observe protocol, 4 died, 4 heparin alluce after reactionary haemorrhage). b) Incidence of DVT: 118 legs in 84 patients showed positive FUT. c) In patients undergoing bladder, prostate and miscellaneous surgery (n=79): i) Neither EMS nor subcutaneous heparin reduced incidence if DVT compared to control (34.8% and 33.3% vs 34.4%, respectively). d) In patients undergoing laparotomy: i) Heparin significantly reduced incidence of DVT vs control (3.4% vs 43.8%, p<0.001). EMS did not significantly reduce the incidence of DVT vs controls (28% vs 43.8%, p<0.10). e) Both heparin (p<0.001) and EMS (p<0.01) effectively reduced the incidence of DVT in laparotomy for benign disease. f) Only heparin (5%, p<0.001) effectively reduced the risk of DVT in patients with malignant disease (59% in control, 61% in EMS).
Pollock(Pollock 1977)	1977	Randomised control trial 3 groups Group 1: Heparin 8hrly Group 2: Bilateral ES calf Group 3: No thromboprophylaxis (control)	Intra-operative patients	Thromboprophylactor Galvanic current Square waveform reversal polarity Potential difference 45V Pulse duration 30-50ms Frequency 12-30 per minute (5-20Hz)	125I-fibrinogen uptake test (FUT) daily a) DVT	a) Incidence of DVT in benign disease: control 35%, ES 16%, heparin 9% b) Incidence of DVT in malignant disease: control 59%, ES 61%, heparin 5%
Lindstorm(Lindstrom *et al.* 1982)	1982	Randomised control trial a) Control group Standard treatment. Plasma/ whole blood to replace blood loss. b) Stimulation group Intraoperative electrical stimulation c) Dextran 40 (D40) group: 500ml Dextran 40 given preoperatively and days 1 and 3 postoperative.	Intra-operative patients (n=112)	Device not specified. Pulse duration 50ms, Amplitude 40-50mA, 6 impulses per group, Impulse frequency 8 per sec group frequency 8 groups/min. Electrode Two pre-gelled electrodes attached below the knee joint and above the ankle posteriorly.	I125-fibrinogen uptake test (FUT) every alternate day for day 4-6. Phlebography a) DVT b) PE	Incidence PE a) Both EMS (6/37) and D40 (4/35) had a significantly lower incidence of PE compared to control (14/40, p<0.05). Incidence DVT a) There was a no significant difference in the incidence of DVT between the control (12/40), EMS (5/37) and D40 (7/35) groups. b) Incidence of DVT in patients with malignant disease was 15% in the EMS group and 54% in the control group.

Nicolaides (Nicolaides *et al*. 1983)	1983	Randomised control trial Patients risk stratified prior to randomisation 3 groups Group A: Intraoperative electrical stimulation Group B: Heparin 5000iu subcutaneous heparin 12hrly Group C: Intermittent sequential compression (ISC) and Thrombo-embolic deterrent (TED) stockings Calf and thigh compression - 11s compression, decompression every 71s	Intra-operative patients (n=150)	Device Powley Doran Electronic Gaiter (Downs Surgical Ltd) Galvanic stimulus 12 contractions per minute Electrode to gastrocnemius and soleus muscles	I125 fibrinogen uptake test (FUT) preoperatively, day 1 postop and alternate days. a) DVT	Incidence DVT Heparin and ISC/TED significantly reduced the risk of DVT in perioperative patients compared to electrical stimulation. (ES 18% vs Heparin 9% vs ISC/TED: 4%)
Bostrom(Bostrom *et al*. 1986)	1986	Randomised control trial Group A: Heparin (n=65) Heparin 5000u x2 s/c Group B: ES (calf) + dextran70 (n=57) Intraoperative calf muscle stimulation 500ml Dextran70 on days 1,3,5 and 7	Neurosurgical patients (n=122)	Device not specified Stimulus strength 40-50mA Pulse duration 50ms Impulse per group: 6 Impulse frequency within groups: 8 per second Group frequency: 8 per minute	I125fibrinogen uptake test day 5-8 postoperatively. Phlebography performed to confirm suspected DVT. a) DVT	a) Eighty nine patients completed screening for postoperative DVT. b) In group A, 5 patients developed DVT (10%) c) In group B, 5 patients developed DVT (13%) - One bilateral DVT d) Both methods reduce the incidence of postoperative DVT and can be used with safety.
Merli (Merli *et al*. 1988)	1988	Randomised control trial 3 groups Group A: Placebo (n=17) Group B: Heparin (n=19) 5000iu subcutaneous heparin Group C: Heparin + ES	Acute (<2/52) spinal injury patients	Device Myocare Tm (no 6280) Pulse duration 50µs Frequency 10Hz Contraction 4s, rest 8 sec, 23 hours per day	I125 fibrinogen uptake test (FUT) Venography if positive a) DVT	a) 34 pts excluded as had DVT on admission b) DVT was present in 8/17 (47%) Group A, 8/16 (50%) Group B and 1/15 (6.7%) Group C. c)Combination of low-dose heparin with EMS significantly decreased the incidence of DVT -ARR 43.3% (11.4, 66.0) compared to heparin alone. ARR 40.4%(9.2,63.0) for EMS and heparin compared to placebo

Pambianco(Pambianco *et al.* 1995)	1995	Randomised control trial 4 groups: Group A: Electrical stimulation (n=8) Group B: Heparin (n=120) 5000iu subcutaneous sodium heparin Group C: Intermittent Pneumatic compression (IPC) (n=117) 8 hours per night Group D: Control (n=115)	Partially ambulatory stroke rehabilitation patients (n=508)	Mederomic Functional Electrical Stimulation Device Current 35-45mA Electrode 4 electrodes to lower limb	Ultrasound twice weekly to discharge/28 days a) DVT	EMS arm was discontinued 4 months into the pilot study due to lack of compliance.
Velmahos(Velmahos *et al.* 2005)	2005	Randomised control trial 2 groups: Group A: Electrical muscle stimulation (EMS) (n=30) 2x30 minutes treatment a day for minimum 7 days maximum 14 days. Group B: Control (n=30) Standard prophylaxis (heparin / sequential compression device) after contraindication for use was no longer present.	Major trauma patients (ISS>9) not suitable for heparin (n=60)	Lymphavision (Bexley trading, San Rafael Calif) Voltage 0-120V 3ms stimulus at frequency 1.75Hz (105/minute) Inversion polarity every 5secs. Electrodes Calf and medial thigh of both extremeties	Ultrasound (baseline) a) primary outcome: i) proximal DVT ii) total DVT	A further 4 EMS and 9 control were excluded due to lack of outcome evaluation. a) Primary outcome: i) No significant difference in incidence of DVT between groups. Proximal DVT: EMS (3/26), control (3/21) Total DVT: EMS (7/26) and Control (6/21) EMS was not effective in reducing DVT rates in major trauma patients, ARR 1.7% (-22.5, 26.8)
Lobastov(Lobastov *et al.* 2014)	2014	Non randomised controlled trial 2 groups: Group A: Electrical stimulation Group B: Control Both groups received graduated compression bandage and low dose unfractionated heparin (5000iu tds)	Post-operative high risk surgical patients (n=80)	Veinoplus 9V battery Low frequency (250Hz) Alternating electrical current Muscle contraction 50ms Stimulation rate: 60-105 bpm Minimum 5 x 20min sessions per day. Electrodes VeinPack self adhesive electrodes over gastrocnemius.	Ultrasound (Compression sonography) within first 24 hrs and every 3 days postoperative a) DVT	Baseline US DVT in 20 patients a) 8 with proximal extension excluded from study b) 12 (ES (n=5) and control group (n=7)) received prophylactic dose LDUH. Incidence of postoperative DVT a) was significantly lower in ES group vs control (2.5% vs 25%, p=0.007). c) In patients without preoperative DVT , incidence of DVT was 3% vs 21% (p=0.025) c) Proximal DVT: ES 0% , Control 5% d) Pulmonary embolus: ES 0%, Control 5%.

Table 52 - Comparing Intermittent Pneumatic Compression/ Sequential Compression Device and Electrical Muscle Stimulation

Author	Year	Trial design	Population	Device/ electrodes	Outcome measure	Results
Laverick(Laverick *et al*. 1990)	1990	Case series 2 groups Group A: Electrical stimulation Group B: Intermittent pneumatic compression (IPC) of foot Impulse system (Novamedix Ltd) Inflatable plastic sleeve midfoot. Inflation every 20s. Deflation 3s.	Healthy volunteers (n=10)	Belfast calf stimulator 70 volt stimulus at 10secs intervals.	Ultrasound a) Popliteal vein Maximum velocity mean venous velocity	a) Mean velocity significantly increased by a factor of 3.43 (1.65 – 5.12) for IPC and 3.52 (1.40 – 5.11) for calf stim. b) Peak venous velocity increased significantly by a factor of 3.3 (range 1.82 – 3.40) for IPC and 4.36 (range 2.51 – 6.79) for calf stim.
Jorgensen(Jorgensen *et al*. 1994)	1994	Randomised trial Group A: Intermittent Pneumatic Compression (IPC) 35-55mmHg one inflation per minute Group B: Electrical Stimulation	Intra-operative laparo-scopic chole-cystect-omy (n=6)	Biomec 527B Calf Muscle Stimulator 35V 4 stimulations per minute Electrode not specified	Ultrasound Femoral vein a) Peak venous velocity at baseline, before and after pneumoperitoneum, and at the end of surgery before release of pneumoperitoneum.	a) Effect of insufflation (12mmHg) - baseline femoral venous velocity was significantly reduced (20.12cm/s to 12.17cm/s; p=0.002) b) Effect of releasing pneumoperitoneum - only 4 patients had their venous velocity measured, which showed a brief increase in blood flow by 192%, 100%, 60% and 167%. c) Effect of IPC on peak venous velocity - was not significantly different with and without pneumoperitoneum (22.5cm/s vs 29.8 cm/s). d) Effect of electrical stimulation on peak venous velocity - was not significantly different with and without pneumoperitoneum (21.66cm/s vs 31.67 cm/s). e) The mean peak blood flow velocities achieved by IPC and EMS were similar with and without pneumoperitoneum.

Czyny(Czyrny *et al.* 2010)	2010	Case series 2 Protocols, 48 hrs washout sessions: a) Electrical stimulation b) Intermittent Pneumatic Compression (IPC) Knee high Tyco Healthcare Kendall Novamedix A-V Impulse system 130mmHg impulse pressure with 3s impulse duration Participants seated for 4 hrs. Limited to 2 visits to toilet, 16 ounces of fluid and a normal lunch. One leg treated (randomly allocated).	Healthy (n=40)	Focus Neuromuscular Stimulation System Biphasic square wave Phase duration 300µs. Ramp up 2s, ramp down 2 secs. Frequency 50Hz Stim cycle 12s on 48s off per minute. Intensity increased to cause muscle twitch. Electrodes: Surface electrodes on plantar aspect of foot.	Ultrasound Popliteal and femoral peak venous velocities at 0, 15, 120 and 240 minutes. a) Primary analysis: paired t-test accounting for non-inferiority index of 5.0cm/s b) Secondary analysis: Accounts baseline differences using mixed model method.	a) Primary analysis: Electrical stimulation is non-inferior (<5cm/s) to IPC, achieving noninferior flow values at 120 minutes for the femoral vein (p=0.005) and 120min (p=0.004) and 240min (p=0.014) for the popliteal vein. b) Secondary analysis: Electrical stimulation is non-inferior to IPC when measured at the femoral vein (time=120 minutes, p=0.08) and popliteal vein (time =120 min, p=0.18; time=240 min, p=0.043). c) Treatments were uncomfortable: 92.5% IPC and 82.5% of electrical foot stimulation. d) 62.5% found IPC more comfortable than ES.
Williams(Williams *et al.* 2015)	2014	Cross over trial 2 groups: Group A: Sequential Compression device (SCD) (n=10) SCD Express TM, Covidien Compression 11s Pressure 45mmHg at anke, 40mmHg at calf Group B: Electrical Stimulation (n=10)	Healthy volunteers (n=10)	Geko TM T-1 FirstKind Ltd 27mA Frequency 1Hz Pulse width 70-560µs	Ultrasound Femoral vein a) Peak venous velocity (PVV) b) Time averaged mean velocity (TAMV) c) Volume flow (VF)	a) Peak venous velocity (PVV) significantly increased by 51% (p=0.002) with SCD and 103% (p=0.002) with electrical stimulation. b) Time averaged mean velocity (TAMV) increased by 101% with electrical stimulation (p=0.002) but only 5% with IPC (NS). c) Volume flow (VF) increased by 101% with electrical stimulation (p=0.002) but only 3% with IPC (NS).

Table 53 - Effect of electrical stimulation in patients with impaired calf muscle pump

Author	Year	Trial design	Participants	Device/ electrodes	Outcome measure	Results
vann Beekvelt(van Beekvelt *et al.* 2000)	2000	Non-randomised control trial 3 groups SCI patients performed 10 tiptoe movements with the aid of ES a) ESav: electrical stimulation at average current used by ABS b)ES60: electrical stimulation with a current of 60mA c) ABS performed 10 tiptoe movements with ES and voluntary contraction (VC). Experiment carried out in supine and upright position. Pressure of cuff was varied at 50mmHg, 60mmHg and 70mmHg.	Spinal cord injury (SCI, n=10) and able bodied subjects (ABS, n=10) SCI patients with T3-T12 complete lesions. Time of onset fo SCI 1-18years.	Device Elpha2000 (Danica, Nijkerk) Biphasic pulse 200µs Frequency 30Hz Stimulation time 2s, 500ms rise time, 2s pause Pattern lasts for 45s Electrodes gastrocnemius	Strain gauge plethysmography a) Muscle pump activity Pressure of cuff was varied at 50mmHg, 60mmHg and 70mmHg.	Muscle pump activity a) There was no significant difference between voluntary and ES-induced MPA [58.1 (18.4%) vs 67.7 (8.7%)] in ABS group. b) SCI individuals showed a significantly lower ES induced MPA than ABS [21.5 (15.9%) vs 67.7 (8.7%)]. c) Although the MPA in SCI subjects was significantly higher using high current (ES60) in the supine position [30.8 (18.7%) vs 21.5 (15.9%)], it remained significantly lower than in ABS [67.7 (8.7%)]. Venous capacity c) The venous capacity (pressure volume curve) was 50% lower in SCI patients compared to ABS.

