

The role of formula low energy diets in Type 2 diabetes mellitus patients treated with insulin

A thesis submitted for the degree of Doctor of Philosophy
Imperial College London

Adrian Brown BSc MSc

2018

Nutrition and Dietetic Research Group

Section of Investigative Medicine

Faculty of Medicine

Abstract

Type 2 diabetes mellitus (T2D) and obesity are two of the key health challenges of modern society. With an ageing population and increasing life expectancy, advanced T2D, including those treated with insulin, is now frequent within clinical practice. Effective treatment of glycaemic control in those treated with insulin relies at present on insulin intensification, that although effective at managing blood glucose, is associated with weight gain and an increased risk of cardiovascular disease (CVD) mortality.

Bariatric surgery has been shown to be one of the most effective treatments for obesity and T2D. One key mechanism by which bariatric surgery aids diabetes control is through energy restriction. The use of formula low energy diet (LED) to replicate this and attendant significant weight loss has been observed to bring about T2D remission in those with recently diagnosed T2D patients. However, evidence for the use of these formula diets is lacking in those T2D patients concurrently treated with insulin.

The work presented in this thesis aimed to investigate the role of formula low energy diets in obese type 2 diabetes patients concurrently treated with insulin.

Investigation 1 studied the effect of a formula LED, over a 24-week period, on weight loss, insulin usage, and glycaemic control in obese T2D patients treated with insulin compared with gold standard clinical care (GSC). The presented data demonstrates that those on an LED had greater weight loss, improvement in HbA1c, and reduction in insulin usage compared to GSC.

Investigation 2 studied the effect of weight loss induced by an LED on surrogate markers of CVD. Compared with GSC, there were key reductions in triglycerides, diastolic blood pressure and high sensitivity C-reactive protein. Based on CVD risk algorithms, there may be a CVD benefit of weight loss through LED, at least in the short term.

The work presented in the thesis helps to support the use of formula LED as a therapeutic treatment for complex T2D patients. The exact mechanisms by which the LED brings about the observed improvements in glycaemic control and reduction in insulin usage remains to be determined.

Copyright Declaration

The copyright of this thesis rests with the author and is made available under a 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, that they do not use it for commercial purposes and that they do not alter, transform or build upon it. For any reuse or redistribution, researchers must make clear to others the licence terms of this work.

Declaration of Contributors

I confirm that the author performed all the work described in this thesis, except where referenced. All collaboration and assistance are detailed below:

Statistical analysis of the study data was performed with the kind assistance of Mr Omar Omar (Clinical Research Core, Weill Cornell Medicine – Qatar).

The formula low energy diet products were provided by Cambridge Weight Plan Ltd free of charge.

Clinical studies were performed with the help of the research staff at both the NIHR Wellcome Trust Imperial College Clinical Research Facility and at the Diabetes Centre at Guy's and St Thomas' Hospital NHS Trust.

All radioimmunoassays were performed under the guidance Dr Edward Chambers, Dr Paul Bech and Dr Georgina Becker, with assistance from Dr Claire Byrne, Dr Neomi, Gonzalez Abuin, Katerina Petropoulou, Anna Cherta Murillo and Mai Khatib. Glucose assays were performed with the assistance of Katerina Petropoulou, Dr Ben Jones and Dr Edward Chambers.

List of publications

Reviews

Brown, A. & Taheri, S. (2018) Very low energy diets for weight loss in patients with kidney disease *Journal of Kidney Care* Vol 3, no.1

Brown, A, Guess, N., Dornhorst, A., Taheri, S., Frost G. (2017) Insulin-associated weight gain in obese type 2 diabetes mellitus patients: What can be done? *Diabetes, Obesity and Metabolism*, May 16 [Epub ahead of print]

Brown, A., Frost, G., Taheri, S. (2016) Using low energy diets in weight management: is there a place? *British Journal of Obesity*, 1, No. 3, pages 84-119

Abstracts

Brown AC, Taheri S, Dornhorst A, McGowan B, Leeds AR, Frost G. (2016) Low energy formula diet produces greater improvements in weight and glycaemic outcomes in obese patients with long-standing insulin-treated type 2 diabetes at 24-weeks compared to gold standard intensive lifestyle management

Brown AC, Taheri S, Dornhorst A, Kapoor N, McGowan B, Leeds AR, Frost G. (2016) Using a low energy formula diet in obese patients with long-standing insulin-treated Type 2 diabetes produces significantly greater weight loss, improvement in glucose control and insulin reductions compared to gold standard clinical care over a 12 week period, *Diabetic Medicine*, Vol: 33, Pages: 69-69

Acknowledgements

I would firstly like to thank Professor Gary Frost for all his help and support over the last four years, for being a true inspiration as a leading academic dietitian and supervisor, and for allowing me to work independently while also being supportive. A special thank you to my second supervisor Professor Shahradsad Taheri for the opportunity to pursue a PhD. You have been incredibly supportive throughout my PhD and over the last 10 years. Thank you for offering constant guidance and advice while enabling me to grow both as a researcher and person. I also wish to thank my other supervisor Professor Anne Dornhorst who has provided medical expertise, reviewing my work and being always ready with advice and help with any situation. Your enthusiasm for diabetes care is inspiring. I am also very grateful to Cambridge Weight Plan for helping to fund this work and supporting me to present my results at international conferences. Of note, I would like to thank Professor Anthony Leeds for all his expertise and extra support. You are all inspirational and have taught me more than you imagine.

I am grateful to Dr Barbara McGowan for all her help and extra support while working at Guy's Hospital and facilitating the recruitment of patients in South London. Without this, the study would not have reached recruitment target, so thank you so much. I would also like to thank Dr Edward Chambers for his help and guidance over the last 4 years and always providing a listening ear.

Huge thanks go to Omar Omar for all his support with the statistical analysis of the study; you made statistics understandable and making sure that at the end all the hard work was analysed correctly. I would like to acknowledge the following MRes students for their help with data collection and the running of study visits; of particular note, I would like to thank Natasha Kapoor who helped right at the start of my PhD and without whom I am not sure the study would not have been a success.

Thank you to all the participants for sticking with me throughout the study, as without your participation this thesis would not have been possible.

A special mention goes to all the members of staff within the NIHR Imperial College Clinical Research Facility and within the Diabetes Research Team at Guy's Hospital for all their

support. This thesis would not have been possible without your help and assistance. I can't mention everyone, but I would like to highlight a couple of people, Janette, Elizabeth and Bev for booking in all the participants, giving me a laugh and bearing with me when things didn't go to plan. Thank you to Oky for being a good friend and for helping with all the laboratory work throughout my thesis; your knowledge and help has been truly appreciated. Finally, to Richard for being a true expert and deftly cannulating people that no-one else could. At Guy's Hospital, a particular shout out goes to Dr Nikolaos Fountoulakis and Dr James Crane whose knowledge and support with the patients was incredible and without whom no cannula would have been placed.

Many thanks to my colleagues and friends Claire, Anya, Katerina, Yateen, Phil, Maria, Milena, Neomi, Anjali, Mariana and everyone else on the 6th floor and Research Unit for being with me throughout this journey and helping to bring some fun into the day when at times I struggled, and maybe a drink or two.

A final thank you goes to all my friends, both new and old, who have been through this journey with me over the last few years. There have been a lot of ups and downs, but with your help and listening ears it has been possible to keep moving forward. Thank you to Matt and Joanne for always being there and a big thank you to John for giving your invaluable feedback - your drive and intelligence are a constant motivation.

Finally, I would like to thank my parents for all their support and unwavering belief throughout my time at university. Without you, I would not have been able to get where I am and for that I will be eternally grateful; I love you.

Abbreviations

A160	A160 subunit of protein kinase B
α -cell	Alpha-cell
α subunits	Alpha subunits
ACCORD	Action to Control Cardiovascular Risk in Diabetes
ACR	Albumin to creatinine ratio
ADHD	Attention deficit hyperactivity disorder
ADD	Attention deficit disorder
ADVANCE	Action in Diabetes and Vascular Disease: Preterax and Diamicon Modified Release Controlled Evaluation trial
AgRP	Agouti related peptide
AHEAD	Action for Health in Diabetes
AHI	Apnoea hypopnoea index
Akt/PKB	Protein kinase B
Alk Phos	Alkaline phosphatase
ALT	Alanine transaminase
ANOVA	Analysis of variance
AS160	Protein kinase B substrate 160
ATP	Adenosine triphosphate
AUC	Area under the curve
β -cell	Beta-cell
β subunits	Beta subunits
BDA	British Dietetic Association
BIA	Bioelectrical impedance analysis
BMI	Body mass index
BMR	Basal metabolic rate
bn	Billion
BP	Blood pressure
°C	Degrees centigrade
Ca ²⁺	Calcium
CART	Cocaine and amphetamine regulated transcript
CAS	Composite appetite score
CHD	Coronary Heart Disease

CHO	Carbohydrate
CI	Confidence intervals
CKD	Chronic kidney disease
cm	centimetres
CoA	Coenzyme A
CONGA	Continuous overlapping net glycaemic action
CRN	Clinical Research Network
CRF	Clinical Research Facility
CV	Co-efficient of variation
CVD	Cardiovascular Disease
DAG	Diacylglycerol
DARE	Diabetes Alliance for Research in England
DAWN	Diabetes Attitudes, Wishes and Needs
DCCT/EDIC	Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study
DBP	Diastolic blood pressure
DEBQ	Dutch Eating Behaviour Questionnaire
DEXA	Dual-energy x-ray absorptiometry
DI _o	Oral Disposition index
DIOGENES	Diet, Obesity and Genes
DiRECT	Diabetes in Remission clinical trial
DKD	Diabetes kidney disease
DNA	Deoxyribonucleic acid
DPP	Diabetes Prevention Programme
DRI	Daily recommended index
DRN	Diabetes Research Network
ECG	Electrocardiogram
ED	Energy deficit
EDTA	Ethylenediaminetetraacetic acid
EE	Energy Expenditure
EFSA	European Food Safety Authority
eGFR	Estimated glomerular Filtration Rate
EGP	Endogenous glucose production
EPIC	European Prospective Investigation into Cancer

EQ5D	EuroQol 5 dimensions
ER	Endoplasmic reticulum
ESS	Epworth sleepiness scale
FADH ₂	Flavin adenine dinucleotide
FBC	Full blood count
FBG	Fasting blood glucose
FFA	Free fatty acid
FFM	Fat free mass
FM	Fat mass
FOXO	Forkhead box 0
FP	1 st phase insulin response
FPG	Fasting plasma glucose
FSI	Fasting serum insulin
δ-cells	delta-cells
g	Grams
G6P	Glucose-6-phosphate
GCP	Good clinical practice
GI	Glycaemic index
GIP	Gastric inhibitory polypeptide
GIIS	Glucose induced insulin secretion
GLP-1	Glucagon-like peptide-1
GLUT	Glucose transporter
GP	General practitioner
GS	Glycogen synthesis
GSC	Gold standard clinical care
GSK-3	Glycogen synthase kinase 3
GV	Glucose variability
HbA1c	Glycated haemoglobin
HDL-C	High density lipoprotein-cholesterol
HGP	Hepatic glucose production
HOMA-IR	Homeostatic model assessment – Insulin resistance
HR	Hazard ratio
hsCRP	High sensitive c-reactive protein
IAWG	Insulin associated weight gain

ICF	Informed consent form
ID	Identification
IGI	Insulinogenic index
IMCL	Intramyocellular lipids
IN	Intermittent
INR	International Normalising Ratio
IQR	Interquartile range
IR	Insulin resistance
IRS	Insulin receptor substrate
ISGU	Insulin stimulated glucose uptake
ISRCTN	ISR controlled-trial database
IT	Insulin therapy
K _{ATP}	Potassium Adenosine Triglyceride
Kcal	Kilocalories
Kg	kilogram
Kg/m ²	kilogram per metre squared
LC-CoA	Long chain fatty acyl-CoA
LDL-C	Low density lipoprotein cholesterol
LED	Low Energy Diet
LFT	Liver function tests
μl	microliters
MACE	Major adverse cardiovascular events
mg/dL	milligrams per decilitre
mmHg	millimetre of mercury
mmol/l	millimoles per litre
mmol/mol	millimoles per mole
MMTT	Mixed meal tolerance test
MODIFY	Management of Diabetes in Future Years
NADH	Nicotinamide adenine dinucleotide
NASH	Non-alcoholic steatohepatitis
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NIHR	National Institute for Health Research
NPH	Neutral Protamine Hagedorn

NPY	Neuropeptide Y
NS	Non-significant
OA	Osteoarthritis
ODI	Oxygen desaturation index
OECD	Organisation for Economic Co-operation and Development
OHA	Oral Hypoglycaemic Agents
OSA	Obstructive sleep apnoea
P	Phosphorylation
PAL	Physical activity level
PDH	Pyruvate dehydrogenase
PDK	Phosphatidylinositol-dependent protein kinase
PI-3K	Phosphatidylinositide-3 kinase
PIP2	Phosphatidylinositols-4,5-bisphosphate
PIP3	Phosphatidylinositols-3,4,5-triphosphate
PIR	Psychological insulin resistance
PIS	Subject information sheet
pmol/L	picomole/litre
POMC	Pro-opiomelanocortin
PRI	Population recommended intake
PSQI	Pittsburgh Sleep Quality Index
PTH	Parathyroid hormone
QoL	Quality of life
QUICKI	Quantitative insulin sensitivity check index
RCT	Randomised Control Trial
RE	Regular
RIA	Radioimmunoassay
RMR	Resting metabolic rate
RPM	Revolutions per minute
RR	Relative risk
RYGB	Roux-en-Y gastric bypass
SBP	Systolic blood pressure
s.c.	Subcutaneous administration
SD	Standard deviation
SEM	Standard error of the mean

SGLT2	Sodium glucose co-transporter-2
SOS	Swedish Obese Subjects Study
SP	2 nd phase insulin response
SUR1	Sulfonylurea receptor
T2D	Type 2 diabetes mellitus
TC	Total cholesterol
TCA	Tricarboxylic acid
TDR	Total diet replacement
TEE	Total energy expenditure
TG	Triglyceride
TNF- α	Tumour necrosis factor-alpha
U	Units
U+E	Urea and electrolytes
U/kg	Units per kilogram
UK	United Kingdom
UKCRN	National Institute for Health Research Clinical Research Network
UKPDS	United Kingdom Prospective Diabetes Study
VAS	Visual analogue scale
VDCC	Voltage dependent calcium channels
VLED	Very Low Energy Diet
VLED-E	Very low energy diet with exercise
VO ₂ peak	Peak oxygen uptake
UKPDS	United Kingdom Prospective Diabetes Study
WC	Waist circumference
WHO	World Health Organization
WHR	Waist to hip ratio

Table of Contents

Abstract	2
Copyright Declaration.....	3
Declaration of Contributors	4
List of publications	5
Acknowledgements.....	6
Abbreviations.....	8
Table of Contents.....	14
Index of Figures	19
Index of Tables	21
Chapter 1: Introduction	22
1.1. Diabetes - Type 2 diabetes and Obesity	23
1.1.1. Definition and classification of diabetes.....	23
1.1.2. Definition and classification of obesity	25
1.2. Diabetes challenges within Patients with T2D treated with insulin	27
1.3. Intensive Glycaemic Control	31
1.4. Insulin associated weight gain	33
1.4.1. Predictors of Insulin associated weight gain.....	34
1.4.2. Mechanisms of insulin associated weight gain.....	35
1.4.3. Exogenous Insulin Versus Normal Insulin Physiology	37
1.5. Lack of focus on Advanced Diabetes	38
1.6. Insulin	39
1.6.1. Glucose-induced insulin secretion	40
1.6.2. Insulin stimulated glucose uptake	41
1.6.3. Insulin resistance.....	41
1.6.4. Aetiology of T2D and impaired insulin secretion.....	42
1.6.5. Altered Free Fatty Acid Metabolism: abnormal fat accumulation	43
1.7. Inflammation, obesity and T2D	46
1.8. Treatment for T2D	47
1.8.1. Weight reduction.....	47
1.8.2. Benefits of weight loss within T2D and specifically those treated with insulin....	48
1.8.3. Formula Very Low and Low Energy Diets.....	50
1.8.3.1. Weight Loss	51
1.8.3.2. Weight Maintenance	52
1.8.3.3. Side effects of formula low energy diets	54
1.8.3.4. The current role of formula very low and low energy diets in clinical practice	56
1.8.3.5. Formula low energy and very low energy diets in type 2 diabetes, CVD and other obesity associated co-morbidities	56
1.8.3.6. Very low and low energy diets in T2D patient treated with insulin	57
1.8.3.7. Other obesity related co-morbidities.....	60
1.8.3.7.1. Obstructive sleep apnoea	60

1.8.3.7.2. Kidney disease and other conditions.....	62
1.8.3.8. VLED and nutritional status	62
1.8.3.9. Body Composition Changes	63
1.8.3.10. Changes in Appetite Hormones following weight loss using VLED	64
1.9. Conclusion	64
1.10. Overall Hypothesis:	65
1.10.1. Objectives	65
Chapter 2: Methodology	66
2.1 Methodology	67
2.2 Ethics Approval	67
2.2.1 Declaration of Interest.....	67
2.2.2 Clinical Trial Registration.....	67
2.3 Study Design.....	67
2.3.1. Intervention Phase (0-24 weeks).....	68
2.3.1.1. Low Energy Formula Diet Group	68
2.3.1.1.1. Side Effects and Adverse Events with LED	69
2.3.1.2. Gold Standard Clinical Care Group	69
2.2.1.3. Behavioural Change.....	71
2.3.1.3. Physical Activity.....	72
2.4 Recruitment.....	72
2.5 Informed consent, Privacy and Confidentiality	74
2.6 Eligibility Criteria	74
2.6.1. Inclusion Criteria:	74
2.6.2. Exclusion Criteria:	74
2.6.3. Female subject and pregnancy	75
2.7. Withdrawal Criteria	75
2.8. Treatment allocation	76
2.8.1. Blinding.....	76
2.8.2. Sample Size Calculation	76
2.8.3. Statistical Analysis.....	77
2.9. Anthropometric Measure	78
2.9.1. Additional anthropometric measures	79
2.10. Study Visits.....	79
2.10.1. Screening and Baseline Measurement Visit (Visit 1).....	80
2.10.2. Baseline Visit (Visit 2).....	81
2.10.3. Insulin Titration	84
2.10.3.1. Up-titration of insulin	85
2.10.4. Subject Adherence	85
2.10.5. Telephone Consultations.....	85
2.10.6. Subsequent Visits.....	86
2.11. Visual Analogue Scales	86
2.12. <i>Ad Libitum</i> Meal Test.....	86
2.13. Questionnaires.....	87