10.2 Tables relating to NMES in peripheral arterial disease

Table 54 - Results of systematic review evaluating the use of non-invasive medical devices in the management of peripheral arterial disease, grouped according to indication

Device	Paper	Year	Setting/program	Subject profile	N (PAD/control)	Outcome measure	Outcome time period	Finding
Stable Claudicants								
Circulator boot	Dillon(Dillon 1980)	1980	Combination of laboratory studies and case series. Reclining, applied to whole leg. 55-80mmHg applied in late diastole. One 40min session for lab study,	Claudicants, severe PAD non-reconstructable	29/6	Lab - subcutaneous pO2, pulse volume, ABPI Clinical - ulcer healing, presence rest pain, ICD	40 mins	Oscillometry readings from the leg increased during therapy in normal and diseased limbs after one session, changes more pronounced after series of treatment. TcPO2 increased in diseased limbs during treatment. 22/25 severe legs benefitted clinically from therapy (claudication distance, ulcer healing, rest pain). Best results in 3-4 sessions/day for >1wk. Two stopped therapy due to pain
ArtAssist	Eze(Eze *et al.* 1996)	1996	Alternatively foot+/-calf, sitting position, 120mmHg 10s 2/min	Stable claudicants, SFA occlusion	10/22	Laser Doppler (great toe) Duplex popliteal artery	Mean of 6 IPC cycles	Flux (PAD/healthy) 288/428% of baseline with combination IPC. Arterial flow (PAD/healthy) 150/273% of baseline with combination IPC Combination IPC more effective than IPC calf/foot alone.
ArtAssist	Delis(Delis *et al.* 2000)	2000	Recovery position, IPC foot, 1-120mmHg, 4s, 3/min, 5 mins on, 10 min rest, 5 mins on etc	Claudicants (fontaine 2)	40/25	Duplex popliteal artery velocities and flow	Lab	Mean popliteal artery flow 211% p<0.001 in healthy, 151% p<0.001 in PAD. Mean popliteal artery velocity 215% p<0.001 in healthy, 149% p<0.001 in PAD Post compression baselines of both were significantly higher than pre-compression.
ArtAssist	Delis(Delis *et al.* 2000)	2000	Sitting position, IPC foot/calf/combination, 1-120mmHg, 4s, 3/min, 5 mins on, 10 min rest, 5 min on etc	Claudicants (fontaine 2)	31/25	Duplex popliteal artery velocity and flow	Lab	IPC combination mean velocity 263% (p<0.001) in controls, 170% (p<0.001) in PAD IPC combination volume flow 278% in (p<0.01) controls, 174% (p<0.001) in PAD IPC combination was more effective than IPC foot or IPC calf in PAD
ArtAssist	Delis(Delis *et al.* 2000)	2000	Device versus no device. Sitting position, 1-120mmHg, 4s, 3/min, >4 hr/day	Claudicants (fontaine 2) stratified for smoking and diabetes	25/12	ICD, ACD, ABPI rest and post-exercise, duplex popliteal artery flow	4.5months treatment, follow-up at 12 months	No improvement in parameters for those randomised to no device. Popliteal artery flow 136% of week 0 baseline at 4.5 months. IPC foot at 4.5m – ICD 246% baseline, AWD 206% (p<0.001 for both).

								ABPI (pe) significantly greater than control group at 4.5 and 12m. Significant benefit over controls persisted at 12m
ArtAssist	Delis(Delis *et al.* 2002)	2002	Sitting position, IPC calf/foot/combination, 1-120mmHg, 4s, 3/min, 5 mins stim, 10 min rest, 5 min stim etc	Stable claudicants	22 IC, 36 bypass	Laser Doppler great toe	Minutes, unspecified	IPC increases limb skin blood flux in controls and claudicants. IPC combination and IPC foot produced the biggest flux differences over IPC calf ($p<0.004$).
ArtAssist	Ramaswami(Ramaswami *et al.*	2005	Device versus no device. Sitting, IPC foot+calf, 120mmHg, 3/min, 1hr twice a day	Stable claudicants (matched for smoking, diabetes)	15/15	ICD, AWD, ABPI	12 months	ICD compared to baseline (device/no device) at 4, 6 and 12 months was 237/102%, 241/103%, and 250/104%. AWD was 184/102%, 196/105%, and 201/106% (difference $p<0.01$ for all) No significant change in ABPI shown in either group
ArtAssist	Delis(Delis *et al.* 2005)	2005	Device versus no device. Sitting, IPC foot and calf, 1-120mmHg, 4s, 3/min, 3+ hours/day	Stable claudicants (AWD 35-350m)	20/21	ICD AWD ABPI (rest and post-exercise) US popliteal artery volume flow QoL (SF-36) Compliance	17 months	ICD IPC at 5 months 197% ($p<0.005$), BMT no significant difference AWD IPC at 5 months 212% ($p<0.005$), BMT no difference Resting ABPIs not changed either group pe-ABPI IPC higher at 5 months ($P<0.005$), BMT no difference No significant resting artery flow volume changes either group Improved quality of life in IPC group at 5 months, BMT unchanged. All reported gains with IPC sustained 12 months after treatment. 85% compliance with home IPC (defined as ≥2.5h/day)
DVT-30	Morris(Morris *et al.* 2002)	2002	Supine , unilateral , thigh and calf, 60mmHg, 10 secs, 1/min	Stable claudicants	11/18	Duplex common femoral artery frequency. Temperature limb.	10 minutes	PAD subjects arterial frequency 94%/129% of baseline during compression/deflation. Controls 85%/121% of baseline. Hallux temperature changes -0.1°C for controls and +2.2°C PAD
AV impulse	Morgan(Morgan *et al.* 1991)	1991	Seated, non-weight bearing, 100mmHg, 3s, 3/min	Claudicants	10/12	Doppler popliteal artery flow	Lab	Flow 193% and 184% of baseline in healthy and PAD ($p<0.0001$ and $p<0.03$). Increase reduced by supine position and limb cooling. Flow increase more persistent in PAD. Contralateral limb flow not affected.

FM220 (IMC)	de Haro(de Haro *et al.* 2010)	2010	Calf 65mmHg for 3secs, 3/min	Stable claudicants	14/16	ICD, AWD and ABPI (pre- and post-exercise) Compliance	3 months	ICD 185%, (p=0.002), ACD 176% (p=0.002), in IMC group, no significant changes in controls. ABPI(pe) 197% (p=0.003) in IMC group, no significant changes in controls. Changes sustained after 3 months. Compliance with device 78%
IPC (unspecified)	Anthonysamy(Anthonysamy *et*	2012	10mins, settings not specified	Stable claudicants (fontaine 2)	15 /0	Duplex popliteal artery peak systolic flow	Lab	Flow with IPC 175% of baseline (p<0.05)
NMES	Loubser(Loubser *et al.* 1988)	1988	Unilateral, common peroneal nerve 2Hz, intensity to produce muscle contraction, 60 mins	Stable claudicants	8/8	BP, HR Hallux photoplethysmographic waveform Skin temp	60 minutes	BP and HR changes not significant for either group Hallux photoplethysmographic waveform significant change in PAD, not control Skin temperature significant rise in PAD, not controls.
NMES (Medicompex,)	Tsang(Tsang *et al.* 1994)	1994	NMES versus sham (TENS) Popliteal and anterior tibial nerves, 8Hz, 350microsecs	Stable claudicants, ABPI<0.9, AWD<500m, peABPI drop>30mmHg	13/13 sham	Treadmill ICD/AWD ABPI, Ankle flexion fatigue index	8 weeks	ICD with NMES 126% of baseline (p<0.003), control 122%, at 8 weeks. AWD with NMES 134% (p<0.004), control 127% at 8 weeks. Differences between control and IPC not significant after 4 weeks therapy cessation. Neither group improved ABPI. Fatigue index improved in both groups, NMES more than sham, but returned to baseline after treatment cessation
NMES (Medicompex)	Hudlicka(Hudlicka *et al.* 1994)	1994	Unilateral, tibialis anterior and gastrocnemius muscles. 8Hz, 330microsecs, voltage to produce muscle contraction. 20mins, 3/day, 28 days	Claudicants	12/12 sham	AWD ABPI Ankle flexion fatigue index	4 weeks	AWD with 4 weeks NMES 161% baseline (p<0.05), sham 102% (ns) Fatigue index NMES 200% baseline (p<0.05), sham 111% (ns) ABPI did not change significantly in either group
EMS (MediCompex)	Anderson(Anderson *et al.* 2004)	2004	EMS versus sham (TENS) Unilateral gastrocnemius 250microsecs, 100V, 6Hz, 20 mins. 3/day, 7/wk.	Stable claudicants, pe-ABPI<0.8, AWD 50-350m	15	Leucocyte activation, vascular permeability, ICD/AWD Compliance	4 weeks	No evidence of activated neutrophils, increased vascular permeability, or increased cardiovascular event incidence. ICD EMS/sham 182%/208% of baseline (p<0.01) AWD 250%/163% (p<0.05) 95% compliance

EMS (Veinoplus)	Abraham(Abraham *et al.* 2013)	2013	Gastrocnemius and soleus. Rectangular pulse <25microC, 50Vpeak, 1-250Hz, max duration 240microsecs, 20mins increasing contraction rates 60-100bpm	Claudicants (Fontaine 2). Most symptomatic leg	15	Duplex SFA, NIRS TcO2 O_2Hb (compared to treadmill test)	Baseline, during, 10mins after	Flow 240% baseline with stimulation at 100bpm ($p<0.01$) but wide variation. No change in NIRS or O_2Hb signal with stimulation No induction of ischaemic pain
Critical Limb Ischaemia								
Circulator Boot	Dillon(Dillon 1997)	1997	Reclining. Cardiosynchronous end-diastolic single chamber pneumatic compression boot, 55-80mmHg	Limb lesions (peripheral arterial, venous, diabetic, and neuropathic disease)	1517	Healing rate Relapse rate	Variable	80.5% healed or improved Relapse rate of 21.6%. Smoking and distance of home from treatment centre sig affected healing rates
Circulator Boot	Dillon(Dillon 1997)	1997	Reclining. Cardiosynchronous end-diastolic single chamber pneumatic compression boot, 55-80mmHg	CLI for limb salvage	2/0	Limb salvage	Variable	Limb salvage described in both PAD cases, also diabetes and osteomyelitis. Intensive treatment regime using injected antibiotics, soaks, dressings and boot Tx. Reversal of peripheral neuropathy loosely described
Circulator boot (then home programme)	Vella(Vella *et al.* 2000)	2000	No unified protocol. Circulator boot - 55-80mmHg timed with end-diastole, 45 mins, 1-2/day. Home boot - foot+/-leg, 100mmHg 3s, 3/min, 2-6h/day, mean duration 8wks	CLI, non-reconstructable with ischaemic ulcers	98/0	Ulcer healing, ulcer size (static or smaller), amputation rate, mortality	Average 40 days	79% "favourable outcome" (ulcer decrease in size, complete healing, revascularised) 83% limb salvage (15 major amputations, 2 deaths) Authors claim can be used to bridge to revascularisation or skin grafting.
ArtAssist	Louridas(Louridas *et al.* 2002)	2002	Not specified	CLI	25/0	Ulcer healing. Rest pain Amputation rate Toe pressures Mortality	Mean 3month	26% ulcers healed Rest pain improved in 40% 58% limb salvage Toe pressures improved p=0.03 Mortality rate 12%.
ArtAssist	Labropoulos(Labropoulos *et al.* 2005)	2005	Foot and calf IPC. Semi-erect, 120mmHg, 3s, 3/min	CLI (fontaine 3-4)	20/0	Duplex flow volume popliteal, medial gastrocnemius, genicular collateral artery. Laser doppler foot	5 IPC cycles	Popliteal – 205% of baseline ($p<0.01$) Gastrocnemial – 170% of baseline ($p<0.01$) Collateral – 156% of baseline ($p<0.01$) Laser Doppler flux increased significantly with IPC use ($p<0.03$)

ArtAssist	Sultan(Sultan *et al.* 2011)	2011	Upright. 0-120mmHg, 4sec, 3/min, 2x3h/day, 3 months. Protocol repeated if of significant benefit	CLI (Rutherford≥4)	171/0	Ulcer healing Limb salvage Mortality Doppler popliteal arterial flow Toe pressure. QoL Cost-effectiveness.	3 months, repeated for 18% Median F/U 13 months	94% clinical improvement (Rutherford4 --> 3), 30% at 3 years Rest pain resolved in all over treatment period 94% resolution of ulcers and limb salvage at 3.5 years, median amputation-free survival 18 months. 30-day mortality 0.6%. All-cause survival 31% at 4 years Toe pressures 146% of baseline (p<0.0001), sustained at 12m. Popliteal artery flow 158% of baseline (p<0.0001), sustained at 12m. Regression showed DM, chronic renal failure, and hypercholesterolemia had no significant effect on limb salvage. Cost per quality-adjusted life year for IPC was €2953
Arterial flow, DJO	Kavros(Kavros *et al.* 2008)	2008	IPC calf, 85-95mmHg, 2s, 3/min, 3x2hours/day	CLI (non-reconstructable)- non-healing ulcer or amputation wound	24/24	Limb salvage, wound healing	18m	58% (IPC) vs 17% (control) had complete healing and limb salvage (p<0.01)
IPC (unspecified)	Montori(Montori *et al.* 2002)	2002	Home use, 6h/day	CLI non-healing wounds	107/0	Wound healing, amputation rate	Median 6m	Wound healing seen with 40% patients $TcPO_2$<20, 48% in osteomyelitis/active infection, 46% in IDDM, 28% with a previous amputation. 7 patients discontinued use due to pain
IPC (unspecified)	Beirne(Beirne C 2009)	2009	Not specified	CLI	149/0	Limb salvage, mortality. Limb digital pressure measurement.	30 days, 90 days, 6m intervals (mean 18m)	72% "Sustained clinical improvement" 3% amputation rate 21% mortality
NeuroTrac 3 TENS (Verity Medical, UK)	Seenan(Seenan *et al.* 2012)	2012	TENS versus sham Unilateral Gastrocnemius 200msecs biphasic pulses, 120Hz, in a continuous pattern	Tourniquet induced ischaemic pain in healthy legs	13	Time to pain threshold, pain tolerance, total pain endurance Reported pain (21-NRS, McGill Pain Questionnaire)	To maximal pain able to be endured.	Time to pain threshold (p<0.05), tolerance (p=0.002) and total endurance (p=0.003) increased with device compared to sham controls. Reported pain lower with device compared to sham
Post revascularisation								
AV impulse	White(White *et al.* 1996)	1996	IPC foot. 50-200mmHg, 3sec, 3/min. 7 post-operative days, 4-6 hours/day	Post ischaemic leg reconstruction (femorotibial bypass)	5/5	Calf and ankle circumference. Duplex femoral/popliteal venous velocity	7 days	Significantly less swelling in experimental patients compared to controls, which peaked on day 5 (no figure given). Significant increase in experimental patients' femoral and popliteal venous velocities (no figure given). Cessation of treatment to less than 4 hours per day led to

								recurrence of symptoms, oedema, and a worsening of ulcers.
ArtAssist	Delis(Delis *et al.* 2002)	2002	Sitting position, IPC calf/foot/combination, 1-120mmHg, 4s, 3/min, 5 mins stim, 10 min rest, 5 min stim etc	Infra-inguinal arterial revascularization	36/20	Laser Doppler great toe	Minutes, unspecified	IPC increases limb skin blood flux in controls and grafted arteriopaths ($p<0.001$), although no difference between these groups. IPC combination and IPC foot produced the biggest flux differences over IPC calf ($p<0.004$).
Misc								
Galvanic electrical stimulation (Micro Z)	Peters(Peters *et al.* 1998)	1998	Device on diabetics, with and without impaired TcO_2 foot. Unilateral, 100Hz (twin peak monophasic), delivered via a silver nylon mesh stocking, 4x60mins. No muscle contraction	Diabetics (Foot TcO_2 <40mmHg).	11/8	Laser Doppler foot TcO2 foot	1 day	No difference in fluximetry readings, either group. Oxygen perfusion rose with stimulation during first five minutes ($p<0.04$). No changes in controls. No significant difference from baseline after cessation of device
Galvanic electrical stimulation (Micro Z)	Peters(Peters *et al.* 2001)	2001	Device to placebo 50V, twin peak monophasic pulses, pulse width 100microsecs, delivered via a silver nylon mesh stocking. 8 hours, delivered at night. Alternating 10 minutes 80Hz, 10 minutes 8Hz, 40 minutes no stimulation.	Diabetics with foot ulcers (Tc02> 30mmHg)	20/20	Healing rate Complete wound healing Complications Compliance (>20hours/week)	12 weeks	No significant differences in wound healing rate between groups. Device/sham total wound surface area healed 86.2%/71.4% (ns) Complete healing 13/7 subjects (ns) Time to complete healing 6.8/6.9 weeks (ns) One amputation, in sham group 70% device compliance

(PAD – peripheral arterial disease, ICD – intermittent claudication distance, AWD – absolute walking distance, TcPO2 – transcutaneous oxygen, SFA – superficial femoral artery, IPC – intermittent pneumatic compression, ABPI – ankle brachial pressure index, pe – post-exercise, US – ultrasound, NIRS – near-infrared spectroscopy, CLI – critical limb ischaemia, DM – diabetes mellitus

10.3 Tables pertaining to Exercise and Electrical Stimulation in Diabetes

Table 55 - The use of electricity in the management of diabetic peripheral neuropathy

Year	Author	Population	Exclusion	Intervention	Training length	N / control	Outcomes	Results
2013	Bosi	Diabetic peripheral neuropathy (NCS confirmed) - amplitude <6mV, latency >6.5ms, conduction velocity <40m/s	Pacemaker, cardiac defibrillator, foot ulcer, limb amputation	FREMS. "Aptiva", Italy. Biphasic, 1-1000Hz freq-mod, 0-255V, pulse width, 1-1000ms. Bilateral legs, no movement	30 treatments 30-mins Daily	54/56 "placebo" no stimulation	Nerve conduction study Thermal, vibration and tactile thresholds. HbA1c	No change NCS or thermal endpoints. Transient reduction night pain. No change in HbA1c. Large drop-out rate
2007	Khalil	Diabetic peripheral neuropathy Clinical diagnosis Age >55y	AKA/BKA, smoker, foot ulcer	TENS. Purpose built. 5Hz, 4mA, pulse width 0.2ms. Unilateral, common peroneal nerve.	12-weeks 5-mins Twice daily	35/31 ("placebo" machine - no output)	Capsaicin response, perception threshold, laser doppler (?where), transcutaneous oxygen tension	No difference between intervention and placebo.
2010	Moharic	Diabetic peripheral neuropathy (MNSI>2) Type 2 DM	Peripheral vascular disease	TENS. "MedioTens", Slovenia. 0-14mA, 100Hz, pulse width 200ms. Across ankle/foot	3-weeks 3-hours Daily	46/0	Temp, vibration, and light touch thresholds foot. Pain score	Improvements in temperature thresholds, but not other modalities.

(DM – diabetes mellitus, IHD – ischaemic heart disease, BMT – best medical therapy, MNSI – Michigan Neuropathy Screening instrument, MDNS – Michigan Diabetic Neuropathy Scale)

Table 56 – Summary table of trials using an exercise protocol in the management of diabetes mellitus

End-point	Year	Author	Population	Exclusion	Intervention	Training length	N / control	Outcomes	Results
Fitness and function	2002	Maiorana	Type 2 DM	Smokers Renal impairment HTN High cholesterol	Circuit training Supervised	8-weeks 1-hr 3/week	16, cross-over trial	Resting heart rate Skin fold thickness Strength and endurance tests Fasting glucose, HbA1c Lipid profile	Resting HR significantly lower after 8 weeks exercise. Increase in VO2max (7%), strength and endurance. Decreased HbA1c and fasting glucose. No differences in lipid profile
	2014	Taveggia	Diabetic peripheral neuropathy >3yrs	MSK impairment. Bone instability. Cardiovascular or respiratory failure. Foot ulcers	Exercise class (treadmill, strength, balance). Supervised	4-weeks 1-hr 5/week	13/14 control – "less intensive exercise"	6-min walk test 10m walking test "Functionality" scale HbA1c	Both groups improved on functionality scales, no difference between groups. Intervention group stamina improved.
	2008	Hordern	Type 2 DM	IHD, pregnancy	Individualised programme of moderate intensity exercise	4-weeks 150-min/week	68/64	VO2max BMI Plasma lipids, fasting glucose, insulin sensitivity, β-cell function	Intervention increased VO2max and reduced BMI over control group Exercise intervention decreased triglyceride levels, but had no effect

									on insulin sensitivity or β-cell function.
	2010	Morton	NIDDM	Diabetic complications (eyes, kidneys, nerves) IHD Cancer	Treadmill walking (heart rate prescribed) Supervised	7-weeks 35-min 4/week	11/10	V02max Blood glucose	Intervention group increased V02max (5% vs -3%) and time to exhaustion (50% vs 8%) over control. Blood glucose not different between groups.
	2010	Hey-Mogensen	Type 2 DM obese men		Stationary bike training. Partially supervised	10-weeks 30-mins 3/week	13 DM /14 non-DM	Cell respiratory function and ROS release during insulin clamp	Aerobic training improves muscle oxidative function (~63%), regardless of DM status. Increased markers of mitochondrial biogenesis and activity
	2010	Allet	Type 2 DM Walk >500m	Foot ulcer Non-diabetic neuropathy	Gait and balance exercise, functional strength training. Supervised	12-weeks 60-mins 2/week	35/36	Walking speed Stride length	Walking speed and stride length increased in intervention group, more noticeable on uneven terrain
	2009	Cheung	Type 2 DM Unable to walk >30-min		Home based Exercise-band resistance training	4-months 40-mins/day	21/19	Grip strength, "time up and go", "chair sit and	No significant improvement in any outcome measure

								reach", "single leg balance" SF-36 HbA1c	
Nerve function	2010	Madden	Diet-controlled DM Age >65y		Treadmill and stationary bike Supervised	12-weeks 60-mins 3/week	20/20 control - light strength exercises	Blood pressure ECG (R-R interval) Baroreflex sensitivity	Intervention group sig increase in BRS. Exercise can reverse functional impairments of autonomic nervous system
	2012	Kluding	DPN	MSK limitations, foot ulcers HTN	Personalised aerobic and strength exercise. Supervised	10-weeks 30 - 50-mins 3-4/week	30 (19 completed) NO CONTROL	BMI HbA1c Pain – VAS, MNSI NCS and sensory threshold Cutaneous nerve density and terminal branching (biopsy)	No change to BMI. HbA1c improved significantly. Extreme pain and neuropathy scores improved, not significant overall. No significant change in NCS. Significant increase in nerve terminal branching on skin biopsy.
	2006	Balducci	Type 1 and 2 DM Able to walk 1.6km	DPN (MNSI) Ataxia MSK deformity Postural hypotension	Treadmill walking 50-80% heart rate reserve Supervised	4-years 1-hr 4/week	31/47	NCS and sensory thresholds BMI, waist circumference, fat body mass Blood pressure	>90% attendance at exercise classes Significant differences in ΔNCV (peroneal and sural) between test and control.

								HbA1c and urine albumin	Significantly more developed motor neuropathy in control group (30% vs 7%)
	2001	Richardson	Diabetic peripheral neuropathy (NCS confirmed)	Central nervous system dysfunction Limb amputation Arthritis IHD Foot ulcer	Balance and ankle strength exercises	3-weeks Daily	10/10 ("control" upper body exercise)	Functional movements (e.g. walking, bend-and-reach, one-foot balance)	Interventional group improved in functional movement scores.
	2002	Malhotra	Type 2 DM Age 30-60	Nephropathy. Rretinopathy. IHD	Yoga Supervised	40-days 40-min/day	20 /20	Median nerve conduction velocity, latency and amplitude	Slight increase in velocity, decrease in latency, compared to controls. NO STATISTICS GIVEN
Cardiovascular function	2014	Allen	Type 2 DM+PAD vs PAD Stable IC <3m ABPI<0.9	CLI. Lower limb vascular surgery or angioplasty Sympathectomy. Severe neuropathy. Unstable IHD	Exercise classes. Supervised	3-months 1-hr 3/week)	13/14	Endothelial function (hyperaemic arterial dilatation response, plasma NO metabolites) Plasma nitrate Exercise ABPI VO2max	DM attenuates improvements in endothelial function and claudication distance
	2006	Middlebroo	Type 2 DM	Raynaud's, vasculitis IHD CVA<6m	Aerobic exercise	6-months 30-mins 3/week	29/30	Skinfold thickness Sensory thresholds Microvascular function (laser	No significant effects found for any endpoints.

				Pacemaker				doppler response to skin heating and iontophoresis of vasodilators) HbA1c, lipid profile. Insulin tolerance test	
	2008	Wycherley	Type 2 DM Obese	Smokers IHD HTN Pregnancy	Walking/jogging Dietary restriction	12-weeks 50-min 5/week	13/16 (control diet restricted only)	Body weight, Stress hormones Brachial artery flow mediated dilatation (FMD)	No differences between groups
	2009	Hordern	Type 2 DM	IHD	Home based Gym programme and home-based exercises.	4-weeks 150-min/week	111/112	Myocardial velocities (US) body composition HbA1c VO2max	Improvement in metabolic function Failed to improve myocardial function

(DM – diabetes mellitus, DPN – diabetic peripheral neuropathy, MSK – musculoskeletal, PAD – peripheral arterial disease, CLI – critical limb ischaemia, IC – intermittent claudication, IHD – ischaemic heart disease, HTN – hypertension, CNS – central nervous system, CVA – cerebrovascular accident, BMI – body mass index, V02max – maximal oxygen consumption, VAS – visual analogue score, MNSI – Michigan Neuropathy Screening Instrument, NCS – nerve conduction studies, NCV – nerve conduction velocity, V02max – maximal oxygen consumption, ROS – reactive oxygen species, NO – nitric oxide, US – ultrasound, SF-36 – Short Form 36)

10.4 Results tables pertaining to small trials and bench testing of NMES devices

10.4.1 Haemodynamic changes with common peroneal stimulation

Table 57 - Changes from baseline arterial haemodynamic values with ipsilateral common peroneal nerve stimulation

	Change with stimulation			Significance (single T-test)
	Mean (confidence interval)		Percentage	
Diameter (cm)	0.02	(0.002 – 0.03)	3.3%	0.03
Peak velocity (cm/s)	24.6	(22.2 – 27.0)	57.3%	<0.01
TAMV (cm/s)	3.9	(3.4 – 4.4)	159.2%	<0.01
Volume flow (ml/min)	93.4	(81 – 106)	190.3%	<0.01

Table 58 - Changes from baseline venous haemodynamic values with ipsilateral common peroneal nerve stimulation

	Change with stimulation			Significance (single T-test)
	Mean (confidence interval)		Percentage	
Diameter (cm)	-0.2	(-0.2 - -0.2)	-16%	<0.001
Peak velocity (cm/s)	17.2	(15.0 – 19.3)	173%	<0.001
TAMV (cm/s)	2.4	(2.0 – 2.8)	185%	<0.001
Volume flow (ml/min)	51.1	(40.0 – 62.2)	105%	<0.001

10.4.2 Haemodynamic changes with footplate NMES

Table 59 - Haemodynamic changes with footplate stimulation measured in the femoral vein and superficial femoral artery using ultrasound

	TAMV (cm/s)			Volume Flow (ml/min)		
	Baseline	Stimulation	Cessation	Baseline	Stimulation	Cessation
Arterial	5.8 ±3	9.5 ±7	13 ±22	96 ±47	158 ±107	95 ±63
Venous	3.4 ±3	4.6 ±2	2.3 ±1	216 ±209	305 ±224	144 ±84

(mean, standard deviation)

10.4.3 Comparison of footplate and common peroneal stimulation

Table 60 - Data used for analysis in section 6.3.2, comparing common peroneal and footplate stimulation

		Time (mins)	Median	Median difference	% difference
Common peroneal nerve stimulation	Venous TAMV (cm/s)	15 0	1.77 .27	1.5	556
	Venous Flow (ml/min)	15 0	76.6 14.0	62.6	447
	Arterial TAMV (cm/s)	15 0	4.00 2.06	1.94	94
	Arterial Flow (ml/min)	15 0	73.5 40.0	33.5	84

10.4.4 NMES versus IPC in healthy subjects

Table 61 – Haemodynamic ultrasound measurements from the femoral vessels, in the protocol comparing intermittent pneumatic compression to neuromuscular stimulation of the leg via the common peroneal nerve.