2.13.1. Quality of Life (EuroQol EQ5D).....	87
2.13.2. Epworth Sleepiness Scale	88
2.13.3. Pittsburgh Sleep Quality Index	88
2.14. Blood sample preparation	88
2.14.1. Glucose Analysis	89
2.14.2. Radioimmunoassay	89
2.14.2.1. Principles of radioimmunoassay	89
2.14.2.2. Insulin Analysis	90
2.15. Estimation of insulin Sensitivity and Insulin Resistance	90
2.15.1. HOMA-IR	91
2.15.2. Quantitative insulin sensitivity check index (QUICKI).....	91
2.15.3. Matsuda Index.....	91
2.15.4. Insulinogenic Index.....	92
2.15.5. Disposition Index	92
2.16. Additional Assessments	93
2.16.1. Physical Activity	93
2.16.2. Sleep Study	93
2.16.3. Cardiovascular Risk Assessment	93
Chapter 3: The effect of a LED on weight loss, glucose homeostasis and insulin usage in obese T2D patients treated with insulin compared with gold standard clinical care.....	95
3.1. Introduction.....	96
3.2. Hypothesis.....	98
3.2.1. Aims.....	98
3.2.2. Outcomes	98
3.3. Methods.....	98
3.3.1. Recruitment data	99
3.3.2. Attrition rates	99
3.4. Results.....	101
3.4.1. Baseline characteristics of the subjects.....	101
3.4.2. Body composition	103
3.4.2.1. Changes in primary outcome – Weight Loss.....	103
3.4.2.2. Waist and Hip Circumference.....	104
3.4.2.2.1. Waist circumference	104
3.4.2.2.2. Hip circumference.....	105
3.4.2.2.3. Waist to Hip Ratio	105
3.4.2.3. Fat Mass and Fat Free Mass Body composition	106
3.4.2.3.1. Fat mass	106
3.4.2.3.2. Fat free mass	107
3.4.2.3.3. Trunk fat.....	108
3.4.3. Glucose Homeostasis	111
3.4.3.1. HbA1c	111
3.4.3.2. Cessation of insulin treatment.....	112
3.4.3.3. Insulin dose	112
3.4.3.4. Fasting Plasma Glucose	114
3.4.3.5. Fasting serum insulin	114
3.4.3.6. Plasma glucose concentrations during MMTT	115
3.4.3.7. Serum insulin concentrations during MMTT	117

3.4.3.8. Insulin Resistance Insulin Indices.....	118
3.4.3.8.1. HOMA-IR.....	118
3.4.3.8.2. QUICKI.....	119
3.4.3.9. Whole-body insulin sensitivity and beta-cell function	119
3.4.3.9.1. Matsuda Index.....	119
3.4.3.9.2. Insulinogenic index.....	119
3.4.3.9.3. Oral Disposition Index.....	120
3.4.4. Adverse effects of LED & GSC groups.....	123
3.4.4.1. Self-reported hypoglycaemic episodes	124
3.4.4.2. Serious Adverse events	125
3.4.5. Assessment of compliance to dietary recommendations	125
3.4.5.1. Predictive weight losses.....	125
3.4.6. Quality of Life.....	126
3.4.7. Energy intake and appetite.....	128
3.4.7.1. Ad-libitum energy intake	128
3.4.7.2. Appetite during Mixed Meal Tolerance Test – Visual Analogue Scales.....	129
3.4.7.2.1. Composite Appetite score	129
3.4.7.2.2. Hunger.....	130
3.4.7.2.3. Fullness	131
3.4.7.2.4. Desire for a meal.....	132
3.4.7.2.5. Appetite for a meal.....	133
3.4.7.3. Energy intake under free-living conditions.....	133
3.4.8. Physical Activity.....	137
3.4.8.1. Steps.....	137
3.4.8.2. Metabolic equivalents	137
3.5. Discussion.....	138
3.5.1. Summary of findings.....	138
3.5.2 Detailed Discussion	138
Chapter 4: The effect of a LED on short-term surrogate markers of complications associated with T2D and obesity in obese T2D patients treated with insulin compared with gold standard clinical care	160
4.1. Introduction.....	161
4.2. Hypothesis.....	163
4.2.1. Aims.....	163
4.2.2. Outcomes	163
4.3. Methods.....	163
4.3.1. Recruitment data	163
4.4. Results.....	164
4.4.1. Lipid Profile	164
4.4.1.1. Total cholesterol (TC).....	164
4.4.1.2. Low Density Lipoprotein - Cholesterol (LDL-C).....	164
4.4.1.3. High Density Lipoprotein Cholesterol (HDL-C).....	165
4.4.1.4. Non-high density lipoprotein cholesterol.....	166
4.4.1.5. Total Cholesterol to HDL Cholesterol Ratio	166
4.4.1.6. Triglycerides	168
4.4.2. Liver Profile	171
4.4.2.1. Serum Alanine transaminase.....	171

4.4.2.2. Serum Alkaline Phosphatase (ALP)	172
4.4.2.3. Serum Albumin	173
4.4.2.4. Serum Bilirubin.....	174
4.4.2.5. High-sensitive C-reactive protein (hsCRP)	175
4.4.3. Blood Pressure	176
4.4.3.1. Systolic blood pressure	176
4.4.3.2. Diastolic blood pressure.....	177
4.4.4. Measures of Kidney Function	178
4.4.4.1. Glomerular Filtration Rate	178
4.4.4.2. Urinary Creatinine	178
4.4.4.3. Urinary Albumin	179
4.4.4.4. Albumin to Creatinine Ratio	179
4.4.4.5. Microalbuminuria	179
4.4.5. Sleep.....	181
4.4.5.1. Apnoea Hypoxia Index	181
4.4.5.2. Oxygen Saturation	181
4.4.5.2.1. Oxygen Desaturation Index	181
4.4.5.2.2. Average oxygen saturation	182
4.4.5.2.3. Lowest oxygen saturation	182
4.4.5.2.4. Lowest oxygen desaturation	183
4.4.5.3. Epworth Sleepiness Scale	183
4.4.5.4. Pittsburgh Sleep Quality Index	183
4.4.7. Cardiovascular Disease 10-year risk and Coronary Heart Disease 10-year mortality.....	187
4.4.8. Energy intake under free-living conditions.....	189
4.5. Discussion	191
Chapter 5: Conclusions	209
5.1. Summary of main findings of study.....	210
5.2 Conclusions and Limitations.....	210
Appendix 1. Patient Information Sheet.....	214
Appendix 2. Consent Form	222
Appendix 3. Energy summary of test meals	223
Appendix 4. Nutritional Breakdown of Formula Low Energy Diet Products	224
Appendix 5. Questionnaire Booklet.....	226
Appendix 6. Summary of weight loss strategies to aid weight loss with insulin.....	235
References.....	236

Index of Figures

Figure 1. 1 Possible mechanisms of weight gain associated with insulin therapy.....	37
Figure 2.1 CONSORT flow chart for Study	70
Figure 2. 2 Schematic representation of appetite study activities.....	83
Figure 2. 3 Insulin Titration for LED and GSC Interventions	84
Figure 3.1: CONSORT Flow diagram of the study	100
Figure 3. 2 Body weight change comparing LED and GSC at 12, 18 and 24 weeks.	103
Figure 3. 3: Percentage of participants for GSC and LED achieving different categorical weight loss (e.g. maintainer, <5%, ≥10% etc.).	104
Figure 3. 4 Comparison of change in waist & hip circumference between groups at 24 weeks.	105
Figure 3. 5 Comparison of change in waist to hip ratio between groups at 24 weeks.....	106
Figure 3. 6 Comparison of change in body composition between LED and GSC at 24 weeks.	107
Figure 3. 7 Comparison of change in FFM between LED and GSC at 24 weeks.	108
Figure 3.8 Comparison of change in Trunk Fat between LED and GSC at 24 weeks.	109
Figure 3. 9 Comparison of change in HbA1C between LED and GSC at 12 and 24 weeks.	111
Figure 3. 10 Comparison of change in Insulin cessation between LED and GSC at 12 and 24 weeks.....	112
Figure 3. 11 Comparison of change in Insulin dose reduction between LED and GSC at 12 and 24 weeks.....	113
Figure 3. 12 Comparison of change in fasting glucose and insulin between LED and GSC at 12 and 24 weeks.....	115
Figure 3. 13 Plasma glucose concentrations comparing GSC and LED during a MMTT. ...	116
Figure 3. 14 Plasma AUC ₀₋₂₁₀ glucose concentrations comparing GSC and LED.....	116
Figure 3. 15 Serum insulin concentrations comparing GSC and LED during a MMTT.....	117
Figure 3. 16 Serum AUC ₀₋₂₁₀ insulin concentrations comparing GSC and LED.	118
Figure 3. 17 Comparison of effect of LED on changes in different indices of insulin sensitivity and beta-cell function at 12 and 24 weeks.....	120
Figure 3. 18 Comparison of effect of LED to GSC on self-reported hypoglycaemic episodes at 12 & 24 weeks.....	124
Figure 3. 19 Comparison of observed weight loss versus predicted weight loss following the total diet replacement, low energy diet and GSC at 12, 18 & 24 weeks.	126
Figure 3. 20 Quality of life changes following LED and GSC at 12 & 24 weeks.....	127
Figure 3. 21 Reduction in energy intake at <i>Ad-Libitum</i> meal.....	128
Figure 3. 22 Composite appetite score visual analogue scales following LED and GSC at 12 weeks.....	129
Figure 3. 23 Hunger visual analogue scales following LED and GSC at 12 weeks.....	130
Figure 3. 24 Fullness visual analogue scales following LED and GSC at 12 weeks.	131
Figure 3. 25 Desire for a meal visual analogue scales following LED and GSC at 12 weeks.	132
Figure 3. 26 Appetite for a meal visual analogue scales following LED and GSC at 12 weeks.	133
Figure 3. 27 Energy, carbohydrate, protein and fat intakes following LED and GSC at 24 weeks.....	135
Figure 3. 28 Number of Steps and metabolic equivalents following LED and GSC at 24 weeks.....	137

Figure 4. 1 Comparison of total cholesterol between groups at 12 & 24 weeks.	164
Figure 4. 2 Comparison of low-density lipoprotein cholesterol between groups at 12 & 24 weeks.....	165
Figure 4. 3 Comparison of high-density lipoprotein cholesterol between groups at 12 & 24 weeks.....	165
Figure 4. 4 Comparison of non-high density lipoprotein cholesterol between groups at 12 & 24 weeks.....	166
Figure 4. 5 Comparison of total cholesterol to high-density lipoprotein cholesterol ratio between groups at 12 & 24 weeks.	167
Figure 4. 6 Comparison of triglycerides between groups at 12 & 24 weeks.	168
Figure 4. 7 Comparison Alanine transaminase (ALT) of between groups at 12 & 24 weeks.	171
Figure 4. 8 Comparison of alkaline phosphatase between groups at 12 & 24 weeks.....	172
Figure 4. 9 Comparison of albumin between groups at 12 & 24 weeks.....	173
Figure 4. 10 Comparison of bilirubin between groups at 12 & 24 weeks.	174
Figure 4. 11 Comparison of High-sensitive C-reactive protein between groups at 12 & 24 weeks.....	175
Figure 4.12 Comparison of systolic blood pressure between groups at 12 & 24 weeks.	176
Figure 4. 13 Comparison of diastolic blood pressure between groups at 12 & 24 weeks.	177
Figure 4. 14 Comparison of estimated glomerular filtration rate between groups at 24 weeks.	178
Figure 4. 15 Comparison of change in urinary albumin and albumin to creatinine ratio between groups at 12 & 24 weeks.	179
Figure 4. 16 Comparison of apnoea hypoxia index (AHI) between groups at 24 weeks.	181
Figure 4. 17 Comparison of oxygen desaturation index between groups at 24 weeks.	182
Figure 4. 18 Comparison of Epworth sleep scale between groups at 12 and 24 weeks.	183
Figure 4. 19 Comparison of Pittsburgh Sleep Quality Index between groups at 12 and 24 weeks.....	184
Figure 4. 21 Comparison of the change in predicted 10-year cardiovascular risk using QRISK2 between groups at 24 weeks.	188
Figure 4. 22 Comparison of change in predicted 10-year coronary heart disease mortality risk between groups at 24 weeks.	189
Figure 4. 23 Summary of saturated fat, polyunsaturated fat and monounsaturated fat and total fat intake following LED and GSC at 24 weeks.....	190

Index of Tables

Table 1. 1 Classification of overweight and obese in adults and risk of comorbidities	25
Table 1.2 Advantages and Side Effects of use of Very Low and Low Energy Formula Diets within clinical practice.....	55
Table 1.3 Contraindications for the use of VLED	55
Table 2.1 Content of each individual Behavioural Session	71
Table 2. 2 Schedule of Enrolment, intervention, study visits and assessments for both groups	81
Table 3.1 Baseline characteristics of subjects comparing low energy diet and gold standard care groups.....	102
Table 3. 2 Primary Outcome Weight Change comparing LED and GSC at 12 and 24 weeks	110
Table 3. 3 Anthropometric Outcome comparing LED and GSC at 12 and 24 weeks	110
Table 3. 4 Glycaemic outcomes comparing LED and GSC at 12 and 24 weeks.....	121
Table 3. 5 Indices of insulin sensitivity and beta-cell function comparing LED and GSC at 12 and 24 weeks.....	122
Table 3. 6 Self-reported adverse events among the subject in the LED & GSC group at 24 weeks.....	123
Table 3. 7 Quality of Life score from visual analogue scale comparing LED and GSC at 12 and 24 weeks.....	127
Table 3. 8 Energy intake at <i>ad libitum</i> following the mixed meal tolerance test for LED and GSC.....	128
Table 3. 9 Macronutrient and Energy Intake Outcomes comparing LED and GSC at 12 and 24 weeks.....	136
Table 4. 1 Lipid outcomes comparing LED and GSC at 12 and 24 weeks	169
Table 4. 2 Liver function outcomes comparing LED and GSC at 12 and 24 weeks	170
Table 4. 3 Blood Pressure and Renal Function comparing LED and GSC at 12 and 24 weeks	180
Table 4. 5 Sleep Outcomes comparing LED and GSC at 12 and 24 weeks	185
Table 4. 6 Dietary fatty acid intake comparing LED and GSC at 12 and 24 weeks.....	186
Table 4. 7 Predicted 10-year cardiovascular risk using QRISK2 comparing LED and GSC at 12 and 24 week	187
Table 4. 8 Predicted 10-year coronary heart disease morality risk using Framingham risk score comparing LED and GSC at 12 and 24 week.....	188

Chapter 1: Introduction

1.1. Diabetes - Type 2 diabetes and Obesity

The twin pandemics of obesity and type 2 diabetes mellitus (T2D) represent possibly the two greatest public health challenges affecting the developed and developing world. As obesity and T2D frequently co-exist effective prevention, management and treatment strategies are key priorities for healthcare care systems worldwide. As the number of obese individuals with T2D rises the number requiring insulin treatment also rises. This patient population presents their own clinical challenges as insulin treatment can further exacerbate obesity. To optimise insulin management in this cohort of patients is therefore important to better understand the physiology linking T2D and obesity and the factors relating weight gain with insulin treatment.

1.1.1. Definition and classification of diabetes

Type 2 diabetes mellitus has reached epidemic proportions and is a major health and economic burden facing the world. Worldwide, it is estimated that 382 million people currently live with diabetes and this is projected to rise to 592 million by 2035 (International Diabetes Federation, 2013). Within the UK, the total number of adults with both Type 1 and Type 2 diabetes is estimated to be 3.7 million, approximately 90% of whom have T2D and a 80-85% are either overweight or obese (Diabetes UK, 2012, Diabetes UK, 2017).

T2D is a chronic progressive condition with associated beta-cell dysfunction and β -cell atrophy leading to a progressive reduction in insulin secretory capacity (UKPDS Study Group, 1998, LeRoith, 2002). Insulin resistance (IR) in target cells (liver, skeletal muscle, adipose tissue) (LeRoith, 2002, DeFronzo, 2009) usually precedes T2D and is the cause of initial hyperinsulinemia and then chronic hyperglycaemia. Multiple studies have now demonstrated that muscle IR is the key initial step in the development of T2D (DeFronzo and Tripathy, 2009). With the development of IR, the β -cells attempt to compensate by overproducing insulin to maintain euglycemia. Paradoxically, the chronic hyperinsulinemic state reduces glycogen synthase, non-oxidative glucose disposal, and total glucose uptake in further exacerbating the IR (Iozzo et al., 2001). One accepted hypothesis around the pathophysiology of T2D is that β -cell loss continues until the pancreatic β -cell reserve is exhausted and insulin secretory capacity insufficient to maintain euglycaemia (Seino, 2015). The rate of decline of β -cell mass, however, depends on many factors including genetic

predisposition. Traditionally three factors have been highlighted as the key aetiological factors for the glucose intolerance of T2D, they are IR in muscle, IR in the liver and β -cell failure, referred to as the “triumvirate”. These three factors have now become eight distinct pathophysiologic contributors, with the addition of the following factors, as listed below (DeFronzo, 2009).

1. Adipocyte resistance to the antilipolytic effect of insulin leading to increased lipolysis.
2. Decreased incretin effect (Glucagon-like peptide-1 [GLP-1] and Gastric inhibitory polypeptide [GIP]).
3. Increased glucagon secretion by α -cells leading to raised endogenous glucose production.
4. Enhanced renal glucose reabsorption by the sodium glucose co-transporter-2 (SGLT2) transporter.
5. Central nervous system resistance to the anorectic effect of insulin and altered neurosynaptic hormone secretion.

International guidelines currently diagnose T2D if any of the following criteria are met (ADA, 2014a, NICE, 2015, WHO & IDF, 2006):

1. HbA1c $\geq 6.5\%$ (48mmol/L) OR
2. Fasting plasma glucose ≥ 7.0 mmol/L (≥ 126 mg/dL) OR
3. Plasma glucose ≥ 11.1 mmol/L (≥ 200 mg/dL) 2 hours after the ingestion of 75g oral glucose OR
4. Plasma of ≥ 11.1 mmol/L (≥ 200 mg/dL) with classical symptoms of hyperglycaemia (ADA, 2014a).