Arterial - PV

	Baseline	IPC on	IPC on%	IPC just off	IPC just off%	10mins post IPC	10mins post IPC%	Geko on	Geko on%	Geko just off	Geko just off%	10mins post geko	10mins post geko%
25% Percentile	62	56.51	-12.46	53.49	-17.09	56.61	-18.84	69.96	4.675	57.25	-12	58.17	-9.379
Median	63.01	59.13	-8.387	61.05	-3	65.29	0.6996	72.97	10.79	62.08	-7.461	62.17	-4.681
75% Percentile	72.45	67.55	3.633	67.48	0.8489	69.48	7.218	78.14	15.33	66.49	0.3928	66.44	9.461
Mean	65.64	61.54	-6.002	56.9	-13.49	63.47	-3.07	73.24	12.01	62.15	-5.052	63.12	-3.5
Std. Deviation	5.726	7.074	10.06	18.58	27.37	9.005	13	5.212	9.155	5.475	7.821	8.356	12.19
Wilcoxon Signed Rank Test													
Theoretical median	0	0	0	0	0	0	0	0	0	0	0	0	0
P value (two tailed)	0.002	0.002	0.084	0.002	0.0781	0.002	0.625	0.002	0.002	0.002	0.084	0.002	0.625
Significant (alpha=0.05)?	Yes	Yes	No	Yes	No	Yes	No	Yes	Yes	Yes	No	Yes	No

Arterial - TAMV

	Baseline	IPC on	IPC on%	IPC just off	IPC just off%	10mins post IPC	10mins post IPC%	Geko on	Geko on%	Geko just off	Geko just off%	10mins post geko	10mins post geko%
25% Percentile	5.344	3.643	-35.97	4.396	-30.52	3.387	-42.89	8.818	32.21	4.053	-41.67	4.142	-34.61
Median	5.855	5.94	-11.58	5.309	-5.829	5.563	-14.87	10.58	84.25	5.11	-9.881	5.034	-14.82
75% Percentile	7.642	7.744	28.16	7.332	-1.403	6.832	14.97	13.43	97.64	7.22	35.15	7.311	36.46
Mean	6.367	5.669	-8.895	5.613	-10.69	5.597	-10.31	10.71	70.17	5.904	-3.766	6.06	-1.541
Std. Deviation	1.647	2.408	36.86	1.928	25.58	2.109	30.72	3.279	42.29	2.68	39.81	2.838	41.55
Wilcoxon Signed Rank Test													
Theoretical median	0	0	0	0	0	0	0	0	0	0	0	0	0
P value (two tailed)	0.002	0.002	0.5566	0.002	0.0781	0.002	0.4316	0.002	0.0039	0.002	0.625	0.002	0.8457
Significant (alpha=0.05)?	Yes	Yes	No	Yes	No	Yes	No	Yes	Yes	Yes	No	Yes	No

Arterial - Flow

	Baseline	IPC on	IPC on%	IPC just off	IPC just off%	10mins post IPC	10mins post IPC%	Geko on	Geko on%	Geko just off	Geko just off%	10mins post geko	10mins post geko%
25% Percentile	117.4	85.82	-27.01	116.2	-27.22	91.33	-41.77	194	27.06	95.05	-38.51	104.9	-36.18
Median	158.1	168	-12.98	134.7	-3.545	149.1	-8.29	258.5	74.97	132.1	-4.954	119.8	-17.7
75% Percentile	188.6	187.6	57.02	182	17.95	180.4	38.36	334.3	123.2	171.2	38.19	164	64.22
Mean	152.9	151	4.522	145.7	0.4596	143.8	0.7932	255.9	75.62	147.2	2.939	147.7	5.194
Std. Deviation	48.28	64.26	48.99	46.82	34.84	53.34	43.47	84.82	61	76.73	50.78	81.22	58.81
Wilcoxon Signed Rank Test													
Theoretical median	0	0	0	0	0	0	0	0	0	0	0	0	0
P value (two tailed)	0.002	0.002	1	0.002	0.9004	0.002	0.9219	0.002	0.0059	0.002	0.9219	0.002	1
Significant (alpha=0.05)?	Yes	Yes	No	Yes	No	Yes	No	Yes	Yes	Yes	No	Yes	No

Venous - PV

	Baseline	IPC on	IPC on%	IPC just off	IPC just off%	10mins post IPC	10mins post IPC%	Geko on	Geko on%	Geko just off	Geko just off%	10mins post geko	10mins post geko%
25% Percentile	9.489	14.57	0.7863	10.1	-20.89	9.824	-28.36	33.34	177.6	11.31	-19.16	11.03	-11.22
Median	14.8	22.66	50.63	12.75	-5.184	11.71	-14.23	47.85	267.1	13.84	10.06	15.44	6.215
75% Percentile	17.4	31.82	130.6	14.71	0.7452	14.7	7.629	64.67	342.6	17.21	22.88	17.81	32.21
Mean	14.2	22.75	74.17	12.68	-7.723	12.17	-8.365	50.82	259	14.21	4.972	14.77	8.504
Std. Deviation	3.975	7.786	84.99	2.648	17.38	2.495	31.36	22.41	103	3.138	27.81	3.8	29.23
Wilcoxon Signed Rank Test													
Theoretical median	0	0	0	0	0	0	0	0	0	0	0	0	0
P value (two tailed)	0.002	0.002	0.0195	0.002	0.1934	0.002	0.2324	0.002	0.002	0.002	0.7695	0.002	0.375
Significant (alpha=0.05)?	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	No	Yes	No

Venous - TAMV

	Baseline	IPC on	IPC on%	IPC just off	IPC just off%	10mins post IPC	10mins post IPC%	Geko on	Geko on%	Geko just off	Geko just off%	10mins post geko	10mins post geko%
25% Percentile	3.289	4.192	-2.516	3.058	-29.44	3.033	-26.98	5.016	15.14	3.791	-19.44	3.486	-1.463
Median	4.598	4.992	4.814	3.792	-16.37	3.696	-2.574	6.076	59.69	4.223	1.063	4.409	8.67
75% Percentile	5.977	6.052	37.33	5.361	10.85	5.573	21.02	9.354	89.73	6.035	31.13	6.198	17.66
Mean	4.829	5.224	14.32	4.125	-9.031	4.213	-2.957	7.083	55.95	4.797	8.291	4.786	5.153
Std. Deviation	1.949	1.471	24.2	1.216	25.55	1.222	36.23	2.438	45.16	1.448	41.24	1.535	24.92
Wilcoxon Signed Rank Test													
Theoretical median	0	0	0	0	0	0	0	0	0	0	0	0	0
P value (two tailed)	0.002	0.002	0.2754	0.002	0.2754	0.002	0.8457	0.002	0.0098	0.002	0.8457	0.002	0.1602
Significant (alpha=0.05)?	Yes	Yes	No	Yes	No	Yes	No	Yes	Yes	Yes	No	Yes	No

Venous - Flow

	Baseline	IPC on	IPC on%	IPC just off	IPC just off%	10mins post IPC	10mins post IPC%	Geko on	Geko on%	Geko just off	Geko just off%	10mins post geko	10mins post geko%
25% Percentile	131.5	145.9	-23.88	118.6	-37.87	107.2	-34.2	203.8	3.632	139.6	-24.7	132.3	-5.07
Median	150.2	171.9	2.579	130.8	-15.75	159.4	-0.3165	267.5	84.17	159.8	-6.149	173.6	8.146
75% Percentile	281.9	214.4	43.79	169.9	8.299	204.9	34.33	361.8	128.6	190.7	20.14	269.1	43.53
Mean	192	185.6	8.199	147.8	-13.51	158.2	-5.269	288	71.02	171.7	0.2017	190.2	11.6
Std. Deviation	98.06	48.26	32.15	36.24	25.61	53.5	37.91	111.9	68.87	53.4	33.89	75.77	42.61
Wilcoxon Signed Rank Test													
Theoretical median	0	0	0	0	0	0	0	0	0	0	0	0	0
P value (two tailed)	0.002	0.002	0.625	0.002	0.1602	0.002	0.7695	0.002	0.0137	0.002	0.8457	0.002	0.2324
Significant (alpha=0.05)?	Yes	Yes	No	Yes	No	Yes	No	Yes	Yes	Yes	No	Yes	No

10.4.5 PAD trial

Table 62 - Temperature and fluximetry changes with use of the geko in the PAD trial

		Temp						Flux					
Subject	Week	HandBL	HandON	Hand%change	FootBL	FootON	Foot%change	HandBL	HandON	Hand%change	FootBL	FootON	Foot%change
1	0	30.4	30.4	0.00	28.4	29.9	5.28	18.6	42.8	130.11	34.8	39.1	12.36
2	0	30.1	30.9	2.66	29.3	30.9	5.46	147.2	69.1	-53.06	38.7	86.8	124.29
3	0	32.1	31.8	-0.93	29.7	29.4	-1.01	59.1	148.8	151.78	22.1	54.4	146.15
5	0	31.8	33.1	4.09	28.4	28.5	0.35	81.7	52.1	-36.23	12.7	242	1805.51
7	0	30.9	30.9	0.00	30.8	30	-2.60	174.4	176.3	1.09	42.1	73.8	75.30
10	0	32.5	32	-1.54	31.8	33.4	5.03	51.4	40.8	-20.62	94.4	105.5	11.76
11	0	29.2	30.8	5.48	32.6	34.9	7.06	28.3	157.8	457.60	35.3	50.5	43.06
12	0	29.3	30.2	3.07	30	30.4	1.33	28.9	21.8	-24.57	17.2	26.3	52.91
14	0	31.9	32.1	0.63	30.1	31.7	5.32	96.7	73.8	-23.68	27.3	31.3	14.65
15	0	26.5	27.1	2.26	28.7	29.4	2.44	100.7	186.2	84.91	51.9	93.8	80.73
1	6	29.1	29.3	0.69	29	30.4	4.83	43	41.3	-3.95	16.6	18.2	9.64
2	6	32.7	33.2	1.53	26.8	27.2	1.49	57.5	105.9	84.17	14	14.4	2.86
3	6	31.6	31.3	-0.95	30.1	30.1	0.00	234.9	174.1	-25.88	22.6	67.4	198.23
5	6	28.8	30.9	7.29	29.2	29.4	0.68	86.2	89.7	4.06	13.4	40.9	205.22
7	6	29.6	30	1.35	27.3	27.4	0.37	80.6	171.5	112.78	14.2	65.6	361.97
10	6	30.1	30.4	1.00	31.4	31.6	0.64	124.7	136.8	9.70	131.3	137.2	4.49
11	6	29.4	30.4	3.40	30.6	31.1	1.63	96.4	49.9	-48.24	33.5	44.4	32.54
12	6												
14	6												
15	6	27.1	27.7	2.21	29	30.2	4.14	35.7	86.8	143.14	16.7	136.3	716.17
16	0	30.9	33.1	7.12	29.6	29.7	0.34	100.7	104	3.28	23.4	184.3	687.61
16	6	28.5	29.6	3.86	28.4	32.8	15.49	12.4	148.2	1095.16	99.1	128.9	30.07

10.4.6 NERVES trial

Table 63 - Nerve conduction study results (sensory)

Subject		Sural SNAP amplitude (µV)		Sural CV (m/sec)		Superficial peroneal SNAP amplitude (µV)		Superficial peroneal CV (m/sec)	
		Week0	Week10	Week0	Week10	Week0	Week10	Week0	Week10
1	Control	A	3.0	A	41.2	A	A	N/A	N/A
2	Device	4.0	4.1	47.2	42.6	A	A	N/A	N/A
3	Control	3.1	3.1	48.3	54.4	N/A	2.3	N/A	53.2
4	Device	A		A		A		A	
5	Device	A	0.9	A	43.1	A	A	A	A
6	Device	A		A		A	A	A	A
7	Device	A	3.8	A	44.6	A	3.2	A	50.3
8	Control	A	A	A		A	A	A	A
9	Device	A		A		4.9		44.1	
10	Device	1.5		51.1		3.6		43.6	
11	Control	A	3.4	A	41.3	A	A	A	
12	Device	A		A		A		A	
13	Control	A		A		A		A	
14	Device	A		A		A		A	

A=Absent; N/A= Not Applicable; CV= conduction velocity; µV= microVolts; m/sec= meter/second; mV= milliVolts; msec=milliseconds; SNAP= sensory nerve action potential; CMAP= compound muscle action potential; NEMR= No Evokable Motor Response; F-Wave Min= F Wave Minimum latency

Table 64 - Nerve conduction study results (motor)

Subjec		Peroneal CMAP amplitude (mV)		Peroneal CV (m/sec)		Peroneal F Wave-Min (msec)		Tibial CMAP amplitude (mV)		Tibial CV (m/sec)		Tibial F Min (msec)	
		Week0	Week10	Week0	Week10	Week0	Week10	Week0	Week10	Week0	Week10	Week0	Week10
1	Control	2.9	1.5	51.9	47.5	A	57.8	1.1	0.4	41.5	44.8	A	A
2	Device	2.5	2.3	41.4	46.5	A	51.2	2.5	2.5	40.6	45.1	A	53.2
3	Control	N/A	0.9		44.0		A	2.8	2.0	N/A	48.8	A	A
4	Device	NEMR				A		NEMR		NEMR		A	
5	Device	NEMR	NEMR	42.2	41.0	A	A	NEMR	0.2	NEMR	40.1	A	A
6	Device	NEMR	NEMR		NEMR	A	A	NEMR	NEMR	NEMR	NEMR	A	A
7	Device	1.9		41.3	44.9	A	A	2.9	5.3	40.6	42.7	A	59.1
8	Control	NEMR	0.5	NEMR	41.1	A	A	NEMR	NEMR	NEMR	NEMR	A	A
9	Device	0.8		45.4		A		2.4		43.9		A	
10	Device	1.7		40.4		A		2.5		42.5		A	
11	Control	1.6	1.5	49.9	43.7	A	A	NEMR	NEMR	NEMR	NEMR	A	A
12	Device	NEMR				A		NEMR		NEMR			
13	Control	NEMR				A		NEMR		NEMR		A	
14	Device	0.8		36.6		A		0.9		36.7		A	

A=Absent; N/A= Not Applicable; CV= conduction velocity; µV= microVolts; m/sec= meter/second; mV= milliVolts; msec=milliseconds;
SNAP= sensory nerve action potential; CMAP= compound muscle action potential;
NEMR= No Evokable Motor Response; F-Wave Min= F Wave Minimum latency

Table 65 - Nerve conduction study results (H-reflex latency)

Subject		Soleus H-Reflex latency (msec)	
		Week 0	Week 10
1	Control	35.4	37.6
2	Device	38.1	38.0
3	Control	A	A
4	Device	A	
5	Device	A	34.4
6	Device	A	A
7	Device	31.9	32.0
8	Control	A	A
9	Device	A	
10	Device	A	
11	Control	A	A
12	Device	N/A	
13	Control	A	
14	Device	40.4	

A=Absent; N/A= Not Applicable; µV= microVolts; m/sec= meter/second; mV= milliVolts; msec=milliseconds; CV= conduction velocity; SNAP= sensory nerve action potential; CMAP= compound muscle action potential; NEMR= No Evokable Motor Response; F-Wave Min= F Wave Minimum latency

10.5 Trial subject information sheets

Muscle Pump

Information Sheet

You are being invited to take part in a research study at Imperial College London. Before you decide it is important for you to understand why the research is being done and what it will involve.

Please ask if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for your time.

What is the purpose of the study?

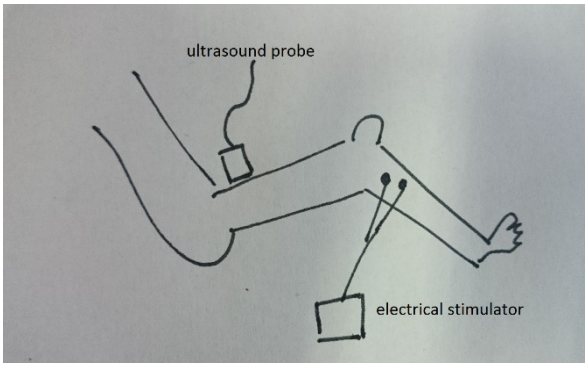
People with cardiovascular problems can be helped by machines that make the leg muscles contract. We want to improve current machines to optimise the beneficial effect by finding the best settings.

Why have I been chosen?

You have been given this sheet as you are a healthy person, with healthy blood vessels. We would like to explore the best settings in healthy people before testing it in patients.

What will happen to me if I take part?

The trial involves one visit to the university campus at Charing Cross (4N13C). It will take roughly one hour, and during this time you will be asked to lie on an examination couch. Adhesive pads will be attached to your leg, and an ultrasound machine will take pictures and video at the top of your leg.



Small pulses of electricity will act on one of the nerves on your leg causing your ankle to flex. We aim to find the optimum setting for doing this.

What are the possible risks of taking part?

We do not anticipate any harm will come to you as a result of this trial.

The device delivering the electricity is licensed for use, and the settings have been deemed safe to use if used according to manufacturer's instructions. If the electrical impulses become too strong then you must tell us, and we will stop the protocol. You are free to withdraw at any time.

Our insurance requires that we exclude pregnant women (screening includes a urinary pregnancy test).

What are the possible benefits of taking part?

There is no financial benefit to taking part.

What if something goes wrong?

You should not join the study if you have an implantable pacemaker or defibrillator device. You may be withdrawn from the study if the investigators feel it is best for you not to participate or if you do not comply with the requirements of the study. If during the health screening tests any abnormal results are found, you will be referred for clinical review as appropriate.

If you feel any discomfort or distress during the study you must say so immediately and we will stop straight away. If for any reason during the study and you do not wish to continue, than we will stop the tests immediately.

Imperial College London has insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the research team (see below). If you are not satisfied with the response, you may contact the Imperial College Joint Research Office.

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the study will be kept strictly confidential. The data collected for the study will be looked at by the research team. They may also be looked at by representatives of regulatory authorities to check that the study is being carried out to the highest professional and ethical standards.

The company that make the device will have access to your trial data for device monitoring purposes.

What will happen to the results of the study?

We will publish the overall results of the study, whatever the findings, to allow others to learn from our findings. As a participant, you will not be personally identifiable in any publication.

Who is organising and funding the research?

The project is organised and funded by Imperial College London.

Special note for students of Imperial College London

The School of Medicine would like to explicitly express that they will not accept participation in the study as mitigation in the event of exam failure.

Further information

We are happy to provide you with any other information, now or later on. You can think about the study and come back to us at any time.

Please contact:

Miss. Kate Williams

k.williams@imperial.ac.uk

0203 311 7335

Academic Section of Vascular Surgery, 4 North, Charing Cross Hospital, London W6 8RF

The Chief investigator for this trial is Prof. Alun Davies, Consultant Vascular Surgeon, Imperial College London. He can be contacted through his secretary at Charing Cross – 020 3311 7320.

Information Sheet for Research Participants

"Revitive" Pilot Trial

You are being invited to take part in a research study. Please ask if there is anything that is not clear or if you would like more information.

What is the purpose of the study?

We would like to evaluate the effect of an electronic device on the legs of healthy subjects. We want to measure its effect on the blood flow in the legs, how safe it is, and how it feels to use it.

Do I have to take part?

Participation in this trial is voluntary.

Tell me some more about the device

The device used for stimulation is the Revitive™ (Actegy Ltd, UK), which delivers a program of 15 preset electrical stimulation patterns (pulse width 450-940µs, 8-69V, 20-50Hz) for 30 minutes, and is CE marked. It consists of two large footpads through which electrical stimulation is delivered to the soles of the feet whilst the user remains in the seated position. The level of stimulus used in each individual will be determined by doubling the minimum stimulus intensity required to evoke visible calf muscle twitching. This is thought to elicit a reasonable yet comfortable compressive force on the underlying blood vessels.

What will happen to me if I take part?

The study lasts approximately 50minutes. The screening process includes:

- Baseline characteristics: height, weight, age
- Medical history
- Short medical examination (vascular examination, ABPI)
- Urinary pregnancy test for females
- Ultrasound examination of both legs to exclude DVT

The protocol requires you to wear shorts to allow ultrasound access to the skin overlying the blood vessels at the top of your leg. Ultrasound measurements will be taken at the beginning, during and after the device use. We will measure your blood pressure, pulse and oxygen levels throughout the study. We will ask you how comfortable the device is.

What are the possible risks of taking part?

We do not anticipate any risk. The device has been through the national testing process and has a CE mark. Its use in this trial is under licence.

If you are pregnant or planning a pregnancy then you should not take part.

What are the possible benefits of taking part?

There are no benefits to you to take part in this trial.

What if something goes wrong?

You should not join the study if you have an implantable pacemaker or defibrillator device. You may be withdrawn from the study if the doctors feel it is best for you not to participate or if you do not comply with the requirements of the study. If during the health screening tests any abnormal results are found, you will be immediately referred for clinical review as appropriate.

Imperial College London has insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the research team (see below). If you are not satisfied with the response, you may contact the Imperial College Joint Research Compliance Office.

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the study will be kept strictly confidential. If you join the study, the data collected for the study will be looked at by the research team. They may also be looked at by representatives of regulatory authorities to check that the study is being carried out to the highest professional and ethical standards.

If an abnormality is found, which does not relate to your veins, a referral to an appropriate specialist will be made, with your permission, and your General Practitioner will be informed.

The company that makes the device will have access to your anonymised trial data.

What will happen to the results of the study?

We will publish the overall results of the study, whatever the findings. As a participant, you will not be personally identifiable in any publication.

Who is organising and funding the research?

The project is organised and funded by Imperial College London.

Further information

We are happy to provide you with any other information, now or later on. You can think about the study and come back to us at any time.

Please contact:

Miss. Kate Williams

k.williams@imperial.ac.uk

0203 311 7335

Academic Section of Vascular Surgery, 4 North, Charing Cross Hospital, London W6 8RF

The Chief investigator for this trial is Prof. Alun Davies, Consultant Vascular Surgeon, Imperial College London. He can be contacted through his secretary at Charing Cross – 020 3311 7320.

Information Sheet for Research Participants

You will be given a copy of this information sheet to keep

Venous occlusion and limb swelling

Short title – "SWELLING"

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve.

Please ask if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for your time.

What is the purpose of the study?

Problems affecting the veins in the legs can cause discomfort and swelling. We want to see how artificially and reversibly blocking the leg veins affects swelling in the lower limb.

Why have I been chosen?

You have been chosen because you are a healthy person with healthy blood vessels.

Do I have to take part?

Participation in this trial is voluntary. Even if you start the trial and change your mind, you are free to withdraw at any point without giving a reason.

What will happen to me if I take part?

The study lasts 20 minutes. If you would like to take part then you will have a medical history and basic medical examination taken by a doctor. If you pass screening by ultrasound to show that your leg blood vessels are healthy then you will be enrolled into the protocol.

You will be required to attend Charing Cross Hospital only once. You will be asked to stand upright whilst a tight tourniquet is applied to your leg. This will be tight enough to stop the veins pumping blood out, but not so tight as to stop your arteries letting blood in. Repeat ultrasound scans of your legs will be taken. We shall also shine a light on your leg to measure the swelling that occurs. The tourniquet will be removed at 10 minutes, or before if you become too uncomfortable to continue. The swelling will resolve fairly quickly as your blood vessels are healthy.

What are the possible risks of taking part?

We do not anticipate any risk. The tourniquet will be uncomfortable but bearable. We will stop the protocol at any time if requested to do so by you.

What are the possible benefits of taking part?

This study should help us to understand the basic science underlying leg swelling in vascular disease.

If you request, we can send you the results of the trial once it is finished.

You will not receive any money for taking part.

What if something goes wrong?

You may be withdrawn from the study if the doctors feel it is best for you not to participate or if you do not comply with the requirements of the study. If during the health screening tests any abnormal results are found, you will be immediately referred for clinical review as appropriate.

If you feel any discomfort or distress during the study you must say so immediately and we will stop straight away. If for any reason during the study you do not wish to continue, than we will stop the tests immediately.

Imperial College London has insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the research team (see below). The normal National Health Service complaints mechanisms are also available to you. If you are not satisfied with the response, you may contact the Imperial College Joint Research Office.

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the study will be kept strictly confidential. If you join the study, some parts of your medical records and the data collected for the study will be looked at by the research team. They may also be looked at by representatives of regulatory authorities to check that the study is being carried out to the highest professional and ethical standards.

If an abnormality is found, which does not relate to your veins, a referral to an appropriate specialist will be made, with your permission, and your General Practitioner will be informed.

What will happen to the results of the study?

We will publish the overall results of the study, whatever the findings, to allow doctors to learn from our findings. As a participant, you will not be personally identifiable in any publication.

Who is organising and funding the research?

The project is organised and funded by Imperial College London.

Further information

We are happy to provide you with any other information, now or later on. You can think about the study and come back to us at any time.

Please contact:

Miss. Kate Williams

k.williams@imperial.ac.uk

0203 311 7335

Academic Section of Vascular Surgery, 4 North, Charing Cross Hospital, London W6 8RF

The Chief investigator for this trial is Prof. Alun Davies, Consultant Vascular Surgeon, Imperial College London. He can be contacted through his secretary at Charing Cross – 020 3311 7320.

Information Sheet for Research Participants

You will be given a copy of this information sheet to keep

Autonomic Activation

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve.

Please ask if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for your time.

What is the purpose of the study?

The purpose of this study is to ascertain the changes that occur in the human nervous system whilst electrical therapy is being applied to the leg. This electrical therapy is currently being used in patients that have problems with the blood vessels in their legs, and it seems to be working better than expected. We would like to explore why this may be.

Why have I been chosen?

You have been chosen as a healthy volunteer. We hope to look at you results and then compare them with the tests we have done on vascular patients to better understand the underlying science.

Do I have to take part?

No – you are under no pressure to take part.

What will happen to me if I take part?

If you take part you will have everything explained to you before starting the protocol. We will ask you to sign a consent form to show that you understand. If you are female we will ask for a urine sample for a pregnancy test. The whole thing will take ~1 hour.

You will be sat on an examination couch and attached to some machines. Some shine laser light on the skin, others measure the activity of your sweat glands. You will also have a small medical cuff attached to monitor your blood pressure, oxygen levels and pulse rate. Please wear comfortable loose fitting clothes.

What are the possible risks of taking part?

There are no risks anticipated. All the equipment is safe to use and has been electrically tested.

What are the possible benefits of taking part?

You are helping ongoing research into cardiovascular diseases at Imperial College London. We cannot offer any financial compensation for your time. If you require transport, a taxi can be arranged.

What if something goes wrong?

You should not join the study if you have an implantable pacemaker or defibrillator device. You may be withdrawn from the study if the investigators feel it is best for you not to participate or if you do not comply with the requirements of the study. If during the health screening tests any abnormal results are found, you will be referred for clinical review as appropriate.

If you feel any discomfort or distress during the study you must say so immediately and we will stop straight away. If for any reason during the study and you do not wish to continue, than we will stop the tests immediately.

Imperial College London has insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the research team (see below). If you are not satisfied with the response, you may contact the Imperial College Joint Research Office.

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the study will be kept strictly confidential. The data collected for the study will be looked at by the research team. They may also be looked at by representatives of regulatory authorities to check that the study is being carried out to the highest professional and ethical standards.

The company that make the device will have access to your trial data for device monitoring purposes.

What will happen to the results of the study?

We will publish the overall results of the study, whatever the findings, to allow others to learn from our findings. As a participant, you will not be personally identifiable in any publication.

Who is organising and funding the research?

The project is organised and funded by Imperial College London.

Special note for students of Imperial College London

The School of Medicine would like to explicitly express that they will not accept participation in the study as mitigation in the event of exam failure.

Further information

We are happy to provide you with any other information, now or later on. You can think about the study and come back to us at any time.

Please contact:

Miss. Kate Williams

k.williams@imperial.ac.uk

0203 311 7335

Academic Section of Vascular Surgery, 4 North, Charing Cross Hospital, London W6 8RF

The Chief investigator for this trial is Prof. Alun Davies, Consultant Vascular Surgeon, Imperial College London. He can be contacted through his secretary at Charing Cross – 020 3311 7320.

Information Sheet for Research Participants

HEALTHY VOLUNTEERS

You will be given a copy of this information sheet to keep

Venous Insufficiency and Neuromuscular Stimulation

Short title - "VeINS"

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve.

Please ask if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for your time.

What is the purpose of the study?

Problems affecting the veins in the legs can cause discomfort, pain and swelling. A new therapy option has recently become available to increase blood flow in the legs, and the aim of this study is to look at its effect on people with vein problems.

Why have I been chosen?

You have been chosen because you are a healthy person with healthy blood vessels. We have shown that we can increase circulation in the legs of people who wear our device on their legs. We think that this may be helpful in people with blood vessel problems, and comparing them with healthy "controls" such as you will help to identify how exactly it does this.

Do I have to take part?

Participation in this trial is voluntary. Even if you start the trial and change your mind, you are free to withdraw at any point without giving a reason. We would be interested in your reasons as we may be able to address similar problems in the future, but you do not have to tell us. By taking part in this study you are helping us to develop an exciting treatment for people with vein problems.

Tell me some more about the device

The device is called Geko™ and is made by Firstkind Ltd, a company based in the UK. It is the size of a wrist watch, self-adhesive, and generates gentle electrical impulses that stimulate your common peroneal nerve (runs underneath the skin by the outside of the knee). These impulses cause the calf muscle pumps to twitch, and it is this twitching that increases the flow of blood in the lower legs – mimicking the blood flow normally achieved by walking but

without having to move or exert energy. We believe it is this increased blood flow that will help the symptoms of venous insufficiency.





What will happen to me if I take part?

The study lasts 8 weeks. If you would like to take part then you will have a medical history and basic medical examination taken by a doctor. If you pass screening by ultrasound to show that your leg blood vessels are healthy then you will be enrolled on the trial.