The exact aetiology of T2D is complex and multifaceted and remains incompletely understood. This said, T2D is associated with a strong familial predisposition. A family history of T2D in any-first degree family member is associated with a 2.7-3.5 fold higher risk for developing T2D (Meigs et al., 2000, Scott et al., 2013). Among individuals where both parents have diabetes this risk increases to 6.1-fold (Meigs et al., 2000). Even after adjustment for BMI and waist circumference this was only moderately attenuated to an adjusted hazard ratio of 2.44 (Scott et al., 2013). This suggests that body mass index (BMI)

and waist circumference may only play a marginal role in the associated risk related to genetically predisposed individuals. However, in a further analysis of the European Prospective Investigation into Cancer (EPIC) Interact Study, being obese was the highest risk for developing T2D at any level of genetic risk compared with waist circumference and physical activity (Langenberg et al., 2014). It can therefore be seen that a complex interaction exists between environmental and genetic susceptibility to obesity. Not only is the absolute degree of obesity important but also the distribution of body fat with those with increased waist circumference and waist to hip ratio having the highest risk (Ohlson et al., 1985, Meisinger et al., 2006).

1.1.2. Definition and classification of obesity

The World Health Organisation (WHO) defines overweight and obesity as an “abnormal or excessive fat accumulation that may impair health”. The presence of overweight and obesity is assessed by using a simple index namely body mass index (BMI), which is calculated by dividing an individual’s weight in kilograms by their height in metres, squared (kg/m^2). Simply put, overweight is a BMI greater than or equal to 25kg/m^2 , and obesity is a BMI greater than or equal to 30kg/m^2 . Obesity itself is separated into different classifications, increasing with severity (Table 1). These classifications are intended to reflect the risk of type 2 diabetes and cardiovascular disease (CVD) and other obesity-related co-morbidities (WHO, 2004).

Table 1. 1 Classification of overweight and obese in adults and risk of comorbidities

(WHO, 2004)

Classification	BMI Range (kg/m^2)		Risk of comorbidities
	Principal Cut-off	Asian Cut-off	
Underweight	<18.5		Increased
Normal Weight	18.5-24.9		Nadir
Overweight	25.0-29.9	23.0-27.4	Increased
Obese: Class I	30-34.9	27.5-32.4	Moderately increased
Obese: Class II	35-39.9	32.5-37.4	Severely increased
Obese: Class III	≥ 40	≥ 37.5	Very severely increased

BMI remains the most widely used index for defining obesity. The National Institute for Health and Care Excellence (NICE) though urges caution in the interpretation of BMI as it may not be an accurate indicator of adiposity particularly in adults who are highly muscular

(NICE, 2014b). Thus, clinical judgement is required. It is important to also appreciate that certain ethnic groups compared to Caucasian adults have a higher metabolic risk and greater body fat despite having the same age, gender and BMI (WHO, 2004, NICE, 2012, SIGN, 2010a). This prompted the WHO to lower the BMI threshold among Asians so that overweight is defined as 23-27.5kg/m² and obesity as 27.5kg/m² and higher (WHO, 2004). Despite these disadvantages, BMI is easy to calculate, inexpensive, associated with the risk of obesity-related disease and can be performed at a population level. Waist circumference has been suggested to provide a better index of adiposity and particularly visceral fat mass, which has been linked to the greater risk of developing T2D and CVD (Ohlson et al., 1985, Klein et al., 2007). NICE has suggested that this should be used alongside BMI in those with a BMI <35kg/m² (NICE, 2006, NICE, 2014b). BMI therefore should not be used solely as an indicator of an individual's clinical risk (NHMRC, 2013).

Epidemiological studies have identified that a high BMI is associated with an increased risk of a variety of non-communicative chronic diseases, including T2D, CVD, cancer, chronic kidney disease (CKD) and non-alcoholic steatohepatitis (NASH) (Wormser et al., 2011, Williams et al., 2011, Singh et al., 2013). In addition, obesity is associated with an increase in all-cause mortality (Whitlock et al., 2009, Berrington de Gonzalez et al., 2010). Published data shows that compared with a BMI of 22.5-25kg/m², those with a BMI 30-35 kg/m² lose between 2-4 years of life, while for an individual with a BMI of 40-45kg/m², life will be cut short by 8-10 years (Whitlock et al., 2009).

The global burden of disease related to a high BMI is substantial. In 2015, 4.0 million deaths were directly associated with obesity, representing 7.1% of deaths from any cause (Afshin et al., 2017). In the same study, cardiovascular disease and diabetes were reported to be the two leading causes of death worldwide related to a high BMI, accounting for 3.3 million deaths (Afshin et al., 2017). The association between BMI and all-cause mortality is J-shaped with overweight and obesity increasing this risk compared with an ideal BMI of between 22.5-25 kg/m² (Whitlock et al., 2009, Berrington de Gonzalez et al., 2010).

While the pathophysiology of obesity is complex and remains poorly understood, obesity is fundamentally a result of an excessive intake of calories (energy intake) compared to calories expended (energy expenditure). Therefore, the increased rate of obesity may just reflect a state of chronic positive energy balance. Though this does not have to be significant

overeating, with as little as 50kcal per day in theory accounting for an average 1.8 to 2.0lbs per year (0.82-0.91kg/year) (Hill et al., 2003). Interestingly our evolutionary biology actually protects us more against weight loss than weight gain, with greater counter-regulatory responses to preserve our weight in energy restriction than in positive energy balance (Hill, 2006). With today's 'obesogenic' environment likely to be one major driver for this weight gain where high-fat, energy dense, processed and affordable food more freely available (Swinburn et al., 2011), we now live in an environment where weight gain is easier than weight loss.

In 2008, one in every three adults was overweight and one in every nine were obese, with the absolute number at that time estimated to be approximately 1.46 billion, of which 508 million were obese (Stevens et al., 2012). These figures have continued to rise. In a 2015 report, it was estimated that 603.7 million adults were obese worldwide representing an overall adult prevalence of 12% (Afshin et al., 2017). The prevalence of obesity in 73 countries including the UK has doubled since 1980, with many nations showing a continuous increase during this time. Once, the obesity epidemic was only considered to be an issue with the developed world, however, overweight and obesity rates are now rapidly increasing within the developing countries.

The United Kingdom has now become the "fattest" nation in Western Europe and is sixth in the world, with 26.9% of people now being obese (OECD, 2017). Of particular concern is the predicted steady increase of obesity rates until at least 2030, with figures for England expected to reach 35% (OECD, 2017). The 2007 Foresight report predicted that by 2050 60% of the UK population could be obese (Butland, 2007). It is apparent from today's data that primary care prevention has not made a significant enough impact to slow obesity prevalence both within the UK and worldwide.

1.2. Diabetes challenges within Patients with T2D treated with insulin

Treatments for T2D have traditionally focused on improvements of glycaemic control for achieving and maintaining euglycaemia and thereby preventing the associated complications of hyperglycaemia (Le Floch, 2010). With treatment consisting of lifestyle management and pharmacotherapy (Inzucchi et al., 2012, Garber et al., 2013). With national guidelines setting glycated haemoglobin (HbA1c) and glycaemic targets (ADA, 2014b, NICE, 2015), to

maintain these stringent HbA1c and glycaemic targets more medications are being introduced earlier (Stratton et al., 2000, Meneghini, 2009).

However, with progressively worsening β -cell function and declining β -cell mass, glucose tolerance continues to decline. Often resulting in lifestyle and oral hypoglycaemic agents (OHA) not achieving to meet these glycaemic targets resulting in the majority of patients requiring insulin therapy (IT) at some point (Turner et al., 1999).

Over the last few decades the burden associated with T2D has significantly changed. Once a disease diagnosed late in adult life and previously perceived as a 'mild' form of diabetes rarely requiring exogenous insulin management. Today T2D is considered as serious as Type 1 diabetes (T1D). Increasingly T2D is diagnosed among young age groups and as the duration of T2D increases so does the requirement for IT and diabetes related complications. As life expectancy increases not only has the number of individuals with TD2 increased but their associated burden on different healthcare systems (Brown et al., 2004).

Within the UK the number of people with T2D treated with insulin has increased considerably from 37,000 in 1991 to over 227,000 people in 2010 (Holden et al., 2014). The increase of patients treated with insulin and their accompanying complication has placed a substantial financial burden on the NHS. In the UK in 2010/2011 it was estimated that diabetes cost in total £23.7 billion, approximately 10% of total NHS resource expenditure (Department of Health, 2010). £9.8bn was for direct costs and £13.9bn in indirect costs with the majority being accounted for by patients with T2D (£8.8bn direct & £13.0bn indirect [£21.8bn]) (Hex et al., 2012). Among patients with T2D, medications were estimated to account for approximately £702million and complications £7bn representing 7% and 80% of spending, respectively.

Strict intensive early glycaemic control has been shown to be effective in significantly reducing diabetes micro- and macro-vascular complications, as shown by the United Kingdom Prospective Diabetes Study (UKPDS, 1998). Metformin is the recommended first line treatment for T2D (NICE, 2015, ADA, 2016). The mechanism of action of metformin is highly complex, but primarily involves suppression of hepatic gluconeogenesis, enhancing insulin suppression of endogenous glucose production, reduced intestinal glucose absorption, and improved insulin sensitivity (Foretz et al., 2014). A substantial proportion of patients,

with T2D do not achieve glycaemic targets with metformin monotherapy over time with 42% of people failing to achieve glycaemic control within two to five years (Hoerger et al., 2008, Brown et al., 2010). Therefore, additional OHA (Turner et al., 1999) and eventually insulin is required.

IT is now routinely recommended for the management of T2D, despite this it is typically delayed and too often considered last resort for those not achieving or maintaining glycaemic targets. However, more recently guidelines have started advocating the early use of insulin in T2D management (Inzucchi et al., 2012, Garber et al., 2013, Harper et al., 2013, ADA, 2014b). Basal insulin analogues such as Detemir and Glargine, along with Neutral Protamine Hagedorn (NPH) insulin have now been highlighted as possible treatment options with metformin alone. With the advent of improved insulin analogues, the more recently introduced classes of OHA, and the glucagon-like-peptide-1 (GLP-1) agonists the use of insulin in the T2D management pathway has changed. Where once the majority of patients were treated with insulin monotherapy, now many of these patients are using at least one concurrent OHA (Holden et al., 2014). Exogenous insulin is classified into different categories, which is based on their pharmacokinetic profile. For T2D patients, a variety of different insulin regimes and combinations are prescribed, biphasic, basal insulin, rapid acting and ultra-long acting insulins. Overall, between 1991 and 2010, the prevalence rate for insulin treated patients with T2D in the UK increased 6.5 fold from 0.67 per 1000 people to 4.34 per 1000 (Holden et al., 2014). Despite this, approximately a third of all T2D patient (33.9%) did not achieve the national glycaemic targets of $\leq 6.5\%$ (≤ 48 mmol/mol) recommended by NICE (Health and Social Care Information Centre, 2015). This figure is even lower for insulin treated patients with only 26.6% achieving target (Calvert et al., 2007), highlighting further that T2D patients on IT represent a very challenging group to manage effectively.

Contrary to national and international guidelines and the fact that only a fraction of T2D patients achieve these glycaemic targets, the initiation of insulin continues to be delayed despite poor glycaemic control. In a study of 5064 T2D patients on multiple OHA, general practitioners (GP) delayed insulin initiation for 7.7 years, this was despite a mean HbA1c of $9.85 \pm 1.96\%$ (95mmol/mol) (Calvert et al., 2007). This is a recurrent theme within the literatures with a more recent study reporting a similar HbA1c of $9.5 \pm 2.3\%$ (80mmol/mol) before initiation (Paul et al., 2016). This apparent reluctance to initiate and escalate IT has

been defined as ‘psychological insulin resistance’ (PIR) and has been reported among both clinicians and patients alike (Peyrot et al., 2010, Russell-Jones et al., 2018).

A number of studies have attempted to further unpick patients’ and physicians’ attitudes towards insulin initiation and intensification (Peyrot et al., 2005, Polonsky et al., 2005, Brod et al., 2009). The primary two obstacles appear to be the fear of weight gain (UKPDS Study Group, 1998, Morgan et al., 2012)) and the fear of hypoglycaemia (UKPDS Study Group, 1998, Sorli and Heile, 2014). Within the Diabetes Attitudes, Wishes and Needs study (DAWN study) that surveyed 2713 T2D patients reported the concern about weight gain and hypoglycaemia in approximately 50% of patients (IDF, 2002). Another key belief was that patients felt that they had somehow failed to effectively manage their diabetes (IDF, 2002, Polonsky et al., 2005), which resulted in them not wishing to commence IT. Interestingly physicians were found to underestimate this as a genuine concern in their patients (Peyrot et al., 2010). Other patient concerns included loss of freedom, daily need for injections, fear of needles, social stigma, inconvenience and the expected failure of insulin to actually reduce blood glucose (Brod et al., 2009, Peyrot et al., 2010).

Physicians’ attitudes towards insulin initiation are similar to those of the patients including increased hypoglycaemia, weight gain and perceived low efficacy of insulin (Russell-Jones et al., 2018). Additional concerns included patient anxiety about insulin, perceived adherence, pain of injections, training the patients to inject and the need for extra monitoring (Nakar et al., 2007, Hayes et al., 2008). This was further highlighted in an online survey (Management of Diabetes in Future Years [MODIFY]) of over six hundred physicians that aimed to gain an insight into the physician’s role, current practice and challenges of IT. Key barriers identified were the lack of experience in available insulins (49%), the time required to educate patients (49%) and belief that patients would be unable to cope (39%). Within the UK roughly equal numbers felt the responsibility for insulin initiation was that of primary care (29%), secondary care (36%), or both (35%). Of interest is the apparent discord between general physicians and specialists and their belief on the responsibility of insulin intensification, with 30% of GPs never or rarely intensify insulin, compared to 4% of specialists ($p<0.0001$) (Cuddihy et al., 2011). This data demonstrates the movement of NHS management of T2D care towards the primary care and out of specialist secondary care services but also is worrying that a significant number of GPs won't intensify IT. Thus, this has the potential of

delaying the timely intensification of IT and resulting in an increase in glycaemic burden and possible complications (Korytkowski, 2002).

1.3. Intensive Glycaemic Control

The United Kingdom Prospective Diabetes Study (UKPDS) showed that aggressive management of blood glucose control is associated with a substantial reduction in microvascular complications (UKPDS Study Group, 1998), risk of cardiovascular disease and mortality (Stratton et al., 2000, Holman et al., 2008). Patient therapy was rapidly increased with IT being taken by 44% of patients by 6 years after diagnosis (Matthews et al., 1998, Turner et al., 1999). In spite of these potential benefits, intensification of therapy was also associated with the greatest weight gain, with the insulin patients gaining 4kg more than those assigned to conventional therapy. There is a suggestion among patients with T2D that an increase in central adiposity may negate those benefits of improved by glycaemic control (Purnell et al., 2013). Within the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study (DCCT/EDIC), excessive weight gain was shown to increase central adiposity, insulin resistance, dyslipidaemia, blood pressure and increase subclinical markers of atherosclerosis (Purnell et al., 2013). However, it is also argued that this weight gain is offset by the improvements in glycaemic control seen with intensification of IT (Laville and Andreelli, 2000). Indeed, the 10-year data from UKPDS shows that there is an extended effect of improved glycaemic control, a so-called “legacy effect”, with a post-trial risk reduction for myocardial infarction (MI) of 15% and all cause mortality of 13% (Holman et al., 2008).

The cardiovascular improvements shown with the UKPDS have however been challenged in three, landmark randomised control trials (Gerstein et al., 2008, Patel et al., 2008, Abaira et al., 2009, Duckworth et al., 2009) suggesting that there is no reduction in either all-cause or cardiovascular mortality when targeting intensive glycaemic control is compared with conventional treatment. The Action to Control Cardiovascular Risk in Diabetes (ACCORD) (Gerstein et al., 2008) trial was prematurely terminated due to higher mortality within the intensive-therapy group (1.41 vs. 1.14% per year: HR 1.22; 95% CI 1.01-1.46; P=0.04). This was however not confirmed within the Action in Diabetes and Vascular Disease: Preterax and Diamicon Modified Release Controlled Evaluation trial (ADVANCE) (Patel et al., 2008) or Veterans Affairs Diabetes Trial (VADT) (Abaira et al., 2009, Duckworth et al., 2009), which

found no increase in mortality with intensive therapy. With the inclusion of all 4 of these trials, two systematic reviews (Hemmingsen et al., 2011, Hemmingsen et al., 2013) concluded that there was no statistically significant difference between intensive glycaemic control compared to conventional control for all-cause mortality or cardiovascular mortality. Further analysis did reveal however that intensive control reduced the risk of non-fatal myocardial infarction, amputation of lower extremities, as well as risk of developing nephropathy (Patel et al., 2008), retinopathy and risk of retinal photocoagulation (UKPDS Study Group, 1998) and that the potential benefits seen with intensive glycaemic control on coronary events and all-cause mortality are only observed after extended follow-up, up to 30 years after intervention (Holman et al., 2008).

During these trials the risk of hypoglycaemia was increased during the intensification of treatment (UKPDS Study Group, 1998, Gerstein et al., 2008, Holman et al., 2008, Patel et al., 2008, Abaira et al., 2009, Duckworth et al., 2009). It has been suggested that this increased risk of hypoglycaemia contributed to the heightened mortality risk among patients with T2D (Currie et al., 2010). Furthermore, recent observational evidence has indicated that there is an increased relative risk (RR) all-cause mortality of 1.95 (CI 1.70-2.25) associated with insulin and metformin therapy versus sulphonylurea and metformin (Mogensen et al., 2014). Other studies have reported similar associations (Currie et al., 2010, Roumie et al., 2014). In T2D patients, a U-shaped relationship has been observed between HbA1c and adjusted hazard ratios (HR) for all-cause mortality for those treated with either OHA or insulin. In which, an HbA1c less than 6% or greater than 8% results in greater mortality with an HbA1c of approximately 7.5% showing the lowest all-cause mortality (Currie et al., 2010, Colayco et al., 2011). Further data proposes a dose-response relationship between insulin and risk of death in subjects with T2D, showing adjusted HR nearly tripling with greater exposure to insulin (HR 2.79, CI 2.36-3.30); particular in older patients, with better glucose control (Gamble et al., 2010, Holden SE, 2013). With both T2D and obesity being linked with an increased risk of CVD (Kannel and McGee, 1979, Hubert et al., 1983, Haffner and Cassells, 2003, Pi-Sunyer, 2009, Diabetes UK, 2012) and the cumulative effect resulting in a 13% increase in the risk of fatal and nonfatal CHD for every 1 unit increase in body mass index (BMI) (Eeg-Olofsson et al., 2009), the use of insulin within this population potential poses a significant risk, particularly in those with more longer disease duration. Thus, although intensification of treatment to near euglycaemia (HbA1c $\leq$ 6.5%; 48mmo/mol) is potentially beneficial for those with a longer life expectancy, fewer co-morbidities and no known history

of severe hypoglycaemia (ADA, 2014b); there is a clear need for more individualised, conservative glycaemic targets and insulin management plans for obese T2D with multi-comorbidities. Evidence from epidemiological studies has suggested that there is an increased association between all-cause mortality, major adverse cardiovascular events (MACE), cancer and other outcomes in people with T2D. A study looking at all-cause mortality of nearly 85,000 patients with T2D on differing medications found insulin monotherapy was associated with an adjusted hazard ratio (HR) of 2.197 compared to metformin alone (Currie and Holden, 2014). In the initial study insulin dose was not accounted for however in a subsequent study an insulin dose response effect and all-cause mortality was observed (Holden et al., 2015). The adjusted HR for all-cause mortality was 1.54 for each 1-unit increase in insulin dose in those patients with T2D. One criticism related to the interpretation of the risk of mortality and CVD in these types of epidemiological studies, is that patients treated with insulin are at a more advanced stage of disease progression and maybe phenotypically different to those on OHA alone and one is therefore not comparing like with like.

1.4. Insulin associated weight gain

Insulin remains the most effective diabetes treatment for glycaemic control, however despite this it is also associated with significant weight gain. Within the literature an insulin associated weight gain (IAWG) of between 3 to 9kg has been reported over the first year following initiation, with the majority occurring within the first 3 years (UKPDS Study Group, 1998, Larger et al., 2001, Brown et al., 2017). Any weight gain is an undesirable outcome for the majority of patients are T2D who are already overweight or obese (Barratt et al., 2008) and particularly in those treated with insulin. The amount of IAWG has been shown to vary between individuals with some gaining and others losing weight (Jansen et al., 2010) though the reasons for this remains unresolved. Alongside the increase in mortality, weight gain following insulin initiation has been directly correlated with total exogenous insulin dose ($r = 0.62$, $P < 0.02$) and the mean daylong serum insulin level ($r=0.67$, $P<0.01$) (Henry et al., 1993). Suggesting that it is those with the higher HbA1c and requiring further insulin intensification to meet recommended glycaemic targets that could have the greatest positive impact on their weight and mortality risk.

Studies have shown that the distribution of the weight is relevant to cardiovascular risk and insulin resistance (Turkoglu et al., 2003, Yusuf et al., 2004) and particularly central visceral fat. Though weight gain is commonly observed following insulin initiation, the distribution between lean and fat body mass is not fully established; with an answer potentially impacting on any potential benefit seen from improved glycaemic control. Studies have shown that IAWG in T2D is predominantly fat mass (60%), particularly visceral fat (Sinha et al., 1996, Rigalleau et al., 1999) and predominantly within the abdominal region. Although this is not a ubiquitous finding with one study suggested a greater increase in lean tissue than fat mass and using computed tomography, a reduction in visceral fat and a gain in subcutaneous fat (Shah, 2011). Using a 3 and 4-component model, a more even distribution of fat and fat-free mass weight gain was observed, with Salle and colleagues showing 50% of weight gain was fat and 33% from fat-free mass (Salle et al., 2004, Packianathan et al., 2005). These differences have predominantly been reported in newly insulin treated T2D patients only (Salle et al., 2004), suggested that weight gain in those following the initial year of IT might plateau, which has been seen elsewhere (Aas et al., 2009). With substantial methodological heterogeneity between studies, comparison is difficult and definitive answer remains elusive.

An important consideration during IT initiation and up-titration is the risk-benefit relationship between an improvement in glycaemic control and the associated weight gain. In spite of weight gain *per se* being known as an independent risk factor for CVD, the impact of IAWG remains unclear. IAWG has not been found to be associated with a direct increase on CVD mortality or post myocardial infarctions re-infarctions rate (Aas et al., 2009), though surrogate markers of cardio-metabolic risk are negatively impacted. Total and LDL-cholesterol, total body, trunk and subcutaneous fat mass have all been observed to increase (Jansen et al., 2010), in addition to worsening renal glomerular hyperfiltration and increased systolic blood pressure (Yki-Jarvinen et al., 1997, Tomaszewski et al., 2007). This lack of a direct association between IAWG and increase CVD events may be explained by the large number of cardio-protective drugs routinely prescribed today to insulin treated T2D patients (Brown et al., 2017).

1.4.1. Predictors of Insulin associated weight gain

Within the literature several independent predictors of IAWG have been highlighted diabetes-related distress, initial insulin dose, older age, HbA1c change (improvement in HbA1c),

higher baseline body mass index (BMI), pre-treatment insulin resistance and the increase in insulin dose (Jansen et al., 2014, Salle et al., 2005, Jacob et al., 2007). Interestingly a recent study has suggested that greater baseline BMI is associated with less IAWG (Balkau et al., 2014, Paul et al., 2016). In 155,197 T2D patients commencing IT, it was reported that those with a BMI $<30\text{kg/m}^2$ gained significantly more weight than those that were obese. In fact, individuals with a BMI $>40\text{kg/m}^2$ actually lost weight over the course of the 24-month study (-2.2, 95% CI -2.2 to 1.1). This agrees with another study that showed a lower baseline BMI, higher baseline HbA1c and a higher insulin dose at both baseline and 1 years independently predicted IAWG (Balkau et al., 2014). Possible explanations for this finding maybe altered body compositions, reduced compliance to IT, altered pharmacodynamics of the insulin and greater motivation of obese patients to make changes to minimise weight gain and in fact lose weight. Although the exact reason remains unclear and further research is required to see if this paradoxical relationship truly exists.

1.4.2. Mechanisms of insulin associated weight gain

The exact mechanisms augmenting IAWG are not completely understood at present, though several theories have been put forward. This has been reviewed in greater detail elsewhere (Russell-Jones and Khan, 2007, Brown et al., 2017) therefore only a brief summary will be presented in this thesis. These potential causes may include a reduction in glycosuria, an increased energy intake linked to fear of hypoglycaemia, central effects on appetite regulation, anabolic nature of insulin hormone and catch-up weight (Figure 1.1) (Brown et al., 2017). It remains unresolved as to whether there is a dominant mechanism that drives IAWG. From the literature there may be some interconnectability between mechanisms, resulting in interplay between two or three mechanisms but this is likely to be vary between individuals.

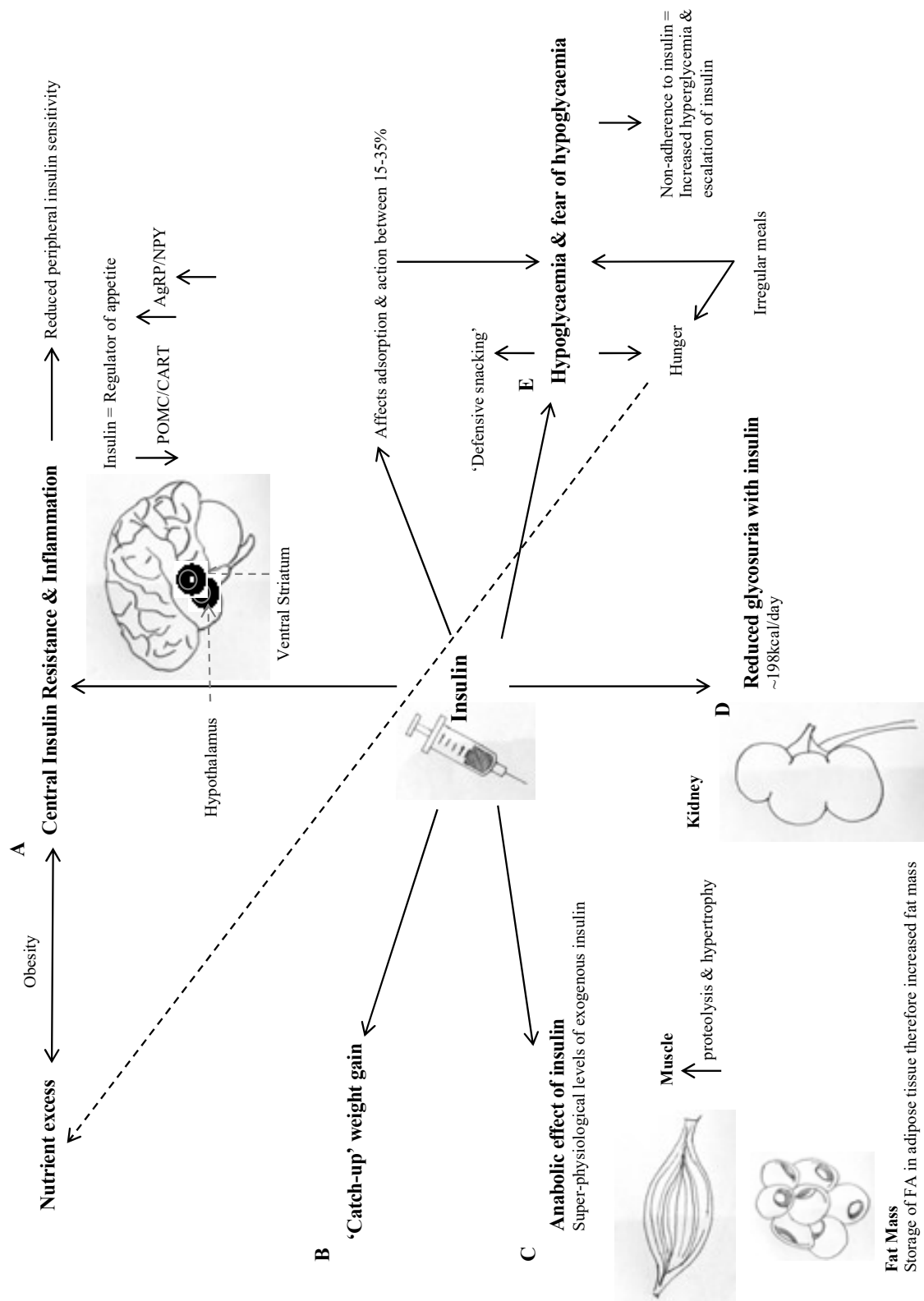


Figure 1. 1 Possible mechanisms of weight gain associated with insulin therapy

(Brown et al., 2017).

Insulin associated weight gain (IAWG) is multifaceted. A) Insulin is key to appetite and reward regulation in the hypothalamus. Nutrient excess can cause hypothalamic IR and inflammation, with TNF- α inhibiting insulin action by suppressing IRS-1. IR reduces insulin activation of POMC/CART neurons and suppression of AgRP/NPY neurons, thus potentially increasing appetite and energy intake. B) Weight gain is seen before T2D diagnosis, but preceding T2D diagnosis and prior to insulin initiation patients often lose weight. IAWG has been proposed to be due to the body 'catching-up' to maximal weight before diagnosis. C) Insulin is an anabolic hormone and as such increases FA (fatty acid) storage in adipose tissue, proteolysis and muscle hypertrophy. Exogenous insulin results in super-physiological levels in the periphery causing fat and lean tissue gains. D) Uncontrolled hyperglycaemia can cause glycosuria within T2D; thus, insulin causes the re-uptake of the energy thus weight gain. E) Hypoglycaemia increases with the IT, the un-physiological nature of exogenous insulin, and irregular meal intake. This can cause weight gain through 'defensive snacking', non-adherence and escalation of IT and may affect hunger. Abbreviations POMC/CART, pro-opiomelanocortin/cocaine and amphetamine regulated transcript; AgRP/NPY, agouti related peptide/neuropeptide Y; TNF- α , tumour necrosis factor-alpha; IAWG, insulin associated weight gain, IR, insulin resistance; T2D type 2 diabetes mellitus; IT Insulin Therapy; IRS-1 insulin receptor substrate-1

1.4.3. Exogenous Insulin Versus Normal Insulin Physiology

It has been further suggested that the variability in glucose excursions, independent of the elevated mean glucose concentration, is a risk factor for diabetes-related complications (Smith-Palmer et al., 2014). Functionally, IT should replicate the physiological release of endogenous insulin avoiding high glycaemic excursions, as occurs physiologically in healthy individuals. Endogenous insulin is secreted by pancreatic β -cells into the portal vein and firstly enters the liver where it suppresses endogenous glucose production and increases glucose uptake and the formation of glycogen, with approximately 50% of it being degraded by the liver (Bergman, 2000). The remaining circulating insulin increases glucose uptake in peripheral target tissues including the skeletal muscle and adipose tissue. Subcutaneous administration (s.c) of insulin is hence non-physiological as it initially circulates systemically resulting in increased peripheral action in skeletal muscle and adipose tissue and has a delayed degradation in the liver (Lindholm, 2002). Insulin s.c. therefore has a delayed effect in reducing endogenous glucose production in the liver.

The effects of exogenous insulin to lower blood glucose (pharmacodynamics) are closely reflected by its physiological pharmacokinetic affects (Vora and Heise, 2013). With s.c insulin, there is a disconnection between insulin pharmacodynamics and pharmacokinetics. The action of exogenous insulin is significantly delayed, possibly reflecting insulin being transported to target tissues and the subsequent signalling and metabolic responses (Vora and Heise, 2013). This can mean that when insulin is administered subcutaneously, there can be

an increased variability of both its absorption and metabolic action of between 15-35% (Heinemann, 2002, Lindholm, 2002). This can result in a lack of reproducibility after every exogenous injection at a given dose, within a given person. Moreover, a slower rate of absorption and a more extended duration of action occurs with both higher insulin doses and BMI (Heise and Pieber, 2007), which are common features of the IR obese T2D patients treated with insulin. Increased glucose variability and reduced suppression of endogenous hepatic glucose production has the potential of escalating insulin requirements further and exacerbating IAWG.

The pharmacokinetic limitations of traditional long-acting exogenous insulin to recreate the low, peak-less overnight plasma profile of endogenous insulin (Owens et al., 2001, Heise and Pieber, 2007), may potentiate nocturnal hypoglycaemia, contributing to glucose variability, compensatory eating behaviour and possibly weight gain. Therefore, basal insulin that produces a flatter and longer profile of action might improve tolerability and safety. Recently, newer basal insulins have been shown to have a more stable steady-state profile, good reproducibility and an ultra-long duration of action (Gough et al., 2013). With evidence demonstrating good HbA1c reductions, similar weight gains and reductions in hypoglycaemic episodes, particularly at night compared with traditional insulins (Zinman et al., 2012, Ritzel et al., 2015). More research is needed to assess the longer-term benefits of these new ultra-long acting insulins in terms of improvements of glycaemic control and IAWG (Bolli and DeVries, 2015).

1.5. Lack of focus on Advanced Diabetes

Despite the issues that have been presented regarding the burden that is placed on insulin treated patients and the surrounding healthcare system, there still remains very limited research on how best to treat obese T2D patients treated with insulin and reduce this substantive burden. Much of the recent research has focus on prevention and remission of recently diagnosed T2D patients who have limited medication burdens and diabetes related complications, under the belief that this is the most important patient group and represent the greatest potential healthcare cost savings.

Within the current literature there is a wealth of evidence that demonstrates that intensive diabetes prevention programmes can help to sway the tide of diabetes diagnosis (Pan et al.,

1997, Tuomilehto et al., 2001, Knowler et al., 2002). At present these impressive results have not been translated into a successful prevention programme outside the clinical trial setting. Despite this lack of successful real-life application, focus has remained on prevention with the recent 2016 Diabetes Prevention Programme (DPP) being rolled out nationally in the UK. This programme focuses on high-risk patients with an HbA1c between 6.0-6.5% (42-47mmol/mol) that represents around 10.7% of the adult population, in the hope that DPP will make an important contribution to incidence of newly diagnosed T2D. One major issue is the idea that DPP is going to be able to permanently change behaviour in these high-risk individuals and on a large enough scale to make a significant impact on T2D incidence. Critiques of the DPP include the assumption that patients will behave like research participants, that accurate identification of high risk patients is possible, clinically relevant improvements are achievable and that the programme will be affordable and cost effective (Barry et al., 2015).

There is no doubt that prevention is better than cure, but there remains a lack of strong evidence that this is possible at a population and public health level. This type of complete commitment on a very select patient population by the NHS, Diabetes UK and Public Health England fails to address the issues of patients with more advanced diabetes. The clinical management of obese patients with T2D treated with insulin remains challenging and therefore research into effective treatment strategies to help manage both their weight and glycaemia are essential to help reduce direct and indirect burden from this disease.

1.6. Insulin

In order to understand further how T2D affects the body to disrupts glucose homeostasis it is important to first review normal glucose physiology. The primary glucoregulatory hormone is insulin, a peptide hormone synthesised in the pancreatic β -cells of the islets of Langerhans and acts to promote tissue uptake of postprandial glucose. The pancreatic β -cells are vital in the secretion of insulin and maintenance of glucose homeostasis. Insulin is composed of two peptide chains, the A and B chains, which are connected by two disulphide bonds. In humans, the A chain consists of 21 amino acids and the B chain is made up of 30 amino acids (51 amino acids in total) (Mercolini et al., 2008). Insulin is synthesised from its precursor proinsulin by the removal of a connecting peptide, C-peptide in equimolar amounts. With the plasma half-life of insulin being only 5 to 10 minutes and with 40-80% being cleared by the

liver before it enters the systemic circulation means that C-peptide is often used as a marker of insulin secretion (Natali, 2015). Other hormones produced from the pancreas include glucagon and somatostatin produced by the α -cell and δ -cells, respectively. Many of glucagon effects are the opposite to that of insulin, in that it stimulates hepatic glucose production usually after a reduction in plasma blood glucose. Whereas, somatostatin acts on both the β and α -cell receptors inhibiting both insulin and glucagon release (Hill, 2015).