Once on the trial you will be required to attend Charing Cross Hospital a total of 4 times, and each visit will last around 1 hour. You will have multiple ultrasound scans of your legs, and you will be asked to wear a small device on your leg. This is to look at the way the device helps blood flow, and see if wearing the device for a long period of time adds any benefit to treatment.

You will have blood (approximately 15ml/1 tablespoon) and urine samples taken for us to look at markers of blood vessel function and clotting. This will be done at the beginning and

end of the trial (2 times in total). We will ask you to fill in a quality of life questionnaire at the beginning of the trial, and at weeks 6 and 8.

In between visits to the hospital you will be given a supply of devices that we would like you wear for 6 hours every day. You will be asked to keep a diary of how long each device is activated for, and how comfortable you find the device.

What are the possible risks of taking part?

We do not anticipate any risk. The device has been through the national testing process and has a CE mark. Its use in this trial is under licence.

Activation of the device causes small impulses to travel through the skin to make your foot and calf twitch. It may be an odd sensation but should not hurt. There are seven levels of intensity which can be adjusted for your comfort.

In the past some people have had a skin reaction to the jelly used to apply the device to your leg. If this happens we recommend using a slightly different application site for the device to give the skin a rest. If you continue to react to the jelly then the device use should be discontinued.

If you are pregnant or planning a pregnancy then you should not take part.

What are the possible benefits of taking part?

We know from previous studies that the Geko^{TM} device increases blood flow in healthy people. It is currently being used in elite athletes, under the trade name “Firefly”, to combat post-exercise fatigue, and the London Olympians found them very useful in summer 2012. You are also helping Imperial College London to carry out important research. You will not get paid for participating in this study.

What if something goes wrong?

You should not join the study if you have an implantable pacemaker or defibrillator device. You may be withdrawn from the study if the doctors feel it is best for you not to participate or if you do not comply with the requirements of the study. If during the health screening tests any abnormal results are found, you will be immediately referred for clinical review as appropriate.

If you feel any discomfort or distress during the study you must say so immediately and we will stop straight away. If for any reason during the study and you do not wish to continue, than we will stop the tests immediately.

All of the previous work using the geko™ device has demonstrated it to be safe. When the geko™ device is applying an electrical stimulation, you will feel some muscle twitching and maybe tingling in your lower legs but this will soon pass unnoticed. There are very few risks or dangers involved in using this type of equipment.

If you become pregnant during the course of the study you must stop using the device and let us know. We value your participation, but would have to end your inclusion in our trial at this point. We do not anticipate use of the device causing any harm to either mother or child – we only ask you to stop using it due to licencing laws.

Imperial College London has insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the research team (see below). The normal National Health Service complaints mechanisms are also available to you. If you are not satisfied with the response, you may contact the Imperial College Joint Research Office.

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the study will be kept strictly confidential. If you join the study, some parts of your medical records and the data collected for the study will be looked at by the research team. They may also be looked at by representatives of regulatory authorities to check that the study is being carried out to the highest professional and ethical standards.

If an abnormality is found, which does not relate to your veins, a referral to an appropriate specialist will be made, with your permission, and your General Practitioner will be informed.

The company that make the device will have access to your trial data for device monitoring purposes.

What will happen to the results of the study?

We will publish the overall results of the study, whatever the findings, to allow doctors to learn from our findings. As a participant, you will not be personally identifiable in any publication.

Who is organising and funding the research?

The project is organised and funded by Imperial College London.

Special note for students of Imperial College London

The School of Medicine would like to explicitly express that they will not accept participation in the study as mitigation in the event of exam failure.

Further information

We are happy to provide you with any other information, now or later on. You can think about the study and come back to us at any time.

Please contact:

Miss. Kate Williams

k.williams@imperial.ac.uk

0203 311 7335

Academic Section of Vascular Surgery, 4 North, Charing Cross Hospital, London W6 8RF

The Chief investigator for this trial is Prof. Alun Davies, Consultant Vascular Surgeon, Imperial College London. He can be contacted through his secretary at Charing Cross – 020 3311 7320.

Information Sheet for Research Participants

PATIENTS

You will be given a copy of this information sheet to keep

Venous Insufficiency and Neuromuscular Stimulation

Short title - "VeINS"

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.

Please ask if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for your time.

What is the purpose of the study?

Problems affecting the veins in the legs can cause discomfort, pain and swelling. A new therapy option has recently become available to increase blood flow in the legs, and the aim of this study is to look at its effect on people with vein problems.

Why have I been invited?

You have been chosen either because you have a problem with the veins in your legs. You should have had an ultrasound scan looking at your veins, allowing us to put you in the correct group in the study.

Do I have to take part?

Participation in this trial is voluntary. Even if you start the trial and change your mind, you are free to withdraw at any point without giving a reason. By taking part in this study you are helping us to develop a new treatment for people with vein problems.

Tell me some more about the device

The device is called a Geko™ and is made by Firstkind LTD, a Sky Medical Technology Ltd Company. It's about the size of a man's wrist watch and when attached to the back of the knee and switched on it produces gentle electrical impulses. These impulses cause the calf muscle pumps to twitch, and it is this twitching that increases the flow of blood in the lower legs – mimicking the blood flow normally achieved by walking but without having to move or exert energy. We believe it is this increased blood flow that will help the symptoms of venous insufficiency.





What will happen to me if I take part?

The study lasts 8 weeks. You will be required to attend Charing Cross Hospital a total of 4 times, and each visit will last around 1 hour.

During this time you will have a medical history and examination taken by a doctor. You will have multiple ultrasound scans of your legs, and you will be asked to wear a small device on your leg. This is to look at the way the device helps blood flow, and see if wearing the device for a long period of time adds any benefit to treatment.

You will have blood (around 15ml/1 tablespoon) and urine samples taken at the beginning and end of the trial (2 times in total), for us to look at markers of blood vessel function and clotting.

In between visits to the hospital you will be given a supply of devices that we would like you wear every day for 6 hours. You will be asked to keep a diary of how long each device is activated for, and how comfortable you find the device. We will ask you to fill in a quality of life questionnaire at the beginning of the trial, and at weeks 6 and 8.

What are the possible risks of taking part?

We do not anticipate any risk. The device has been through the national testing process and has a CE mark. Its use in this trial is under licence.

The product is not licenced for use in pregnancy. If you are a woman and interested in our study, we would need to perform a urinary pregnancy test before progressing any further. If you are pregnant or planning a pregnancy then you should not take part.

Activation of the device causes small impulses to travel through the skin to make your foot and calf twitch. It may be an odd sensation but should not hurt. There are seven levels of intensity which can be adjusted for your comfort.

In the past some people have had a skin reaction to the jelly used to apply the device to your leg. If this happens we recommend using a slightly different application site for the device to give the skin a rest. If you continue to react to the jelly then the device use should be discontinued.

What are the possible benefits of taking part?

We know from previous studies that the Geko™ device increases blood flow in healthy people. We expect it to do the same for you. If you have problems with your veins we hope there to be a direct benefit to you. You will not get paid for participating in this study. We unfortunately cannot provide transport or parking costs at this time.

What if something goes wrong?

You may be withdrawn from the study if the doctors feel it is best for you not to participate or if you do not comply with the requirements of the study. If during the health screening tests any abnormal results are found, you will be referred to the most appropriate specialist.

If you feel any discomfort or distress during the study you must say so immediately and we will stop straight away. If for any reason during the study and you do not wish to continue, than we will stop the tests immediately.

All of the previous work using the geko™ device has demonstrated it to be safe. When the geko™ device is active, you will feel some muscle twitching and maybe tingling in your lower legs but this will soon pass unnoticed. There are very few risks or dangers involved in using this type of equipment.

Imperial College London has insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the research team (see below). The normal National Health Service complaints mechanisms are also available to you. If you are not satisfied with the response, you may contact the Imperial College Joint Research Compliance Office: 0203 311 0205.

Pregnancy

If you become pregnant during the course of the study you must stop using the device and let us know. We value your participation, but would have to end your inclusion in our trial at this point. We do not anticipate use of the device causing any harm to either mother or child – we only ask you to stop using it due to licencing laws.

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the study will be kept strictly confidential. If you join the study, some parts of your medical records and the data collected for the study will be looked at by the research team. They may also be looked at by representatives of regulatory authorities to check that the study is being carried out to the highest professional and ethical standards.

If an abnormality is found, which does not relate to your veins, a referral to an appropriate specialist will be made, with your permission, and your General Practitioner will be informed.

The company that make the device (Firstkind Ltd) will have access to your anonymised records.

What will happen to the results of the study?

We will publish the overall results of the study, whatever the findings, to allow doctors to learn from our findings. As a participant, you will not be personally identifiable in any publication.

Who is organising and funding the research?

The project is organised and funded by Imperial College London. This research forms part of a PhD project.

Further information

We are happy to provide you with any other information, now or later on. You can think about the study and come back to us at any time.

Please contact:

Miss. Kate Williams

k.williams@imperial.ac.uk

020 3311 7335

Academic Section of Vascular Surgery, 4 North, Charing Cross Hospital, London W6 8RF

The Chief investigator for this trial is Prof. Alun Davies, Consultant Vascular Surgeon, Imperial College London. He can be contacted through his secretary at Charing Cross – 020 3311 7320.

Information Sheet for Research Participants

You will be given a copy of this information sheet to keep

"Neuromuscular stimulation in the management of intermittent claudication" Short title – "PAD"

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.
Please ask if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for your time.

What is the purpose of the study?

Narrowing of the arteries (peripheral arterial disease) can cause problems with blood circulation. This can cause pain on walking (intermittent claudication), and lead to serious complications such as leg ulcers and leg amputation. It can also be associated with other serious health problems such as heart attack and stroke. Exercise is used to treat intermittent claudication, with an aim to prevent symptoms from getting worse, cause growth of new arteries around blood blockages, and reduce the risk of limb loss and death.

A new electrical device increases circulating blood in the legs of healthy people, and may work in the same way as exercise. We would like to test this in a clinical trial.

Why have I been invited?

You have been chosen because you have intermittent claudication in your legs when you walk.

Do I have to take part?

Participation in this trial is voluntary. Even if you start the trial and change your mind, you are free to withdraw at any point without giving a reason. By taking part in this study you are helping us to develop a potential new treatment for people with peripheral arterial disease.

Tell me some more about the device



The device is called a geko™ and is made by Firstkind Ltd (http://www.gekodevices.com/en-uk/). It's about the size of a wrist watch, and when attached to the leg and switched on it produces gentle electrical impulses. These impulses cause the lower leg muscles to twitch, and it is this twitching that increases the flow of blood in the lower legs – mimicking the blood flow normally achieved by walking but without having to move or exert energy. We believe it is this increased blood flow to the leg nerves that will help the symptoms of intermittent claudication.

What will happen to me if I take part?

The study lasts 6 weeks. You will be required to attend Charing Cross Hospital twice, alongside your normal "best medical therapy" (BMT).

During the first visit you will have a medical history and examination taken by a doctor. If you are a woman of child bearing age we will perform a urinary pregnancy test.

- You will have an ultrasound scan of your legs to see how your blood vessels respond to the device.
- You will also have a treadmill test to see how far you can walk. We will measure two distances – the distance until you start feeling pain (claudication distance), and the maximum distance you can walk until you can go no further (absolute walking distance). This will be compared to a repeat treadmill test at the end of the trial.
- You will have blood (around 10ml/1 tablespoon) and urine samples taken at the beginning and end of the trial (2 times in total), for us to look at markers of blood vessel function and clotting.
- We will ask you to fill in a quality of life questionnaire (about 15mins long) at the beginning and end of the trial.

You will be put into one of two groups – a "device" group, and a "no device" group. This will be decided at random – a sealed envelope containing the words "device" or no device" will be opened at your first visit.

If you are allocated to the "device group" you will be given a supply of devices that we would like you wear on both legs for 4 or more hours per day for 5 days a week. You will be asked to keep a diary of how long each device is activated for, and how comfortable you find the device.

If you are allocated to the "no device" group then we would like to follow you alongside your normal NHS care. By comparing you to those in the other group will tell us exactly how much change in walking distances and pain is due to the device, if any.



We would ask you to let us know if you become unwell, or get admitted to hospital for any reason.

What are the possible risks of taking part?

The device has been through the national testing process and has a CE mark. Its use in this trial is under licence.

Activation of the device causes small impulses to travel through the skin to make your foot and calf twitch. It may be an odd sensation, you may not feel it at all, but should not hurt.

Very rarely, people have a skin reaction to the adhesive jelly used to apply the device to your leg. If this happens we recommend using a slightly different application site for the device to give the skin a rest. If you continue to react to the jelly then the device use should be discontinued.

We have no long term evidence that this is safe to use in people with peripheral arterial disease, but so far we have had no device-related problems related to blood clots or plaque emboli (little pieces of arterial wall buildup dislodging and causing severe blood flow problems in the feet/toes). It is the opinion of the vascular department at Imperial College that using the device is as safe as exercising.

What are the possible benefits of taking part?

We know from previous studies that the geko™ device increases blood flow in healthy people. We expect it to do the same for you, including the blood supply to the muscles in the legs. There may be a positive effect on your walking symptoms, although we do not know yet if this will be the case.

If you have trouble getting to the hospital, we can provide you with a taxi ride to and from the department when you attend.

What if something goes wrong?

You may be withdrawn from the study if the doctors feel it is best for you not to participate or if you do not comply with the requirements of the study. No treatment will be withheld as a result of being on the study.

If you feel any discomfort or distress during the study you must say so immediately and we will stop straight away. If for any reason during the study and you do not wish to continue, than we will stop the tests immediately. Any information or blood/urine up to that point will be retained for analysis for the purposes of the trial.

All of the previous work using the geko™ device has demonstrated it to be safe. When the geko™ device is active, you may feel some muscle twitching and maybe tingling in your lower legs but this will soon pass unnoticed. There are very few risks or dangers involved in using this type of equipment.

Imperial College London has insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the research team (see below). The normal National Health Service complaints mechanisms are also available to you. If you are not satisfied with the response, you may contact the Imperial College Joint Research Compliance Office: 0203 311 0205.

Pregnancy

The product is not licensed for use in pregnancy. If you are pregnant or planning a pregnancy then you should not take part. If you become pregnant during the course of the study you must stop using the device and let us know. We value your participation, but would have to end your inclusion in our trial at this point. We do not anticipate use of the device causing any harm to either mother or child – we only ask you to stop using it due to licencing laws.

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the study will be kept strictly confidential. If you join the study, some parts of your medical records and the data collected for the study will be looked at by the research team. They may also be looked at by representatives of regulatory authorities to check that the study is being carried out to the highest professional and ethical standards.