Insulin is a potent regulator of metabolism and an anabolic hormone, which promotes synthesis and storage of dietary macronutrients in the post-prandial state. Key actions include glucose uptake in peripheral tissues particularly adipocytes and skeletal muscle thus suppressing free fatty acid release, limiting hepatic triglyceride synthesis (Sattar, 2015) promoting the formation of liver glucagon and inhibiting hepatic glucose production (HGP). Insulin receptors are expressed in a variety of mammalian tissues (Bailey et al., 1997) demonstrating their extensive metabolic role throughout the body.

1.6.1. Glucose-induced insulin secretion

Insulin secretion is stimulated by the intake of macronutrients during a meal while during a period of fasting circulating insulin falls with a corresponding rise in glucagon. The primary nutrient insulin secretagogue is glucose, glucose-induced insulin secretion (GIIS). It must be highlighted that amino acids and acute exposure of free fatty acids also promote insulin secretion in both healthy and T2D patients (Floyd et al., 1966, Crespín et al., 1973, Nuttall et al., 1984, Newsholme and Krause, 2012). GIIS is initiated through the transportation of glucose into the human pancreatic β -cell through the facilitated glucose transporter (GLUT-1), which is predominantly expressed in human β -cells (De Vos et al., 1995). This process occurs independently of insulin. Once inside the β -cell, glucose is sensed by a glucokinase (hexokinase IV), which immediately phosphorylates glucose to form glucose-6-phosphate (G6P). G6P is then metabolised to produce pyruvate, which then enters the mitochondrion, where enters the tricarboxylic acid (TCA; Krebs) cycle and generates adenosine triphosphate (ATP) in the electron transport chain. ATP is then translocated to the cytosol increasing the ATP:ADP ratio resulting in closure of the K_{ATP} dependent channels (Henquin et al., 2003) causes depolarisation of the cell membrane. This causes the voltage dependent calcium channels (VDCC) to open allowing calcium (Ca^{2+}) influx into the cell leading to an increase

in intracellular calcium and exocytosis of the insulin granules and release of insulin from the β -cell into the portal vein where it is transported to target tissues.

1.6.2. Insulin stimulated glucose uptake

Insulin has a key role in maintaining plasma glucose level within a narrowly defined range (4-7mmol/L) within healthy individuals. Skeletal muscle is the primary site for glucose uptake in humans with approximately 80% of insulin stimulated glucose uptake (ISGU) taking place in the skeletal muscle (Thiebaud et al., 1982).

After a meal, insulin binds to the insulin receptor on the cell membrane of target tissue. This causes a conformational change that closes the α subunits of the receptor closer together enabling ATP to bind to the intracellular domain of the β subunits. This causes tyrosine phosphorylation allowing the insulin receptor substrate (IRS-1) to dock and become itself phosphorylated. IRS-1 then attaches to phosphatidylinositol-3 kinase (PI-3K), increasing the action of its subunit P85 and P110. At the plasma membrane PI-3K causes a cascade of reactions resulting in protein kinase B (Akt/PKB) being attracted to the plasma membrane. Akt is activated and inhibits glycogen synthase kinase 3 (GSK3) causing glycogen synthesis. Activated Akt then enables glucose transporter-4 (GLUT4) storage vesicles to move to, dock and fuse with the membrane allowing glucose to enter the cell. Therefore, it is apparent that the IRS-1/PI-3K/Akt pathway is essential in maintaining normal insulin-stimulated glucose uptake in skeletal muscle (Krook et al., 2000) and any alteration to this pathway could affect glucose homeostasis.

1.6.3. Insulin resistance

IR can broadly be defined as an abnormal biologic response to insulin (Sattar, 2015) in target tissues including muscle, fat and liver resulting in a hyperglycaemia state characteristic of obesity and T2D (DeFronzo and Tripathy, 2009). IR is not only associated with impairing glucose homeostasis but also with essential hypertension (Ferrannini et al., 1987), non-alcoholic fatty liver disease (Utzschneider and Kahn, 2006), certain forms of cancer and CVD (Brunzell and Ayyobi, 2003, Reaven, 2004, Wilcox, 2005).

Within individuals with T2D the effect of IR does not just affect glucose homeostasis but also free fatty acid (FFA) metabolism. In groups of obese individuals, hyperinsulinemia causes a

marked increase in de novo lipogenesis (Schwarz et al., 2003). Studies using hyperinsulinemic-euglycemic clamps have shown that IR is associated with reduced stimulation of glucose transport in muscle and adipose tissue (Garvey and Hermayer, 1998). In comparison to insulin-sensitive controls, those with IR require 60% lower glucose infusion to maintain euglycemia with insulin-stimulated rates of peripheral glucose uptake also being 60% lower (Petersen et al., 2005). This difference has been attributed to approximately 70% reduction in non-oxidative muscle glucose metabolism (Petersen et al., 2004).

The abnormalities in insulin signalling in those with IR and T2D studies have shown that there is reduction in insulin stimulated tyrosine phosphorylation of both the insulin receptor and IRS-1 and decreased association of PI-3K with IRS-1 and a decrease in Akt phosphorylation (Cusi et al., 2000). Furthermore, in the hyperinsulinemic state, IRS protein expression is suppressed coupled with a downregulation of the insulin receptor, which together contribute to insulin resistance and worsening glycaemic control. Given the importance of GLUT in glucose transport into peripheral tissue it has also been suggested that impaired GLUT-4 translocation or diminished activity may be part of the mechanisms of IR (Hunter and Garvey, 1998). Indeed in adipocytes, cellular depletion of GLUT-4 transporters is a major mechanism of IR in obesity and T2D (Garvey et al., 1991), while GLUT-4 expression is relatively unchanged in skeletal muscle (Garvey et al., 1992). A variety of other defects in insulin signalling have been suggested but this is beyond the scope of this chapter.

1.6.4. Aetiology of T2D and impaired insulin secretion

The onset of β -cell dysfunction occurs early in the natural history of T2D, even within those exhibiting apparently normal glucose tolerance evidence of IR and β -cell dysfunction is present (Gastaldelli et al., 2004). By the time of diagnosis, which can occur 9 to 12 years later (Harris et al., 1992), β -cell function has deteriorated even further (UKPDS Study Group, 1995), with data from the UKPDS suggesting that approximately 50% of patients already have evidence of diabetic tissue damage at the point of diagnosis (UKPDS Study Group, 1990).

Insulin is known to be secreted in a multioscillatory process with rapid insulin pulses of around 5-10 minutes and slower ultradian oscillations which last between 50-120 minutes,

which appear to be closely associated with plasma glucose concentrations (Simon and Brandenberger, 2002). The release of insulin from the β -cell in response to glucose is characterised by biphasic kinetics. Firstly an initial phase (1st phase, FP) which happens rapidly but ceases after a few minutes followed by a more sustained phase (2nd phase, SP) (Henquin, 2009). The FP is important in optimal glucose homeostasis (Luzi and DeFronzo, 1989) and occurs as soon as intracellular Ca^{2+} increases. It has been proposed that the SP insulin response is the result of the K_{ATP} independent channels pathways (Straub and Sharp, 2002), which acts in cooperation with the K_{ATP} dependent channel pathway.

It is estimated that two separate pools of insulin are presenting in the β -cell, a docked pool and a reserve pool (Proks et al., 1996), in which these granules are then released at the different phases of insulin release. The activation of the K_{ATP} dependent channel pathway results in exocytosis of insulin of an immediately releasable pool that has been associated for the FP of GIIS (Hoenig and Sharp, 1986, Straub and Sharp, 2002). These insulin-containing granules are poised for immediate release. The prolonged stimulation as present in the SP insulin response involves a coordinated movement of granule in the docked pool for exocytosis. Recently it has been suggested that with T2D that the failure for insulin granule docking is an important glucose-dependent step in insulin exocytosis (Gandasi et al., 2018).

Within individuals with T2D there is a dysfunction in β -cell function resulting in a marked impairment in FP insulin response, which is more or less non-existent. The SP or late phase insulin secretion appears to be similar in those with and without T2D, although when glucose concentrations are matched, the response is abridged (Perley and Kipnis, 1967, Ward et al., 1984).

1.6.5. Altered Free Fatty Acid Metabolism: abnormal fat accumulation

There is now considerable evidence that implicates both a deranged adipocyte metabolism and altered fat topography in the development glucose intolerance (DeFronzo, 2015). One key feature of adipocytes is the regulation of lipid and glucose homeostasis. In the adipose tissues of patients with obesity or T2D the expression of GLUT-4 is downregulated (Moraes-Vieira et al., 2016) which has been shown to be key in IR and T2D pathogenesis (Shepherd and Kahn, 1999). Indeed, in mice with adipose-selective deletion of GLUT-4 this results in the development of IR, glucose intolerance and hyperinsulinemia (Abel et al., 2001). While

overexpression of GLUT-4 in GLUT-4 knockout mice normalises fasting hyperglycaemia and glucose intolerance (Carvalho et al., 2005). In T2D and IR the elevation in plasma FFA has been suggested to indicate in part the presence of adipocyte resistance to the antilipolytic effect of insulin (Gulli et al., 1992) which has been shown to lead to high triglycerides and apolipoprotein B levels, low HDL-cholesterol and an increase in small dense low density lipoprotein (LDL) (Sattar et al., 1998).

One key theory involved in glucose intolerance and β -cell decline is lipotoxicity (Del Prato, 2009). Lipotoxicity is the deposition of lipids in the β -cell alongside chronic elevation of plasma FFA that adversely affects the secretion and action of insulin. The chronic exposure of pancreatic β -cells to FFA causes a variety of manifestation including accumulation of malonyl-CoA and the long-chain fatty-actyl-CoA, increased fatty acid oxidation, fatty acid-induced apoptosis and activation of endoplasmic reticulum (ER) stress (Del Prato, 2009). In vitro, human islet cells incubated in FFA for 48 hours causes impairment in both acute and late insulin response, inhibition of insulin mRNA expression and reduced islet insulin content (Lupi et al., 2002). Furthermore there is a dose-response inhibition of muscle receptor tyrosine phosphorylation, IRS-1 tyrosine phosphorylation, PI-3 kinase activity, and Akt serine phosphorylation (Belfort et al., 2005), all key pathway in insulin signalling.

Within peripheral tissues elevated FFA were initially thought to cause IR by substrate competition between FFA and glucose (Del Prato, 2009). Intramyocellular lipids (IMCLs), also known as ectopic fat, proliferate in the presence of extra FFA and a lack of the subcutaneous fat stores to accommodate this surplus results in lipid overflow into non-adipose tissue (Larson-Meyer et al., 2006). Of particular interest within the pathophysiology of T2D is the presence of fat within the liver and secondly the pancreas. The combined role of this infiltration of IMCL within the liver and the pancreas has been jointly called the twin cycle hypothesis (Taylor, 2013). In humans it has been shown that IMCL accumulation within the pancreatic islets is directly associated with impaired β -cells, particularly FP insulin response (Lim et al., 2011). This concept of fat infiltration is not new and has been described in Zucker diabetic fatty rat models or over 20 years (Lee et al., 1994, Unger, 1995), but due to difficulties in non-invasive quantification of triglycerides in human islets has meant until recently this had proved difficult to quantitate (Tushuizen et al., 2007, Lingvay et al., 2009, Lim et al., 2011). Within rodent models the accumulation of peak pancreatic fat in islets appears to correspond with the appearance of hyperglycaemia and reduction in insulin

secretion and IMCL accumulation preceded adipocyte infiltration in Zucker diabetic fatty rodents (Lee et al., 2010). Of particular interest is that individuals with T2D appear to have significantly greater pancreatic and hepatic triglyceride content compared to both lean and obese non-diabetic subjects, even after adjustment for BMI (Gaborit et al., 2015), suggesting that this form of triglyceride accumulation might be unique to those that develop overt T2D.

The presence of IMCLs is not necessarily detrimental, rather the inability to efficiently oxidise IMCLs is (Goodpaster et al., 2001, van Loon and Goodpaster, 2006). In T2D increased IMCLs results in increased FFA availability, reduced β -oxidation, accumulation of intermediates and ultimately reduced insulin transduction/signalling (Snel et al., 2012c). Recent evidence has now shown that elevated IMCL directly inhibit glucose transport due to the accumulation of lipid metabolites, diacylglycerol (DAG), long chain fatty acyl-CoA (LC-CoA) and ceramide. These metabolites have been shown to reduce insulin signalling and glucose transport (Itani et al., 2002, Yu et al., 2002) in both the liver and skeletal muscle and cause a decline in β -cell mass and apoptosis (Shimabukuro et al., 1998, Newsholme et al., 2007).

The subsequent cascade of cellular processes that are suggested to lead to IR in skeletal muscle and liver cells have similarities but differ slightly according to the specific cell type. In the presence of excessive supply of FFA and/or impaired mitochondrial oxidation there is an accumulation of long chain fatty acyl-CoA which are then broken down to DAG and ceramide (Snel et al., 2012c). These metabolites provoke a sustained activation of serine/threonine kinases that phosphorylate IRS on serine residues, so IRS cannot associate with PI-3K leading to subsequent defects in insulin signalling (Morino et al., 2006). Unlike muscle, accumulation of DAG in the hepatic cells activates protein kinase C leading to a decreased activity in insulin receptor kinase, resulting in a reduced IRS-2 tyrosine phosphorylation and PI-3K association. Ultimately, resulting in a reduced glucose uptake, glycogen synthase and increased HGP through decreased phosphorylation of forkhead box O (FOXO) allowing continued gluconeogenesis. Moreover, with a reduced fatty acid oxidation occurring in T2D patients the mitochondrial capacity to oxidise fat maybe impaired, which could also add to the cellular processes that cause IR (Kelley, 2005). In fact, in healthy lean, IR offspring of parents with T2D there is approximately a 30% reduction in the mitochondrial rate of ATP production in skeletal muscle that is associated with an approximately 80% higher content of IMCL (Petersen et al., 2004). This suggests that IR in

these patients may in part be due to an inherited defect in mitochondrial oxidative phosphorylation, which may be linked to an increased ratio of type II (fast twitch) muscle fibres (Petersen et al., 2004).

It has been suggested that individuals susceptible to T2D and overt T2D have a greater propensity to accumulate fat particularly visceral and ectopic fat as opposed to subcutaneous fat (Sattar, 2015), although the exact reasons for this remains a matter of debate. With some suggesting that the ability of peripheral subcutaneous fat to vary its storage capacity is vital (Kim, 2015). In comparison to subcutaneous fat, visceral fat has been shown to have higher rates of lipolysis (Arner, 1995) and that this increases with increasing amounts of fat (Nielsen et al., 2004). This might seem logical, however with intra-individual variation in total FFA release and the fact that only 20% of portal vein FFAs originate from visceral fat in obese subjects, questions whether subcutaneous or visceral fat is more important in the development of hepatic IR (Klein, 2004) and accumulation of IMCLs.

1.7. Inflammation, obesity and T2D

The pathogenesis of T2D and IR and its link with obesity has been intensely researched in order to better treat these two disease states. Sustained exposure to nutrient excess in obesity and T2D has been shown to cause chronic low-grade systemic inflammation in adipose tissue, the liver, skeletal muscle and the pancreas (Hotamisligil, 2006, Lackey and Olefsky, 2016). During the inflammation process there is an increase in proinflammatory macrophages, tissue inflammation and an increase release of cytokines such C-reactive protein, tumour necrosis factor- α (TNF- α), c-Jun N-terminal kinase (JNK) and interleukin-1 β (IL-1 β) (Lackey and Olefsky, 2016, Saltiel and Olefsky, 2017). These inflammatory cytokines have been linked to the development of IR, defectives in insulin signalling and the disruption of energy homeostasis (Koyama and Brenner, 2017, Jais and Bruning, 2017).

Within the pancreas, increased macrophage infiltration has been associated with increases in IL-1 β , believed to impair insulin signalling (Richardson et al., 2009, Rissanen et al., 2012, Eguchi and Nagai, 2017). Indeed, when IL-1 is blocked in patients with T2D there is an associated improvement in glycaemia and β -cell secretion, alongside a reduction in systemic inflammation (Larsen et al., 2007). Within the liver, proinflammatory cytokines, monocyte-

derived hepatic macrocytes and Kupffer cells are reported to be elevated in obesity (Zigmond et al., 2014, Morinaga et al., 2015) and linked to the disruption of glucose homeostasis. Furthermore, it has been shown that inflammatory cytokines impair the hypothalamic response of insulin on satiety, with TNF- α being key to inhibiting IRS-1 phosphorylation (Hotamisligil et al., 1996, Souza et al., 2005).

Previous studies have shown the caloric restriction and weight loss can induce a reduction in CRP in both obese and obese T2D patients, with larger weight loss being associated with greater reduction in inflammation (Selvin et al., 2007). In addition to CRP, TNF- α and IL-6 have also been shown to reduce with weight loss (Bastard et al., 2000, Snel et al., 2011), although during the acute weight loss phase it appears that inflammatory cytokines are not affected (Snel et al., 2011). Therefore, this presents a potentially important mechanism by which reduction in body fat might help to improve glucose homeostasis.

1.8. Treatment for T2D

Achieving and maintaining optimal glycaemic control is a key goal within the management of T2D that has resulted in up-titration of pharmacotherapy to achieve these goals.

1.8.1. Weight reduction

Intensive lifestyle change remains the cornerstone of T2D and obesity management (Nield et al., 2007). Prospective studies have shown that moderate weight losses of between 5-10% improve fasting blood glucose, insulin sensitivity and CVD risk (National Collaborating Centre for Chronic, 2008). Within a meta-analysis data of weight management even with highly intensive research interventions the amount of weight loss was limited to between 5-7kg, equivalent to 5-7% and was rarely maintained in the long-term (Avenell et al., 2004). The Scottish Obesity Guidelines suggested that for individuals with a BMI up to 35kg/m² that a moderate weight loss might be beneficial, but this is not sufficient in those with T2D and a BMI greater or equal to 35kg/m² (SIGN, 2010a). This would suggest at present traditional treatment options being used to treat patients with obesity and T2D are inadequate to result in beneficial long-term change of their body weight and obesity related co-morbidities.

Currently the most effective treatment for managing obesity and T2D is bariatric surgery (Dixon et al., 2008, Buchwald et al., 2009). This has been shown to reverse the metabolic abnormalities that characterise T2D namely normalisation of peripheral insulin sensitivity and enhancing of beta cell sensitivity to glucose (Guidone et al., 2006). At present, approximately only 2% of patients eligible for bariatric surgery are commissioned to have it within the UK. This is despite the 2014 NICE obesity update recommending that bariatric surgery should be considered as a treatment option for newly diagnosed T2D and those with poor glycaemic control and a BMI between 30-35kg/m² (NICE, 2014b).

1.8.2. Benefits of weight loss within T2D and specifically those treated with insulin

The ultimate goals of any intervention for T2D are to reduce the occurrence of macrovascular and microvascular complications and for those with an elevated BMI while encouraging weight loss and maintenance. The benefits of weight loss have been reported in those with less advanced T2D including glycaemic control, blood lipids, blood pressure and observational evidence of CVD events (Goldstein, 1992, Williamson et al., 2000, Ridderstrale et al., 2006, Lean et al., 2017). The benefits however in those treated with insulin have not been as well documented. In fact, there is evidence that patients treated with insulin do not have the same beneficial improvements in HbA1c, remission and weight loss compared with patients treated with diet or OHA (Ridderstrale et al., 2006, Pi-Sunyer et al., 2007, Gregg et al., 2012).

Even with significant weight losses in both patients treated with or without insulin the benefit of weight loss on macrovascular outcomes remains unclear. The only randomised clinical trial to date to assess whether dietary weight loss programmes reduced the risk of CVD mortality or events is the Look AHEAD trial (Pi-Sunyer et al., 2007). Results from the Look AHEAD study are interesting in that they failed to show a benefit of weight loss on cardiovascular events (The Look AHEAD Research Group, 2013). This was despite showing that intensive lifestyle interventions (ILI) lead to significant weight losses, HbA1c improvement, increased T2D remission rates and surrogate markers of CVD risk including blood pressure, lipid profile and urine albumin-to-creatinine ratio (Pi-Sunyer et al., 2007). Suggesting that weight loss or the degree achieved in the study may not prevent CVD events in T2D, however, limitations within the study (Wilding, 2014) means that the lack of difference may not have been attributable to these factors. Importantly the control group

received management of their diabetes and CVD risk factors in routine care, including cardio protective medications, which were greater than the ILI group, therefore may have neutralised the effect of weight loss on CVD outcomes. In addition, as the control group also lost weight, which was greater than usually seen in routine care, which may have contributed to the lower event rate. Added to this the weight regain within the ILI group meant that the relative difference was only modest at the end of the study (Wilding, 2014).

The ideal population to examine whether weight loss is beneficial in those with T2D treated with insulin is the bariatric cohort due to the large sustainable weight loss achieved. One key criticism of the current bariatric surgery literature is the lack of randomised control trials with much of the data being observational, often retrospective, have large numbers of dropouts and traditionally of poor quality. Notwithstanding this, bariatric surgery remains the best treatment for both obesity and T2D at present.

Like traditional medical therapy much of the literature has focussed on non-insulin treated patients, where high remission rates (58-95%) are reported (Buchwald et al., 2009). Even with significant weight losses there is still not a permanent reversal of T2D (Sjostrom et al., 2004, Arterburn et al., 2013, Schauer et al., 2017a), with rises in HbA1c and medication escalation over time and with weight regain. In a subgroup of T2D patients on IT there is a significantly lower rate of remission, both in the short and mid-term, with figure of between 19.3 to 75.2% reported and higher chances of relapse (Casella et al., 2011, Jimenez et al., 2012, Ardestani et al., 2015, Adams et al., 2016, Ghio, 2017). This difference is likely to relate to a reduced residual β -cell function meaning that despite significant improvement in insulin sensitivity and insulin resistance, insulin secretion remains insufficient to compensate for elevated blood glucose levels.

The Swedish Obesity Subjects (SOS) trial was a prospective controlled intervention study of bariatric surgery (Sjostrom et al., 2004) and currently represents the best indicator of how weight loss influences CVD events, cancer and overall morality following surgery (Sjostrom, 2013), despite its substantive methodological weaknesses. In comparison with usual medical care, bariatric surgery was associated with a reduction in all-cause mortality, CVD events (myocardial infarction and stroke) and cancer. With weight loss following bariatric surgery having a positive effect on CVD risk factors at both 2 and 10-years follow up (Sjostrom et al., 1997, Sjostrom et al., 2004, Sjostrom et al., 2011, Sjostrom et al., 2014). Despite surgery

demonstrating outstanding weight loss outcomes and improvements in co-morbidities, it is invasive and associated with its own significant complications. Furthermore, it is reported that only approximately 2% of the eligible population get bariatric surgery each year (Dixon et al., 2011), with surgery being limited by funding, operative capacity and lack of tier 3 and tier 4 services in many geographical areas to prepare patients appropriately for surgery. Therefore, the question remains how to optimally treat the other 98% of patients; clearly there is a need for a clinically effective non-invasive strategy to meet this need and fill the therapeutic void between current medical management and bariatric surgery.

1.8.3. Formula Very Low and Low Energy Diets

The published evidence to support the clinical use of formula diets both very low energy diets (VLED) and low energy diets (LED) in the management of obesity and its associated co-morbidities has grown over the last 20 years. Despite the increasing evidence in both the number and quality of studies the use of VLED and LED remains controversial and are infrequently recommended. In part this is due to concerns over their cost and long-term efficacy once stopped but also due to a lack of awareness by the clinicians treating patients. Within the insulin treated patient group, the risk of hypoglycaemia is significant, and this may be another reason that at present it is not recommended without specialist referral.

VLED are designed to be a total diet replacement (TDR) providing less than 800 calories per day and come in prepared formula usually in the form of liquid shakes, soups and bars (NICE, 2014b). A recent change within the European Food Safety Agency formulation guidelines on Total diet replacement products has resulted in the minimum daily intake of calories being increased from 450kcal/day to 600kcal/day. Furthermore, minimal amounts of protein, fat, essential fatty acids and carbohydrate have also been recommended, but this will not be law until 2022. In comparison LED range between 800 to 1200 kcal/day. This can be through a formula diet only as with the VLED or in combination with product and conventional food. VLED and LED provide between 70-100g/day of dietary protein, which equates to 0.8-1.5g protein/kg ideal body weight (Tsai and Wadden, 2006). This compositional design is intended to induce rapid weight loss while aiming to maintain fat free mass. Due to lack of evidence no exact recommendations are currently made for a fibre within recommended daily amounts (RDA), as a result fibre is often pragmatically provided between 7-10g (Saris, 2001). Within the literature VLED and LED are used typically over an

8-20 week period (TDR phase) (Christensen et al., 2011, Steven et al., 2016a, Lean et al., 2017), but at present there is no consensus on the ideal length of time. NICE however recommends that they should be used for a maximum of 12 weeks, either continuously or intermittently (NICE, 2014b). However, this is likely to vary on an individual's health.

VLED are designed to induce a state of ketosis, through a combination of dietary carbohydrate and energy restriction, which increases fatty acid oxidation resulting in the release of ketone bodies, which have been suggested to suppress appetite. One of the key advantages of both VLED and LED is that they help with the removal of food from the daily decision process, provides meals with greater structure and allows individuals to look at their relationship with food with greater clarity (Leeds, 2014, Brown, 2015).

1.8.3.1. Weight Loss

There is now substantial evidence to show that both VLED and LED are effective at promoting clinically relevant weight loss, at least in the short term (up to 24 weeks). The length of time on these formula diets varies between 4 to 24 weeks within the medical literature, which is different to that recommended in clinical practice. Initially weight losses of between 1.2 to 2.5kg per week are reported in the TDR phase, which can result in weight losses between 10-20kg. Within individuals with early onset T2D, weight loss has been reported to be approximately 15kg up to 6 months (Steven and Taylor, 2015, Steven et al., 2016a, Lean et al., 2017). Following a VLED in patients with T2D treated with insulin weight loss appears more variable (11-25.8kg) (Jazet et al., 2007, Snel et al., 2011, Snel et al., 2012a, Snel et al., 2012b). With longer period of caloric restriction encouraging greater weight losses. Data now suggests that at 12-months VLED together with behavioural programmes can lead to an additional 3.9kg (95% CI -6.89, -1.11) weight loss compared with behaviour change alone (Parretti et al., 2016).

In theory VLED should produce greater weight losses than LED due to the greater energy deficit, however evidence has shown that this might not always be the case, both in the short term and the long term (Saris, 2001, Norris et al., 2005, Christensen et al., 2011). In a meta-analysis, short-term weight loss was significantly greater following VLED compared with a LED (16.1±1.6kg vs. 9.7±2.4%, respectively), which was also seen in other reviews and papers (Wing et al., 1991, Foster et al., 1992, Saris, 2001, Norris et al., 2005). Although few

studies used an intention to treat model, hence analyses may have been biased as only completers were reported on. Recently, however, a more controlled study demonstrated that at both 8 and 16 weeks following a VLED and LED that there was a non-significant difference in weight loss between groups (Christensen et al., 2011). In the long-term there has traditionally been significant weight regain following the TDR phase (Wing et al., 1991, Foster et al., 1992, Saris, 2001, Norris et al., 2005) with the VLED regaining 62% of weight loss compared to 41% for the LED (Tsai and Wadden, 2006). This weight regain results in there being no difference between these two dietary approaches at 12-months, no matter if individuals lose more weight initially.

The question remains whether these results can be translated into a real-life setting; this has in part been addressed with the publication of two studies within the UK commercial and clinical environment (Lean et al., 2013, Rolland et al., 2014). Firstly, a pilot study of the Counterweight Plus programme, which involved the addition of a LED during the initial 12 weeks to the already established programme (Laws, 2004), enabled those with a BMI $\geq 40\text{kg/m}^2$ to lose on average $12.4 \pm 11.4\text{kg}$ at 12-months (Lean et al., 2013). While in a retrospective analysis of a commercially available VLED programmes reported significant weight losses at both 12 and 3-years (18 ± 11.4 and $12.9 \pm 10\text{ kg}$, respectively). In spite of methodological limitations within both studies this shows that there is a potential that formula VLED and LED diets can be effective outside of research studies.

1.8.3.2. Weight Maintenance

Weight maintenance remains one of the most challenging areas of weight management. A plethora of drivers including physiological, psychological and biological cause weight regain and are often unique to the patient. Traditionally there has been concern that following successful weight loss using VLED and LED this is followed by rapid weight regain. Convention has suggested that challenges arise with weight maintenance when conventional food is reintroduced following the TDR phase, with patients often not having addressed the reason for their initial weight gain. Therefore, the use of behaviour change alongside formula diets can help with weight maintenance and help patients address their disordered eating behaviour (Saris, 2001, Mulholland et al., 2012). Indeed, recent well-controlled studies have challenged this previously held belief that weight regain is inevitable following TDR. Four studies, including two systematic reviews have all shown that weight maintenance is possible

with the addition of weight maintenance strategies (Mulholland et al., 2012, Christensen P, 2014, Johansson et al., 2014, Christensen et al., 2017b). The continued use of formula products either continuously or intermittently has been shown to aid weight maintenance at up to 4 years within patients with osteoarthritis (OA) (Christensen et al., 2017b). Within this randomised control trial participants were assigned to either having an intermittent treatment of LED for 5 weeks every 4 months for 3 years (IN) or a daily meal replacement for 1-2 meals for 3 years (RE). Over this period weight regain was kept to a minimal with 0.68 and 1.75kg in the IN and RE groups, respectively, with a non-significant difference between groups. More over the old adage that “slow and steady” weight loss is better than “rapid” has now been challenged, with evidence showing that weight regain is no different at 144 weeks (Purcell et al., 2014)

Other strategies include the use of physical activity during the weight maintenance phase. Compared with diet counselling studies the use of physical activity has shown both positive (Fogelholm et al., 2000, Borg et al., 2002) and negative treatment effects (Christensen et al., 2013). Furthermore, when physical activity studies were included in a meta-analysis of weight maintenance programmes after VLED they showed a non-significant reduction of -0.8kg (95% CI 2.8 to 1.2kg). Similarly results were seen with the use of pharmacotherapy to aid weight maintenance following VLED (Johansson et al., 2014), although it should be noted that two of the three studies used sibutramine, a drug that was withdrawn in 2010 (Brown, 2015).

Within weight management services there is a commonly held belief that “slow and steady” weight loss is better for weight maintenance than weight lost more rapidly however this is questioned by recent evidence. Of interest is that greater weight loss in the first year is predictive of weight loss maintenance in the long-term (Astrup and Rossner, 2000, Wadden et al., 2011, Rolland et al., 2014). A sub-analysis of the Look AHEAD cohort showed that patients losing $\geq 10\%$ body weight at 1 year had a 10.4-times greater odds ratio of maintaining that loss at 4 years, compared to those that lost less than 5% (Wadden et al., 2011). Several other studies have also shown that large initial weight losses are important in maintaining clinically significant weight loss in the long-term (Astrup and Rossner, 2000, Anderson et al., 2001, Saris, 2001). It might be hypothesised that a substantial initial weight loss may help individuals to remain motivated thereby counteracting the counter-regulatory

effects of appetite hormones (Sumithran et al., 2011). The exact mechanism however behind this effect remains unclear and requires further research.

The importance of the composition of diet in preventing weight regain and obesity related risk factors after weight loss was assessed in a multicentre randomised trial, the Diet, Obesity and Genes (Diogenes) study (Larsen et al., 2011, Aller et al., 2014). Following an 8-week weight loss phase, using an LED (800-1000kcal/day), participants were then given a moderate-fat diet with varying protein content and glycaemic index (high GI/protein; low GI/high protein; high GI/low protein; low GI/low protein) or a control diet and followed over a 12-month period. The results at 6-month showed that a moderate increase in protein and reductions in GI led to improvement in weight loss maintenance, although at 12-month only protein content remained significant. This confirms previous studies that a higher protein intake produces better weight maintenance (Westerterp-Plantenga et al., 2004, Lejeune et al., 2005), however with a greater -0.138kg weight loss in favour of the high protein diet (Clifton et al., 2014), this is of questionable clinical relevance. This apparent discrepancy between the results being reported in the short term and during extended follow-up is likely to relate to compliance and returning to previous eating habits. This is evident with studies that have greater supervision over 12-months follow up, with a 5% greater intake of protein compared to those without continued contact (Clifton et al., 2014).

One issue that NICE highlighted in regarding the use of VLED is that of weight cycling, with epidemiological studies suggesting that this causes an increased risk of morbidity and mortality (Lissner et al., 1991) and psychological stress of regaining weight. The difficulty in interpreting these results is identifying the difference between intentional and unintentional weight loss. In spite of this, a systematic review concluded that there was a lack of evidence that there was any negative effect, in addition to no causal association with weight cycling with early mortality or morbidity (Mehta et al., 2014).

1.8.3.3. Side effects of formula low energy diets

During the initial stages of rapid weight loss with VLED and LED, individuals have reported experiencing side effects including constipation, cold intolerance, increased hair loss, fatigue, dizziness, muscle cramps and gallstones (Wadden et al., 1983, Saris, 2001, Lean et al., 2017). Side effects with formula diets are generally mild and easily managed, although if VLEDs are

used without supervision there is a risk of complications (Wadden et al., 1983). Until recently the reporting of side effects has been limited or rarely mentioned within the literature. The first paper to comprehensively cover side effects reported that VLED had significantly greater occurrence of bad breath, intolerance to cold, and flatulence, with trends towards greater hair loss and dry skin compared to LED (Christensen et al., 2011). Of particular concern have been reports of gallstones following VLED, this has been rarely reported in the literature and is has been generally limited to the USA rather than Europe, due potentially to the differing essential fat levels (Saris, 2001).

Table 1.2 Advantages and Side Effects of use of Very Low and Low Energy Formula Diets within clinical practice

Adapted from (Brown, 2015)

Advantages	Side Effects
Accurate calorie management	Hypoglycaemia
Nutritionally complete	Constipation
Significant weight loss	Flatulence
Improvement in glycaemic control	Feeling the cold
Reduced cardiovascular risk markers	Dizziness and hypotension
Improvement in sleep apnoea	Nausea
Improvement in general quality of life	Diarrhoea
Benefits for osteoarthritis	Gallstones
Improvement in psoriasis	Headache
Benefits for mood	Acute gout
Removal of daily decision making process	Halitosis
	Sleep disturbance
	Temporary hair loss

Table 1.3 Contraindications for the use of VLED

(Brown, 2015)

Contraindication of use for a VLED
Infants and children
Pregnancy and lactating women
Unstable cardiac or cerebrovascular disease
Acute and chronic renal failure
Severe or end stage liver failure
Acute psychiatric disorder
Gout
Elderly

Abbreviation: VLED, very low energy diet

1.8.3.4. The current role of formula very low and low energy diets in clinical practice

As mentioned before, VLED and LED are used rarely within current clinical practice. The latest NICE update of the obesity guidelines (CG189) reviewed the evidence for the use of VLED in obese patients, which was only briefly mentioned in the previous guidelines (NICE, 2014, NICE, 2006). The guideline made a key recommendation that VLED should not be used routinely to help manage obesity (defined as BMI $>30\text{kg/m}^2$) and if used only in those who require rapid weight loss, for example people seeking hip operations or fertility treatment. Additional recommendations included VLED should be used in conjunction with a long-term multi-disciplinary component delivered from an obesity service that included psychological assessment. And that the risk, benefits and weight regain are discussed, mirroring the recommendations of many obesity groups position statements (NOF, 2010). Of interest was the lack of mention of LED within the 2014 update, this might however reflect the quality of published papers at the time of the review.

1.8.3.5. Formula low energy and very low energy diets in type 2 diabetes, CVD and other obesity associated co-morbidities

There is now a significant volume of evidence that shows that VLED can improve weight-related co-morbidities including waist circumference, blood pressure and lipid profiles (Tsai and Wadden, 2006, Mulholland et al., 2012).