If an unexpected abnormality is found, a referral to an appropriate specialist will be made. With your permission, your General Practitioner will be informed.

The company that make the device (Firstkind Ltd, UK) will have access to your anonymised records, for device monitoring purposes.

What will happen to the results of the study?

We will publish the overall results of the study, whatever the findings, to allow doctors to learn from our findings. If you request, we will send you the results in the post. As a participant, you will not be personally identifiable in any publication.

Who is organising and funding the research?

The project is organised and funded by Imperial College London. This trial has been reviewed and approved by London South East NHS Research Ethics Committee. This is an educational project for the PhD of Ms Kate Williams.

Further information

We are happy to provide you with any other information, now or later on. You can think about the study and come back to us at any time. Large print versions of written materials are available on request.

Please contact:

Miss. Kate Williams
k.williams@imperial.ac.uk
020 3311 7335
Academic Section of Vascular Surgery, 4 North, Charing Cross Hospital, London W6 8RF

The Chief investigator for this trial is Prof. Alun Davies, Consultant Vascular Surgeon, Imperial College London. He can be contacted through his secretary at Charing Cross – 020 3311 7320.

Information Sheet for Research Participants

You will be given a copy of this information sheet to keep

Electrical Stimulation in Diabetic Foot Ulcers

Short title – "ULCERS"
Ethical approval: NRES South Central, Oxford A (ref - 14/SC/0084)

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.

Please ask if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for your time.

What is the purpose of the study?

Diabetic foot ulcers are estimated to occur in 15% of people with diabetes, which can be slow to heal, difficult to treat, and have a large impact on day to day living. Whilst some heal relatively quickly with good dressings, footcare and the help of your doctor, some are resistant to treatment. Infections of diabetic ulcers are common, and cause over one million leg amputations worldwide every year (60% of all amputations in developed countries).

We are testing a device that increases blood circulation in healthy people. We think it will help diabetic people, especially those with foot ulcers. It may help them heal more quickly, but you may not receive any benefit at all. To provide evidence one way or another we will give half of the people in the trial use of the device, and half will be treated with no changes to normal care.

Why have I been invited?

You have been chosen because you have diabetes and a foot ulcer(s).

Do I have to take part?

Participation in this trial is voluntary. Even if you start the trial and change your mind, you are free to withdraw at any point without giving a reason. By taking part in this study you are helping us to develop a potential new treatment for people with diabetic foot ulcers.

Tell me some more about the device

The device is called a geko™ and is made by Firstkind Ltd. It's about the size of a man's wrist watch and when attached to the leg and switched on it produces gentle electrical impulses. These impulses cause the calf muscle pumps to twitch, and it is this twitching that increases the flow of blood in the lower legs – mimicking the blood flow normally achieved by walking but without having to move or exert energy. We believe it is this increased blood flow to the skin of the leg that will aid ulcer healing.

What will happen to me if I take part?

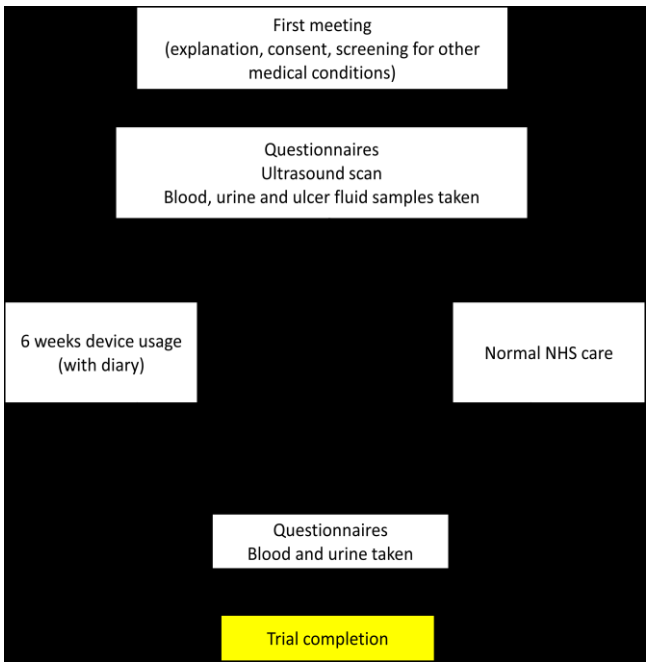
The study lasts 6 weeks. You will be required to attend Charing Cross Hospital for your normal outpatient clinic appointments. During the first visit you will have a medical history and examination taken by a doctor.

- You will have one ultrasound test on your upper leg. This should last about 1 hour.We will monitor the size of your ulcer over the 6 weeks by taking photographs at your normal appointments.
- You will have blood (around 10ml/1 tablespoon) and urine samples taken at the beginning and end of the trial (2 times in total), for us to look at markers of blood vessel function and clotting. We will also take a small amount of fluid from the ulcer itself by using an absorbent piece of sterile paper (2 times total/pea-sized amount)
- We will ask you to fill in a quality of life questionnaire at the beginning and end of the trial.

You will be put into one of two groups – one that wears a device on their leg(s) every day, or one where a device is not used. This will be decided at random – a sealed envelope containing the words "device" or no device" will be opened at your first visit.

If you are allocated to the "device group" you will be given a supply of devices that we would like you wear every day for 4 or more hours per day for at least 5 days a week. Please wear it on the leg with the ulcer. If you have ulcers on both legs please wear a device on each leg. You will be asked to keep a diary of how long each device is activated for, and how comfortable you find the device.

If you are allocated to the "no device" group then we would like to follow you alongside your normal NHS care. By comparing you to those in the other group will tell us exactly how much change in ulcer healing is due to natural causes, and how much is due to the device, if any.



We would ask you to let us know if you become unwell, or get admitted to hospital for any reason.

What are the possible risks of taking part?

We do not anticipate any risk. The device has been through the national testing process and has a CE mark. Its use in this trial is under licence.

The product is not licenced for use in pregnancy (this would take a special application to the MHRA regulatory body). If you are a woman and interested in our study, we would need to perform a urinary pregnancy test before progressing any further. If you are pregnant or planning a pregnancy then you should not take part.

Activation of the device causes small impulses to travel through the skin to make your foot and calf twitch. It may be an odd sensation, you may not feel it at all, but should not hurt. When you try the device for the first time it will be with a qualified doctor in attendance. We do not anticipate any problems.

In the past some people have had a skin reaction to the jelly used to apply the device to your leg. If this happens we recommend using a slightly different application site for the device to give the skin a rest. If you continue to react to the jelly then the device use should be discontinued.

What are the possible benefits of taking part?

We know from previous studies that the geko™ device increases blood flow in healthy people. We expect it to do the same for you, including the blood supply to the skin in and around the ulcer. There may be a positive effect on ulcer healing, although we do not know yet if this will be the case.

If you do not normally get hospital transport, we can provide you with a taxi ride to and from the department when you attend.

What if something goes wrong?

You may be withdrawn from the study if the doctors feel it is best for you not to participate or if you do not comply with the requirements of the study. If during the health screening tests any abnormal results are found, you will be told the result, and referred to the most appropriate specialist and to your GP (with your permission). We do not expect to find any abnormal results. No treatment will be withheld as a result of being on the study.

If you feel any discomfort or distress during the study you must say so immediately and we will stop straight away. If for any reason during the study and you do not wish to continue, than we will stop the tests immediately. Any information or blood/urine/ulcer fluid collected up to that point will be retained for analysis for the purposes of the trial.

All of the previous work using the geko™ device has demonstrated it to be safe. When the geko™ device is active, you may feel some muscle twitching and maybe tingling in your lower legs but this will soon pass unnoticed. There are very few risks or dangers involved in using this type of equipment.

Imperial College London has insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part, you may be eligible to claim

compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the research team (see below). The normal National Health Service complaints mechanisms are also available to you. If you are not satisfied with the response, you may contact the Imperial College Joint Research Compliance Office: 0203 311 0205.

Pregnancy

If you become pregnant during the course of the study you must stop using the device and let us know. We value your participation, but would have to end your inclusion in our trial at this point.

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the study will be kept strictly confidential. If you join the study, some parts of your medical records and the data collected for the study will be looked at by the research team. They may also be looked at by representatives of regulatory authorities to check that the study is being carried out to the highest professional and ethical standards.

Firstkind Ltd will have access to your anonymised records.

What will happen to the results of the study?

We will publish the overall results of the study, whatever the findings, to allow doctors to learn from our findings. If you request, we will send you the results in the post. As a participant, you will not be personally identifiable in any publication.

Who is organising and funding the research?

The project is organised and funded by Imperial College London as part of a PhD project. The devices are supplied by Firstkind Ltd (http://www.gekodevices.com/en-uk/).

Further information

Please contact:

Miss. Kate Williams
k.williams@imperial.ac.uk
020 3311 7335
Vascular Outpatient Reception, 4 North, Charing Cross Hospital, London W6 8RF

The Chief investigator for this trial is Prof. Alun Davies, Consultant Vascular Surgeon, Imperial College London. He can be contacted through his secretary at Charing Cross – 020 3311 7320.

Information Sheet for Research Participants

You will be given a copy of this information sheet to keep

Electrical Stimulation in Diabetic Peripheral Neuropathy

Short title – "NERVES"

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully.

Please ask if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. Thank you for your time.

What is the purpose of the study?

Peripheral neuropathy is a dysfunction of the nerves most commonly affecting the arms and legs that often affects people with diabetes (20-50%). It has a major impact on day to day living, and treatments for it are currently limited to pain control. We think that our device might mimic the effect of exercise, which has been shown to help to slow down progression of peripheral neuropathy.

Why have I been invited?

You have been chosen because you have diabetes and peripheral neuropathy.

Do I have to take part?

Participation in this trial is voluntary. Even if you start the trial and change your mind, you are free to withdraw at any point without giving a reason. By taking part in this study you are helping us to develop a potential new treatment for people with peripheral neuropathy.

Tell me some more about the device

The device is called a Geko™ and is made by Firstkind Ltd. It's about the size of a man's wrist watch and when attached to the leg and switched on it produces gentle electrical impulses. These impulses cause the calf muscle pumps to twitch, and it is this twitching that increases the flow of blood in the lower legs – mimicking the blood flow normally achieved by walking but without having to move or exert energy. We believe it is this increased blood flow to the leg nerves that will help the symptoms of peripheral neuropathy.

What will happen to me if I take part?

The study lasts 10 weeks. You will be required to attend Charing Cross Hospital a total of 2 times, and each visit will last around 1 hour.

During the first visit you will have a medical history and examination taken by a doctor. You will be asked about and tested for other things that can cause similar problems in leg nerves

(such as high alcohol intake, HIV, and syphilis). Any positive findings will be discussed with you directly by a doctor on the team. We can then discuss what you would like done with that information. You would not be able to participate in the trial beyond this point.

You will have an ultrasound scan of your legs, and you will then be put into one of two groups – one that wears a device on their legs every day, or one where a device is not used (i.e. 10 people in the study will wear a device to see if it makes a difference, 10 people will not wear a device). This will be decided by sealed envelope, whih will be opened once you are admitted to the study. Inside the envelope will be the instruction "DEVICE" or "NO DEVICE". We will look at the nerve test of your legs that you have had done in the neurophysiology laboratory, and repeat this at the end of the trial, and compare the two groups to each other.

You will have blood (around 10ml/1 tablespoon) and urine samples taken at the beginning and end of the trial (2 times in total), for us to look at markers of blood vessel function and clotting.

In between visits to the hospital you will be given a supply of devices that we would like you wear every day for 4 or more hours per day. You will be asked to keep a diary of how long each device is activated for, and how comfortable you find the device. We would ask you to let us know if you become unwell, or get admitted to hospital for any reason. We will ask you to fill in a quality of life questionnaire at the beginning and end of the trial.

What are the possible risks of taking part?

We do not anticipate any risk. The device has been through the national testing process and has a CE mark. Its use in this trial is under licence.

The product is not licensed for use in pregnancy. If you are a woman and interested in our study, we would need to perform a urinary pregnancy test before progressing any further. If you are pregnant or planning a pregnancy then you should not take part.

Activation of the device causes small impulses to travel through the skin to make your foot and calf twitch. It may be an odd sensation, you may not feel it at all, but should not hurt. When you try the device for the first time it will be with a qualified doctor in attendance. We do not anticipate any problems.

In the past some people have had a skin reaction to the jelly used to apply the device to your leg. If this happens we recommend using a slightly different application site for the device to give the skin a rest. If you continue to react to the jelly then the device use should be discontinued.

What are the possible benefits of taking part?

We know from previous studies that the Geko^{TM} device increases blood flow in healthy people. We expect it to do the same for you, including the blood supply to the nerves in your leg. There may be a positive effect on your peripheral neuropathy, although we do not know yet if this will be the case.

We can provide you with a taxi ride to and from the department when you attend. We unfortunately cannot provide costs for other transport or parking at this time.

In the event that you are randomised to using the device, and you find the device helpful, the company that makes them has committed that it will do everything in its power to provide

devices to you, for personal use, at a reduced rate. If this is the case please contact the trial team who can initiate this process for you.

What if something goes wrong?

You may be withdrawn from the study if the doctors feel it is best for you not to participate or if you do not comply with the requirements of the study. If during the health screening tests any abnormal results are found, you will be told the result, and referred to the most appropriate specialist and to your GP (with your permission).

If you feel any discomfort or distress during the study you must say so immediately and we will stop straight away. If for any reason during the study you do not wish to continue, than we will stop the tests immediately. Any information or blood/urine collected up to that point will be retained for analysis for the purposes of the trial.

All of the previous work using the geko™ device has demonstrated it to be safe. When the geko™ device is active, you may feel some muscle twitching and maybe tingling in your lower legs but this will soon pass unnoticed. There are very few risks or dangers involved in using this type of equipment.

Imperial College London has insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part, you may be eligible to claim compensation without having to prove that Imperial College is at fault. This does not affect your legal rights to seek compensation.

If you are harmed due to someone's negligence, then you may have grounds for a legal action. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been treated during the course of this study then you should immediately inform the research team (see below). The normal National Health Service complaints mechanisms are also available to you. If you are not satisfied with the response, you may contact the Imperial College Joint Research Compliance Office: 0203 311 0205.

Pregnancy

If you become pregnant during the course of the study you must stop using the device and let us know. We value your participation, but would have to end your inclusion in our trial at this point. We do not anticipate use of the device causing any harm to either mother or child – we only ask you to stop using it due to licencing laws.

Will my taking part in this study be kept confidential?