Over the last few years the use of VLED and LED has gained a lot of interest in relation to T2D remission and whether this is possible within a clinical setting. Indeed, calorie restriction immediately following bariatric surgery has been proposed to play a central role in the early improvements in glucose homeostasis (Lips et al., 2014, Plourde et al., 2014, Steven et al., 2016b), even before any significant weight loss or change in plasma GLP-1 levels post surgery (Steven et al., 2016b). This has been in part explained via the Twin Cycle Hypothesis (Taylor, 2008). The first steps into understanding this in humans was the Counterbalance study, a small pilot study enrolling 11 patients with recently diagnosed T2D that used a VLED for 8 weeks after which a significant reduction in liver and pancreatic triacylglycerol was associated with normalisation of hepatic insulin sensitivity and β -cell function, respectively (Lim et al., 2011). This pivotal proof of concept study resulted in two further papers from the same group looking at subjects with longer duration of diabetes (Steven and

Taylor, 2015) and following patients after 6-months on a VLED. This study identified responders and non-responders (Steven et al., 2016a). Those that responded were younger, had higher fasting plasma insulin, lower fasting plasma glucose and shorter duration of diabetes and on fewer medications, in addition the responders had lower pancreatic fat and total body fat mass (Steven et al., 2016a). Although this published paper suggested that acute energy restriction could help to reverse the abnormalities typical to T2D, due to methodological weaknesses that included lack of controls, small numbers, selection bias and reporting on only completers of the study meant that these results were difficult to inform clinical practice.

The recent Diabetes in Remission clinical trial (DiRECT) looked to answer many of the questions raised by these initial studies. In particular whether the use of a LED within primary care could aid T2D remission in those with recently diagnosed diabetes (<6 years). The primary outcome was a dual focus on T2D remission defined as having an HbA1c <6.5% (48mmol/mol) after at least 2 months off all anti-diabetic medication from baseline to 12-months or achieving a weight loss of 15kg. In this randomised control trial, 49 general practices recruited 151 obese or overweight patients with T2D and randomised them to standard NHS care or an LED weight management programme. At 12-months, 46% of patients in the intervention group had achieved remission compared with 4% in the control group. While 24% achieved a ≥ 15 kg weight loss compared to none in the control group, furthermore those that lost more weight had greater occurrence of T2D remission. These results collaborated previous data from bariatric surgery where the degree of weight loss was found to correlate with T2D remission (Dixon et al., 2008), and in which 15kg appears to be key (Lean, 2011).

1.8.3.6. Very low and low energy diets in T2D patient treated with insulin

The use of VLED in insulin treated patients is less well-studied and there remains to be definitive conclusions regarding their potential benefits. The majority of studies have come from the same group and have focused on exercise, quality of life and mechanisms of improvements in glycaemic control with VLED (Christiansen et al., 2000, Jazet et al., 2005a, Jazet et al., 2007, Jazet et al., 2008, Snel et al., 2011, Snel et al., 2012b, Snel et al., 2012a, Snel et al., 2012d).

In the short-term (2-days, VLED [450kcal/day]) the key mechanism suggested for lowering fasting plasma glucose is via a decrease in basal endogenous glucose production (EGP), without an effect on insulin suppressibility of EGP, peripheral glucose disposal or lipolysis (Jazet et al., 2005a, Jazet et al., 2005b, Jazet et al., 2005c). This improvement in basal EGP has been proposed to be linked to a reduction in glycogenolysis and not a change in gluconeogenesis (Christiansen et al., 2000). Similar to those patients not on insulin, responders (FBG <10mmol/L) were identified as having a preservation of pancreatic β -cell function to secrete insulin, with higher fasting serum insulin and higher HOMA- β (Jazet et al., 2005c). In terms of the molecular pathways, no changes have been seen immediately following VLED in some of the key T2D related pathways including PI-3K, triglyceride distribution and GLUT-4 translocation in skeletal muscle. It should be noted that all patients had ceased all diabetes medications including insulin, although this did not increase plasma glucose levels (11.3 ± 1.3 vs 10.3 ± 1.0 mmol/L), they remained significantly elevated (>10 mmol/L), which may suggest that 2-days might not be sufficient time to see a peripheral molecular benefit.

The long-term effects of a VLED have been suggested to produce a sustained improvement in weight-loss, dyslipidaemia and hypertension (Jazet et al., 2007). Despite the authors suggesting that glycaemic control was improved there was no significant change either statistically or clinically in FPG and HbA1c at both 30 days and 18-months. Possibly signifying that the ceasing of all diabetes medications failed to enable beneficial changes in glycaemia, as pancreatic function remained insufficient to increase insulin production. Alternatively, this may be due to the degree of weight loss achieved while using the VLED, as when patients achieved a 50% excess weight loss (equivalent to 20% body weight loss) there was not only an improvement in EGP but also insulin sensitivity in both peripheral and central target tissues (Jazet et al., 2008). Furthermore, this increase in glucose disposal was reflected in an increase in AS160 and PRAS40 phosphorylation, downstream components of the PKB/Akt pathway causing improved insulin signalling and IR.

In a recent series of studies from Snel and colleagues, twenty-seven obese T2D patients on insulin were given a 16-week VLED, of whom 13 were given an exercise programme (VLED-E), and then followed-up for 14-months on a regular diet to assess a variety of different outcomes (Snel et al., 2011, Snel et al., 2012b, Snel et al., 2012a, Snel et al., 2012d). These included weight loss, low-grade inflammatory markers, body composition, surrogate markers of CVD including BP, plasma lipids, quality of life (QoL) and glycaemic control. As

previously mentioned the addition of exercise remains unclear. In a post-hoc analysis of the effect of additional exercise (70% maximal aerobic capacity) over the initial 16-week VLED it was found to have a beneficial effect on fat mass, waist circumference, maximal aerobic capacity and mitochondrial DNA content but this has no effect on insulin-stimulated glucose disposal rate (Snel et al., 2012a). At the end of the 16-week VLED, pericardial fat, hepatic TG content, visceral and subcutaneous abdominal fat volumes were significantly reduced, however, at 18 months, there was a significant increase in (non) adipose TG compartments apart from pericardial fat volume (Snel et al., 2012b). This was further explored looking at myocardial triglyceride content and function, again both improved following a 16-week VLED suggesting that cardiac ectopic fat can be effectively removed with caloric restriction. In terms of inflammation, there was no change in pro-inflammatory plasma cytokines in the acute weight loss phase of a VLED apart from hsCRP which decreased, although once eucaloric a significant improvement was seen in particularly in hsCRP, IL-6 and TNF α (Snel et al., 2011).

Lastly quality of life (QoL) was positively influenced by the use of VLED and particularly with the addition of physical activity. Compared with matched controls, individuals with T2D previously treated with insulin scored significantly worse on subscales mostly related to physical function and fatigue (Snel et al., 2012d). In the long-term the addition of exercise appears vital to maintaining QoL improvements, which might be related to the maintenance of weight within the VLED-E group.

Although these studies had no control group, only included small numbers of subjects in whom all diabetes medication was ceased, these studies do clearly show that using a formula VLED diet can have positive benefits on clinical outcomes. However, this improvement is transient in nature and if patients regain weight their diabetes will return or medication reintroduced, although admittedly at potentially a lower dose (Jazet et al., 2007).

In terms of medication titration very little data is currently published to advise clinicians. Oral hypoglycaemic medications particularly insulin secretagogues such as sulfonylureas and also insulin require significant reductions when commencing a VLED and LED to reduce the risk of hypoglycaemia (Brown, 2015). This should possibly involve a pre-emptive reduction of insulin dose by 25% (Haslam, 2010). At present the majority of studies using formula

VLED in T2D patients have stopped all hypoglycaemic agents at the start of the VLED including insulin, thus making the assumption that individuals have sufficient residual pancreatic function to compensate for this medication reduction. This key aspect may result in a different degree of remission or glycaemic response being seen within clinical practice and also limit its applicability in more advanced diabetes patients where stopping all agents is not possible due to insufficient residual pancreatic function.

1.8.3.7. Other obesity related co-morbidities

In addition to T2D other obesity related co-morbidities have also been shown to be positively affected by formula VLED and LED diets. CVD remains the largest cause of mortality in patients with T2D therefore the reduction of CVD risk and its associated risk factors is important.

Weight loss using a formula VLED has been shown not only to improve glucose homeostasis but also cardiac function physical fitness measured by VO_2 peak and cardiovascular symptoms, with the improvements being proportional to the degree of weight loss, (Rider et al., 2009, Jonker et al., 2014, Pedersen et al., 2015). Furthermore, in T2D patients treated with insulin, a 16-week VLED has been associated with decreased pericardial fat volume, myocardial triglycerides content and diastolic heart function (Hammer et al., 2008, Snel et al., 2012b). In two recent systematic reviews of VLED it has been suggested there is an improvement in both cardiovascular risk and its associated surrogate markers (Mulholland et al., 2012, Parretti et al., 2016). Although saying this high quality data remains limited and the published studies showed variable results both in the short and long-term. With only surrogate markers being used to identify CVD risk reduction and no data looking at overall mortality risk using VLED and LED, there remain questions regarding the long-term benefits of these dietary methods.

1.8.3.7.1. Obstructive sleep apnoea

Obstructive sleep apnoea (OSA) is a common respiratory condition that is common in obesity, IR and T2D. OSA is a disorder characterized by frequent, complete or partial upper airway obstruction during sleep, with distinctly reduced (hypopnea) or absent (apnea) airflow (McNicholas, 2008). The apnea-hypopnea index (AHI) is the average number of hypopnea or

apnea per hours during sleep and an indicator of OSA severity (McNicholas, 2008), with ≥ 5 event/hour AHI, being diagnostic of OSA.

OSA is associated with increased risk of CVD, metabolic disorders, and mortality (Marshall et al., 2008, Somers et al., 2008, Tasali and Ip, 2008), all conditions already increased within the T2D patient, and particularly in those treated with insulin. The occurrence of OSA in obese insulin treated patients with T2D could potentially negatively impact both glycaemia, CVD risk and weight loss. Overall prevalence of OSA in the adult population ranges from 9% to 38% (Senaratna et al., 2017), with figures as high as 77% being reported (Young et al., 2005). While in those with T2D following official polysomnography, OSA is estimated to be around 71% (Pamidi and Tasali, 2012), with estimates being as high as 86% in the Sleep AHEAD trial (Foster et al., 2009b). The actual prevalence of OSA among insulin treated T2D is unknown, as they are amalgamated within the general T2D population.

Weight loss has been shown to be highly effective in improving OSA symptoms with a 10% weight loss being associated with a 26% reduction in AHI (Peppard et al., 2000). In those individuals with T2D similar changes are seen with a 10kg weight loss reducing AHI by 11.3 events per hours (48.7% reduction), with AHI change being directly associated with changes in weight (Foster et al., 2009a), although the greatest improvements have been reported following bariatric surgery (Sarkhosh et al., 2013, Buchwald et al., 2004).

Several studies have now shown that using VLED and LED in obese patients results in clinically relevant improvement in weight loss, AHI, oxygen desaturation index (ODI) and even resolution of OSA (AHI<5 event/hr) (Kajaste et al., 2004, Johansson et al., 2009, Tuomilehto et al., 2009, Nerfeldt et al., 2010, Johansson et al., 2011). With a dose-response association appearing to exist between weight loss and AHI improvement (Johansson et al., 2011), the use of dietary treatments to elicit significant weight loss appear important. Despite clear evidence for the use of VLED and LED with obesity, there is no clear evidence for their use among the obese T2D population, particularly those treated with insulin aids OSA symptoms.

1.8.3.7.2. Kidney disease and other conditions

Diabetes-related kidney disease (DKD) is the leading cause of end stage renal failure accounting for approximately 40-50% of patients requiring dialysis (Ritz et al., 1999, Gross et al., 2005, Tuttle et al., 2014). Those with nephropathy are especially vulnerable to CVD and all-cause mortality (Valmadrid et al., 2000, So et al., 2006) with an excessively high mortality rate of 15-20% (Kalantar-Zadeh et al., 2006). In those with more advanced diabetes, insulin use itself has been identified as a risk factor for increasing levels of proteinuria (Valmadrid et al., 2000). With the presence of obesity, hypertension and hyperglycaemia being key to the development of DKD, treatment options that offer alleviation of these issues may have a positive outcome on progression of microalbuminuria to end stage renal failure. Despite this the influence of VLED and LED in the CKD population remains unclear (Mulholland et al., 2012, Rolland et al., 2013, Brown and Taheri, 2018). With only six studies having been published in the area to date all of which are of poor quality resulting in difficult applying the results to clinical practice (Brown and Taheri, 2018). In addition, evidence among patients with T2D is limited or lacking especially for those with advanced T2D requiring insulin.

Other physical improvements following the use of formula VLED and LED diets include psoriasis (Jensen et al., 2014), osteoarthritis (Christensen et al., 2011), as a 'liver shrinkage diet' prior to bariatric surgery (Fris, 2004, Colles et al., 2006), fertility treatment (Moran et al., 2011) and idiopathic intracranial hypertension (Sinclair et al., 2010). Although it must be appreciated that non-favourable evidence is also present within the literature (Tsai and Wadden, 2006, Mulholland et al., 2012).

1.8.3.8. VLED and nutritional status

There remain suggestions that using VLED and LED may detrimentally affect both vitamin and mineral status, particularly in those with a higher BMI and during the weight loss phase (Damms-Machado et al., 2012, European Food Safety Authority Panel on Dietetic Products, 2015). Research has suggested that poor diet quality and prevalence of micronutrient deficiencies are higher in obese patients than in normal weight controls (Kimmons et al., 2006). With evidence showing deficiencies in zinc, selenium, folate, vitamin B₁, vitamin B₁₂,

vitamin A, vitamin C, iron, vitamin E and vitamin D (Kimmons et al., 2006, Ernst et al., 2009, Damms-Machado et al., 2012).

In fact, studies have shown that the micronutrient quality of “popular” energy restricted food-based weight loss diets fail to meet the Daily Recommended Intakes (DRI) for many, if not all micronutrients analysed (Calton, 2010, Gardner et al., 2010); suggesting that deficiencies can be further exacerbated. As the formula TDR products provide 100% of the Population Recommended Intakes (PRI) and Daily Recommended Intakes (DRI) for minerals and vitamins in 3-4 products, this may provide a more balanced micronutrient supply than conventional weight loss diets. Though may not be sufficient to correct nutrition deficiencies present before their use.

From the very limited data available, it remains unclear whether there is a detrimental effect with studies having conflicting conclusions. In a study assessing nutritional health after 1-year of weight loss maintenance in patients with OA it was found that serum vitamin D and vitamin B12 increased while there was no change with ferritin levels and a decrease in parathyroid hormone (PTH) (Christensen et al., 2013). In contrast, a study looking at micronutrient status in obese subjects after a 3-month formula LED suggested DRI of LED do not cover the demands of obese individuals (Damms-Machado et al., 2012). In particular deficiencies increased in vitamin C, selenium, iron, zinc and calcium during the TDR phase. However, mean serum concentrations only reduced in iron and calcium, and actually increased in vitamin D, folate, selenium and zinc. Due to deficiencies being present in obese patients prior to the study it is difficult to truly say if these DRI are insufficient for obese patients and particularly within an acute weight loss phase. Ideally these deficiencies would have been corrected prior to the LED. Undoubtedly there is a need for further research.

1.8.3.9. Body Composition Changes

Controversy remains regarding the possibility of excessive loss of lean tissue following VLED and LED. A recent systematic review and meta-analysis using either dual-energy x-ray absorptiometry (DEXA) or underwater weighing with weight losses of greater than 10kg showed a median lean tissue loss of between 14-23.4%, depending on inclusion of exercise (Chaston et al., 2007). Lean tissue loss was suggested to be explained solely by the energy deficit and that rigorously supervised resistance and aerobic exercise limited lean tissue loss.

Lower lean tissue losses are seen using LED compared to VLED after 16 weeks, 1.2 ± 0.4 kg (11%) compared to 2.1 ± 0.2 kg (17%), respectively (Christensen et al., 2011). VLED with the addition of intensive activity programmes can reduce the loss of lean body mass to around 20% (Snel et al., 2012a). Several studies have shown that with the use of LED, the lean body mass: fat-mass ratio of the lost mass is approximately 25:75 (Saris, 2001).

1.8.3.10. Changes in Appetite Hormones following weight loss using VLED

Concurrently, appetite hormones are altered following weight loss and during weight maintenance, driving the body to regain weight. Prospective studies have shown that following weight loss with a VLED several appetite regulating hormones are altered to encourage weight regain (Sumithran et al., 2011). A follow on from this study (Purcell et al., 2014), by the same group confirmed the findings that leptin, ghrelin and hunger play an important role within this counter-regulatory effect. Changes in ghrelin concentration and hunger persisted for at least 3 years, even after most of the lost weight has been regained. Additionally, participants maintaining a substantial weight loss (e.g., weight regain of <25% at the end of the trial) had a persistent reduction in fasting leptin concentration (Purcell et al., 2014). More research is needed to better understand the hormonal changes during weight loss using formula TDR diets. During the TDR phase it is often self-reported that there is a reduction or lack of physical hunger, despite the substantial energy deficit. The exact mechanisms by which VLED and LED bring about their reduction in appetite and satiety to aid weight loss remains relatively unknown. Although it has been suggested that the anorectic effects of ketones help to mediate this affect (Gibson et al., 2014).

1.9. Conclusion

The management of obese T2D patients treated with insulin poses a substantial clinical challenge due in part to their poor glycaemic control, multiple co-morbidities, IR and IAWG. Despite IAWG being a key problem in T2D patients treated with insulin there remains no clear international or national guidelines regarding effective management of this weight gain. With much of today's focus on prevention and remission of recently diagnosed diabetes, the T2D patient group on insulin has been neglected. Often resulting in these patients getting into a perpetual cycle of insulin dose escalation, worsening glycaemia and further weight gain.

Bariatric surgery still represents the most effective treatment for both obesity and T2D, with a variety of mechanisms being proposed to explain the dramatic weight and metabolic improvements. Recently acute caloric restriction has been proposed as being key to the early glycaemic improvements seen after bariatric surgery. Furthermore, data within the insulin treated T2D groups has shown that improvements in whole body glucose homeostasis require both significant caloric restriction and weight loss to initiate mobilization of IMCL; thought to be central in T2D development (Snel et al., 2012). Therefore, the benefits of moderate caloric restriction and weight loss (5-10%) recommended currently in clinical practice appear not to be sufficient to aid improvement in glucose homeostasis.

With the recent publication of the DiRECT study there is now evidence that the use of LED to bring about substantial weight loss can cause T2D remission in recently diagnosed diabetes. There is however no such high quality randomised evidence for those patients with more advanced T2D. It is therefore the intention of this thesis to investigate the use of LED in obese T2D patients treated with insulin to ascertain whether for the first time formula LEDs can be used to effectively aid weight loss, reduce insulin dose and improve glycaemia.