All information that is collected about you during the course of the study will be kept strictly confidential. If you join the study, some parts of your medical records and the data collected for the study will be looked at by the research team. They may also be looked at by representatives of regulatory authorities to check that the study is being carried out to the highest professional and ethical standards.

If an abnormality is found, which does not relate to your veins, a referral to an appropriate specialist will be made, with your permission, and your General Practitioner will be informed.

The company that make the device (Firstkind Ltd) will have access to your anonymised records.

What will happen to the results of the study?

We will publish the overall results of the study, whatever the findings, to allow doctors to learn from our findings. If you request, we will send you the results in the post. As a participant, you will not be personally identifiable in any publication.

Who is organising and funding the research?

The project has been ratified by the National Research Ethics Committee (Dulwich), reference 13/LO/1844. The project is organised and funded by Imperial College London. The devices are being supplied free of charge by the manufacturer.

Further information

We are happy to provide you with any other information, now or later on. You can think about the study and come back to us at any time.

Please contact:

Miss. Kate Williams

k.williams@imperial.ac.uk

020 3311 7335

Academic Section of Vascular Surgery, 4 North, Charing Cross Hospital, London W6 8RF

The Chief investigator for this trial is Prof. Alun Davies, Consultant Vascular Surgeon, Imperial College London. He can be contacted through his secretary at Charing Cross – 020 3311 7320.

10.6 Associated academic activities

10.6.1 Publications

10.6.1.1 Peer-reviewed journal articles

- "Compression stockings versus neuromuscular electrical stimulation devices in the management of occupational leg swelling" J Wou, KJ Williams, AH Davies. Int J Angiol (IN PRESS)
- "The use of a novel neuromuscular electrical stimulation device in peripheral vascular disease."Williams KJ, Davies AH. Int J Case Rep Images 2014;5(11):744–747
- "The effect of footplate neuromuscular electrical stimulation on venous and arterial haemodynamics". Varatharajan, L, Williams, K, Moore, H, Davies, A. H. Phlebology July 2014
- "Haemodynamic changes with the use of neuromuscular electrical stimulation compared to intermittent pneumatic compression". Williams KJ, Moore HM, Davies AH. Phlebology 2014
- "Chronic deep venous valve management in the future". Moore HM, Williams KJ, Onida S, Davies A. H. Italian Journal of Vascular and Endovascular Surgery, March 2014, 21(1):11-5
- "The calf muscle pump revisted". Williams KJ, Ayekoloye O, Moore HM, Davies AH. J Vasc Surg: Venous and Lym Dis, Jan 2014 P1-6
- "Massive localised lymphoedema: a rare vascular malformation". KJ Williams, MN Al-Sakkal, A Alsafi, AH Davies. BMJ Case Rep Jun 2013, PMID 23761611
- "Das massive loklisierte Lymphödem – eine seltene vaskuläre Malformation". KJ Williams, MN Sakkal, A Alsafi, AH Davies. Vasomed 2014 26(1):46-47
- "A guide to oncological management of soft tissue tumours of the abdominal wall". KJ Williams, AJ Hayes. Hernia Sept 2013, PMID: 24043554

10.6.1.2 Published Abstracts

- "Comparative accuracy and reliability of 3D cameras in simulated wound measurement" KJ Williams, AH Davies. BJS 2015; 102(S5):47
- "A comparative study of the effect of graduated compression stockings and neuromuscular stimulation devices in the management of occupational leg oedema" J Wou, KJ Williams, AH Davies **(Prizewinner - Poster of distinction)** BJS 2015; 102(S5):28
- "Can neuromuscular stimulation relieve symptoms of chronic venous disease, and improve quality of life?" R Ravikumar, KJ Williams, A Babber, AH Davies. BJS 2015; 102(S5):9

- "Electrical stimulation of the foot pump: a pilot randomised controlled trial in patients with chronic venous insufficiency" R Ravikumar, KJ Williams, A Babber, AH Davies. BJS 2015; 102(S5):6
- "Potential clinical applications of electrical muscle stimulation in venous disease" R Ravikumar, KJ Williams, AH Davies. BJS 2015; 102(S5):45
- "Investigating peripheral haemodynamics during neuromuscular stimulation" L Varatharajan, KJ Williams, HM Moore, AH Davies. BJS 2014; 101(S4):25
- "Comparison of intermittent pneumatic compression and neuromuscular electrical stimulation using ultrasound and laser Doppler fluximetry" **(Presidents Poster Prize winner)** KJ Williams, HM Moore, AH Davies. BJS 2014;101(S4):1
- "Comparing the venous haemodynamic effect of a neuromuscular stimulation device to intermittent pneumatic compression in healthy subjects" (abstract 1.5). KJ Williams, HM Moore, M Ellis, AH Davies. Phlebology 2013:28:375-394
- "Intermittent pneumatic compression and neuromuscular electrical stimulation of the leg: Venous haemodynamic effects". K Williams, HM Moore, M Ellis, AH Davies. Phlebology 2013 (28):324-338
- "Comparing the haemodynamic effect in healthy subjects of a neuromuscular stimulation device to intermittent pneumatic compression". KJ Williams, M Ellis, H Moore, AH Davies. Int Angiol 2013 (32) Supp 1-5: 33

I also peer-review journal articles for "Ultrasound in Medicine and Biology", "Phlebology", and "Liver".

10.6.1.3 Book chapters

- "Radiofrequency Ablation of Varicose Veins" KJ Williams, IJ Franklin. Advances in Venous Surgery and Phlebology (Prof M Whiteley)
- "The Deep Venous Valve Management in the Future" HM Moore, KJ Williams, AH Davies. Vascular Updates and Insights, CX Symposium 2014 (Prof R Greenhalgh)

10.6.2 Presentations

10.6.2.1 International

- "Pilot Randomised Control Trial: Neuromuscular Electrical Stimulation in Treating Venous Disease."
  Ravikumar R, Williams KJ, Babber A, Lane TRA, Moore HM, Davies AH.
  European Society for Vascular Surgery (ESVS) annual meeting, Porto, Portugal. September 2015
- "Neuromuscular Electrical Stimulation for prevention of venous thromboembolism"
  Ravikumar R, Williams KJ, Babber A, Lane TRA, Moore HM, Davies AH.
  European Venous Forum, St Petersburg, Russia. July 2015. Oral
- "Prevention and management of vascular disease with neuromuscular stimulation"
  Williams KJ, Ravikumar R, Babber A, Davies AH

CX Venous Workshop. 37th Charing Cross International Vascular Symposium 2015, London, UK, April 2015. *Invited oral presentation

- "A pilot trial of neuromuscular stimulation in the management of patients with chronic venous disease"
  KJ Williams, A Babber, R Ravikumar, AH Davies
  Annual Meeting of American Venous Forum, Palm Springs, CA, USA, Feb 2015
- "Potential clinical applications for Electrical Muscle Stimulation in venous disease" (poster presentation)
  Ravikumar R, Williams KJ, Babber A, Davies AH
  Annual Meeting of American Venous Forum, Palm Springs, CA, USA, Feb 2015
- "Electrical stimulation of the foot pump: A pilot randomised control trial in patients with chronic venous insufficiency" (poster presentation)
  Ravikumar R, Williams KJ, Babber A, Davies AH
  Annual Meeting of American Venous Forum, Palm Springs, CA, USA, Feb 2015
- "The use of inferior vena cava filters in percutaneous endovascular intervention for proximal DVT"
  Busuttil A, Williams KJ, Davies AH
  Annual Meeting of American Venous Forum, Palm Springs, CA, USA, Feb 2015
- "Pilot data from a trial of neuromuscular electrical stimulation in chronic venous disease"
  KJ Williams, AH Davies
  Annual Meeting of the Australasian College of Phlebology, Noosa, QL, Australia, Feb 2015
- "Automated quantification of tissue perfusion in contrast enhanced ultrasound images: Initial in vitro and in vivo evaluation of lower limb perfusion"
  WK Cheung, KJ Williams, K Christensen-Jeffries, B Dharmarajah, RJ Eckersley, AH Davies, MX Tang
  20th European Symposium on Ultrasound Contrast Imaging, Rotterdam, The Netherlands, Jan 2015
- "Simulated wound assessment and clinical application" **(Prize winner)**
  KJ Williams, AH Davies
  RSM Section of Surgery Winter Meeting, Champoluc, Italy, Jan 2015
- "Neuromuscular stimulation and its effect on venous haemodynamics of the leg in chronic venous disease"
  KJ Williams, AH Davies
  RSM Section of Surgery Winter Meeting, Champoluc, Italy, Jan 2015
- "Comparison of intermittent pneumatic compression and neuromuscular stimulation using ultrasound and laser Doppler fluximetry"
  KJ Williams, AH Davies
  American Venous Forum, New Orleans, AL, USA, Feb 2014
- "Neuromuscular stimulation in the lower limbs" **(Prize winner)**
  KJ WilliamsRSM Winter Meeting, Champoluc, Italy Feb 2014
- "Comparison of intermittent pneumatic compression and neuromuscular stimulation using ultrasound and laser Doppler fluximetry"
  KJ Williams, AH Davies
  VEITH, New York Dec 2013
- "The Calf Muscle Pump Revisited "

KJ Williams, O Ayekoloye, HM Moore, AH Davies
VEITH, New York, Nov 2013

- "Venous Haemodynamics and Electrical Neuromuscular Stimulation – Preliminary Data"
  KJ Williams, HM Moore, M Ellis, AH Davies
  XVII International Union of Phlebology World Meeting, Boston, MA, USA, Sept 2013
- "Comparative Effectiveness in the Treatment of Venous Disease" (Invited)
  Williams KJ, Davies AH
  XVII International Union of Phlebology World Meeting, Boston, MA, USA, Sept 2013
- "Comparing the haemodynamic effect of a neuromuscular stimulation device to intermittent pneumatic compression in healthy subjects"
  KJ Williams, HM Moore, M Ellis, AH Davies
  14th Meeting of the European Venous Forum, Belgrade, Serbia, June 2013
- "Venous Haemodynamics and Electrical Neuromuscular Stimulation: Preliminary Data" **(Prize winner)**
  Williams KJ, Moore HM, Davies AH
  Australasian College of Phlebology 16th Annual Scientific Meeting, Hobart, Tasmania, Australia, March 2013

10.6.2.2 National

- "Thromboprophylaxis With Neuromuscular Electrical Stimulation (NMES): A Meta-Analysis of Imaging Proven Venous Thromboembolism"
  Ravikumar R, Williams KJ, Babber A, Lane TRA, Moore HM, Shalhoub J, Davies AH.
  Venous Forum, Royal Society of Medicine, London. May 2015 . Oral
- "Neuromuscular Electrical Stimulation in Treating Venous Disease: A Pilot Randomised Control Trial."
  Ravikumar R, Williams KJ, Babber A, Lane TRA, Moore HM, Davies AH.
  Venous Forum, Royal Society of Medicine, London. May 2015. Poster presentation
- "A comparative study of the effect of graduated compression stockings and neuromuscular stimulation devices in the management of occupational leg oedema" **(Poster of Distinction - Prize winner)**
  J Wou, KJ Williams, AH Davies,
  SARS Annual Meeting, Durham, Jan 2015
- "Can neuromuscular stimulation relieve the symptoms of chronic venous disease, and improve quality of life?"
  KJ Williams, R Ravikumar, A Babber, AH Davies
  SARS Annual Meeting, Durham, Jan 2015
- "Comparative accuracy and reliability of 3D cameras in simulated wound measurement"
  KJ Williams, AH Davies
  SARS Annual Meeting, Durham, Jan 2015
- "Potential Clinical Applications for Electrical Muscle Stimulation in Venous Disease"
  R Ravikumar, KJ Williams, AH Davies
  SARS Annual Meeting, Durham, Jan 2015

- "Electrical stimulation of the foot pump: A pilot randomised control trial in patients with chronic venous insufficiency" **(Patey Prize Session)**
  R Ravikumar, KJ Williams, A Babber, AH Davies
  SARS Annual Meeting, Jan 2015
- "Pilot trial of neuromuscular stimulation in the management of chronic venous disease"
  KJ Williams, A Babber, R Ravikumar, M Ellis, AH Davies
  Vascular Society Annual Scientific Meeting, Glasgow, UK. Nov 2014
- "Management of occupational leg oedema - A comparison study of graduated compression stockings and neuromuscular stimulation devices"
  J Wou, KJ Williams, AH Davies
  RSM President's Day, London, Nov 2014
- "A pilot trial using neuromuscular leg stimulation in the management of chronic venous disease" **(Prize winner)**
  KJ Williams, R Ravikumar, A Babber, M Ellis, AH Davies
  RSM President's Day, MIA Trainee Session, Nov 2014
- "Pilot Randomised Control Trial: The Effect of Electric Stimulation in Patients with Chronic Venous Insufficiency"
  R Ravikumar, KJ Williams, AH Davies
  Presidents Day and Venous Forum Meeting, Oct 2014
- "Comparison of intermittent pneumatic compression and neuromuscular stimulation using ultrasound and laser Doppler fluximetry"
  KJ Williams, AH Davies
  SARS, Cambridge Jan 2014, (Abstract - British Journal of Surgery)
- "Intermittent pneumatic compression and neuromuscular stimulation of the leg: venous haemodynamic effects"
  Williams KJ, Moore HM, Ellis M, Davies AH
  Venous Forum, Royal Society of Medicine, London, May 2013
- "The effect of neuromuscular stimulation on peripheral haemodynamics"
  Varatharajan L, Moore H, Williams K, Davies AH
  Venous Forum, Royal Society of Medicine, London, May 2013
- "Abscess pathway – Reducing cancellation rate on CEPOD list" **(Norman Tanner Prize Session)**
  Williams KJ, Blackwood D, Byrne C
  Royal Society Medicine, London, Oct 2012

10.6.2.3 Regional

- "Implementation of a medical device"
  KJ Williams
  Surgery and Anaesthesia BSc Course, Imperial College London Medical School, London, Jan 2015
- "Reading clinical papers – Layers of evidence"
  KJ Williams, A Busuttil

Surgery and Anaesthesia BSc Course, Imperial College London Medical School, London, Sept 2014

- "Evidence appraisal, and practical application"
  KJ Williams
  Surgery and Anaesthesia BSc Course, Imperial College London Medical School, London, Sept 2014
- "Reading a clinical paper"
  KJ Williams, B Dharmarajah
  Surgery and Anaesthesia BSc Course, Imperial College London Medical School, London, Sept 2013
- "Vascular disease and medical devices" **(Prize winner)**
  Williams KJ
  Imperial College International Research Development Conference, UK, Jan 2013

10.6.3 Grants

Graham-Dixon Foundation Research Grant (£5,600)

"Evaluation of Musculoskeletal Microcirculation with Ultrasound"