1.10. Overall Hypothesis:

In obese type 2 diabetes patients treated with insulin, a 24-week dietary intervention with a formula low energy diet (LED) will result in greater weight reduction leading to significant improvements in glycaemic control and a reduction in insulin usage when compared to gold standard clinical care (GSC).

1.10.1. Objectives

The aim was to compare the effect of a LED with gold standard clinical care in a randomised controlled trial on:

1. Weight loss, body composition, glucose homeostasis and insulin dose and appetite regulation.
2. Short-term surrogate markers of complications associated with T2D and obesity – cardiovascular disease (CVD), sleep, renal and liver function.

Chapter 2: Methodology

2.1 Methodology

This study was an open label, pragmatic, parallel group, randomised controlled trial with a 1:1 allocation. The thesis comprises 2 investigations into the use of formula low energy diets (LED) within subjects with type 2 diabetes mellitus (T2D) treated with insulin. This chapter will describe the methodology in detail and highlights common methods in all investigations. Subsequent chapters will refer to this chapter.

2.2 Ethics Approval

The randomised clinical trial was conducted in accordance with the principles of the Declaration of Helsinki. The study was approved by the Solihull NRES Committee (12/WM/0199) and was sponsored by the Imperial College London NHS Trust (14HH2150). The study complied with the International Conference of Harmonisation of Good Clinical Practice ICH GCP (CPMP/ICH/135/95) July 1996, relevant regulations and standard operating procedures.

2.2.1 Declaration of Interest

Funding to help support the running of this study was provided by Cambridge Weight Plan Ltd (UK), through the provision of an investigator initiated educational grant. The formula diet was donated free of by Cambridge Weight Plan Ltd (UK).

2.2.2 Clinical Trial Registration

The clinical trial was registered with the ISR controlled-trial database (ISRCTN 21335883) and also was registered with the National Institute for Health Research Clinical Research Network Portfolio (UKCRN 17600).

2.3 Study Design

This study was an open label, pragmatic RCT employing the following intervention arms (Figure 2.1):

- i. Low energy diet (LED) group, which combined a formula low energy diet (LED, 800-1200kcal/day) with the combination of behavioural support and physical activity advice.
- ii. Gold standard clinical care (GSC) group, which provided focussed individualised weight management advice combined with behavioural support and physical activity. This is based on current dietary management guideline for weight loss and dietary guidelines for patients with T2D.

2.3.1. Intervention Phase (0-24 weeks)

2.3.1.1. Low Energy Formula Diet Group

At randomisation subjects were commenced on a total diet replacement (TDR) formula low energy diet (Riecke et al., 2010, Bliddal et al., 2011, Christensen et al., 2012, Lean et al., 2013, Jensen et al., 2013, Pedersen et al., 2014) (Cambridge Weight Plan Ltd, UK) for an initial 12 weeks followed by a further 12 weeks of structured food. During the initial 12-week TDR phase subject were asked to consume four formula LED products per day to make total calories of between 800 to 820kcal/day. The four products (soups or shakes) were nutritionally complete for vitamin and mineral to Population Recommended Intakes (PRI) (Christensen et al., 2011). Each product was reconstituted with the addition of water to the powder, which was either mixed in a shaker or a hand blender. Subjects received the formula LED at study visits to aid with retention and compliance to the TDR. Subjects were advised to use a fibre supplement (psyllium/inulin) to avoid constipation if required and to drink at least 2.25 litres of additional water or other energy-free beverages (except tea and coffee) each day (Tsai and Wadden, 2006). Subjects were given additional individualised advice based on the clinical assessment by the study dietitian, which followed the British Dietetic Association Specialist Obesity Groups guidelines on dietary advice for weight management in a 1:1 setting (Grace, 2011).

After the initial TDR phase subjects were guided to follow a graded food reintroduction (FR) phase, in which conventional foods were gradually reintroduced using a combination of formula LED products and conventional foods. Subjects initially increased their energy intake to approximately 1000 kcal/day for 6 weeks. Subjects were requested to reduce the formula products to 3 a day with an additional 400kcal of protein rich foods and vegetables

and half a pint of skimmed or semi-skimmed milk, if the subjects wished. If subjects disliked milk, alternatives were suggested. Thereafter, they moved to 1200kcal per day for a further 6 weeks. This involved 2 formula LED products plus 600kcal split between breakfast, lunch and evening meal, half a pint of skimmed milk or semi-skimmed milk and a piece of fruit.

2.3.1.1.1. Side Effects and Adverse Events with LED

The LED used within this study has been shown to have a good safety profile in several studies including a recent randomised study within recently diagnosed T2D patients (Christensen et al., 2011, Lean et al., 2013, Lean et al., 2017). The common side effects reported with the LED are diarrhoea, constipation, headaches, nausea, mild dizziness, gout, bad breath and sleeplessness (Christensen et al., 2011). Observed adverse events during the current trial will be reported in the results section.

2.3.1.2. Gold Standard Clinical Care Group

This was defined as gold standard clinical care (GSC) according to obesity national guidelines (National Institute for Health and Care Excellence, 2006, NICE, 2014c) and diabetes dietary guidelines (Dyson et al., 2011, NICE, 2015, Diabetes UK, 2018). Subjects were asked to consume a 600kcal deficit diet throughout the intervention, based on their calculated basal metabolic rate (BMR) with added physical activity level (PAL), using the Mifflin St-Jeor equation (Mifflin et al., 1990, Seagle et al., 2009) to calculate their total energy expenditure (TEE). From the TEE, 600kcal was subtracted to encourage gradual weight loss of approximately 0.5-1kg per week (Lean and James, 1986, Frost et al., 1991). Individualised diet plans were provided and their estimated BMR and dietary plans were adjusted accordingly for weight loss. Energy intake ranged between 1200 and 2000kcal/day. Energy recommendations were from starchy carbohydrates, protein, unsaturated fats, and vitamins, minerals and trace elements to the UK recommended daily amounts, based around guidelines for healthy eating by the Department of Health, NICE and NHS choices (Grace, 2011, Dyson et al., 2011, NICE, 2014b, NICE, 2015). Subjects were made aware of how their diet affected their diabetes control and techniques to help modify their diet including monitoring postprandial blood glucose and adjusting carbohydrate intake accordingly and minimising processed foods. Subjects were seen with the same frequency as the LED group.

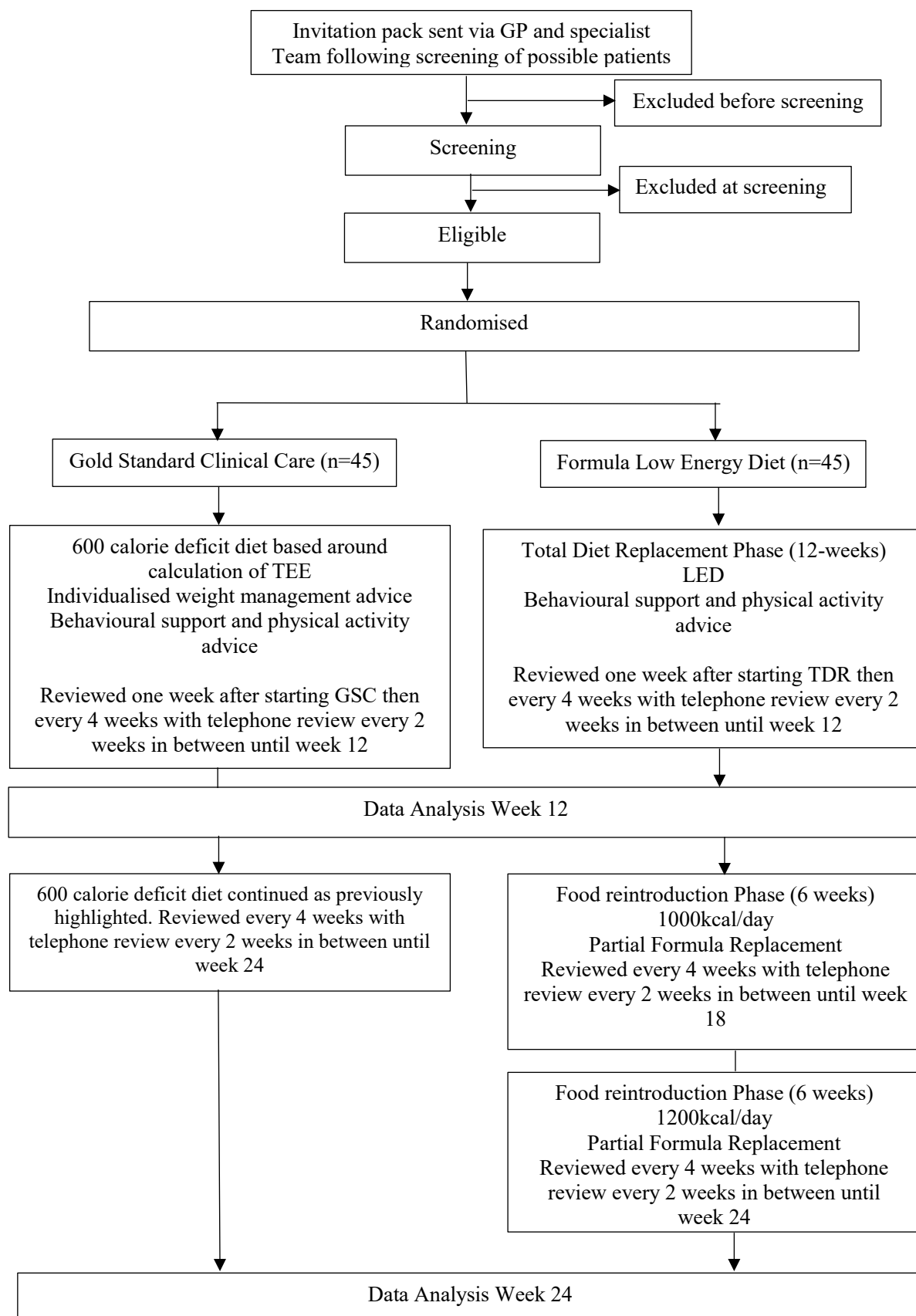


Figure 2.1 CONSORT flow chart for Study

Figure presents the flow of the patients through the study Abbreviations GSC, Gold Standard Clinical Care; LED Low Energy Diet; TDR, Total Diet Replacement

2.2.1.3.Behavioural Change

The intervention comprised of 10 sessions facilitated by a trained member of the research team. Sessions were designed to help the subjects to better understand their relationship with food and greater autonomy of their weight management. The sessions aimed to empower the subjects to make lifestyle and behavioural changes both in the short and long-term (Table 2.1). For this thesis subjects only went to Visit 9 or Session 8 as this represented 24 weeks.

Table 2.1 Content of each individual Behavioural Session

(Brown et al., 2015)

Session	Topics	Description of Behavioural Techniques	Time (weeks)
Session 1 (Visit 2)	Self monitoring Weight loss expectations Goal setting (SMART goals; Specific, Measureable, Achievable, Relevant and Time-bound) Dietary Advice Hunger scale Decisional balance	Food, mood and physical activity diary used to record eating pattern and identify relationship with food Identify triggers to eating and become more conscious about own eating Hunger scale to aid identification satiety/fullness SMART	0
Session 2 (Visit 3)	Healthy Eating Benefit of 5-10% weight loss Healthy snacks of Physical Hunger Meal Ideas	Helping cement concepts of health eating Portions of vegetables and fruit	1
Session 3 (Visit 4)	Emotional and conscious eating Thoughts, feelings and behaviour	Identifying external and internal cues to unhealthy eating and emotionally eating Identify distraction techniques to help with controlling emotional eating	4
Session 4 (Visit 5)	Portions Eat-well plate, serving sizes and weight management Energy balance	Portion size control e.g. using a smaller plate, slowing eating	8
Session 5 (Visit 6)	All of nothing thinking Task and Treat bowl Decisional Balance revisited	Aiding flexibility with goals setting and managing change	12
Session 6 (Visit 7)	Meal planning and shopping lists Healthy eating	Planning meals and physical activity Identifying challenging situations Re-emphasising healthy eating	16
Session 7 (Visit 8)	Physical activity Activities of daily living	Re-emphasising ways to increase activities of daily living	20
Session 8 (Visit 9)	Labels and portions sizes Steps to successful weight loss	Review of progress and plan for next 3-6 months	24

The behavioural modification strategies used were focussed around skills previously successfully used in the obese population (Wing and Hill, 2001, Wadden et al., 2004, Wing and Phelan, 2005, Elfhag and Rossner, 2005, Wing et al., 2008, Butryn et al., 2011, Grace,

2011). These skills were modified from a previously published study within complex obese patients (Brown et al., 2015) for the use in a one to one environment while also ensuring that the Specialist Obesity Group for the BDA (Grace, 2011) guidelines for dietary and lifestyle advice were incorporated (Table 2.1). The theoretical basis of the behaviour change was based around elements of motivational interviewing and cognitive behavioural therapy. Subjects were provided with a written handbook at the end of each session, which included information reinforcing the topics of the session, and also individual goals were written down.

2.3.1.3. Physical Activity

Subjects were advised to increase their physical activity levels by engaging in unsupervised physical activity of at least 30 minutes per day 5 days per week, totalling at least 150 minutes per week in accordance with physical activity guidelines (Haskell et al., 2007, Donnelly et al., 2009b, O'Donovan et al., 2010, NICE, 2014b, NICE, 2015).

Current exercise guidelines highlight that 300 minutes or more of moderate-intensity aerobic activity per week or 150 minutes of vigorous-intensity activity per week is required to induce weight loss (O'Donovan et al., 2010). With a government report highlighting that over 6.3million adults aged 40 to 60 do not achieve 10 minutes of continuous brisk walking over the course of a month (Public Health England, 2014), the focus was decided not be around attempting to achieve weight loss with physical activity. Instead the key focus was to help subjects with little or no experience of exercise to initiate physical activity regularly into their daily routine, while minimising sedentary time. Potential benefits of using a variety of physical activities were highlighted to the subjects using local NHS information. The use of pedometers was recommended to assess subject's current physical activity levels and provide them with goals for achieving the recommended 150 minutes/week. Resistance exercise was also advised in an attempt to minimise the loss of fat free mass seen in previous studies (Saris, 2001, Chaston et al., 2007).

2.4 Recruitment

Potential participants were recruited from both primary and secondary care. Within primary care, participants were identified from local GP practices, community diabetes service and

the Diabetes and Clinical Research Network (DRN and CRN) using the study inclusion criteria. Database searches were completed using local systems and these lists were reviewed for appropriateness. Once an eligible subject was identified, an invitation pack was either sent by the general practice or given to potential subjects when they attended their primary care clinic appointment. The invitation pack included an invitation letter, reply slip, self-addressed envelope and the invitation subject information sheet (PIS). Potential subjects who did not respond to the invitation letter were sent a letter or telephoned approximately 2 weeks after initial contact. Alternatively, if a member of the research team was available at the community participant identification site to discuss the study and the participant agreed to talk to them, the researcher discussed the study with them. Secondary care participants were identified from hospital diabetes clinics, searches of the local databases and by healthcare professionals and were either given an invitation pack or provided with contact information of the research team. The process thereafter was the same as in primary care.

The Diabetes Research Network (DRN) and Clinical Research Network (CRN) supported the recruitment process, as the study was on the National Institute for Health Research (NIHR) portfolio. The DRN searched the Diabetes Alliance for Research in England (DARE) research database and eligible subjects were identified and invitation packs posted. The CRN also contacted GP practices within North West London via the general practitioner (GP) message boards and interested GPs details were passed onto the research team to contact. The research team arranged an appropriate time to visit the interested practices and discussed recruitment and appropriate service support costs. Letters of assurance and access were provided for the study for North West London Clinical Commissioning Group. Interested subjects were contacted to determine if they met the inclusion criteria and then a screening visit was arranged to assess study eligibility. It was planned for recruitment to run over a 15-month period.

Potential participants were identified from the Imperial College London Healthcare NHS Trust, Central London Community Healthcare NHS Trust, primary care within North West London, South London, the Hammersmith and Fulham Clinical Commissioning Group and the Clinical and Diabetes Research Network. Another additional site for recruitment and study visits was Guy's and St Thomas' NHS Foundation Trust.

2.5 Informed consent, Privacy and Confidentiality

All participants provided written informed consent. Informed consent discussions took place in a private setting. The randomised clinical trial complied with the Data Protection Act (United Kingdom, UK). All subject data were coded with a subject identification (ID) number. The code was linked to the identifying information collected at screening. Only authorised personnel had access to the study documents and only coded data was entered into the computerised databases. Access to the secure computerised database was restricted only to the investigative team. All analyses were conducted on coded data.

2.6 Eligibility Criteria

2.6.1. Inclusion Criteria:

- Body mass index (BMI) ≥ 30 kg/m²;
- Men and women;
- Age 18 to 70 years;
- Willing and able to give written informed consent;
- Insulin treatment less than 10 years or greater 10 years with a fasting C-peptide of ≥ 600 pmol

2.6.2. Exclusion Criteria:

- Type 1 diabetes mellitus (based on clinical classification);
- Significant diabetes microvascular complications;
- Experienced a cardiovascular disease (CVD) event in the previous 6 months,
- Stage 4 chronic kidney disease or higher (eGFR < 30 mL/min/1.73 m²);
- Mental incapacity, unwillingness and/or inability to understand and be able to complete the mental health questionnaires in the provided language;
- Pregnant, lactating, or planning pregnancy within the study period;
- Have a clinical diagnosis of binge eating disorder
- Condition precipitating fluid overload such as heart failure (New York Heart Association grade III-IV) and liver cirrhosis;
- Using medication clinically deemed to affect metabolic rate and weight

- Significant psychiatric disorder (e.g. schizophrenia, anxiety, panic disorder, Attention Deficit Hyperactivity Disorder [ADHD], Attention Deficit Disorder (ADD), post-traumatic stress disorder, obsessive-compulsive disorder);
- Uncontrolled depression;
- Participated in a weight management drug trial in previous 3 months;
- Difficult to control International Normalising Ratio (INR) within the therapeutic range;
- Uncontrolled epilepsy;
- Known or suspected of substance use;
- Lactose intolerance;
- Severe musculoskeletal conditions preventing walking;
- Gout;
- Active gallstone disease or known asymptomatic gallstones;
- Clinically assessed hypoglycaemia unawareness;
- Electrocardiographic (ECG) evidence of left bundle branch block.

2.6.3. Female subject and pregnancy

All female subjects of childbearing age were asked when their last menstrual cycle was and if they had missed any periods. A urine pregnancy test was conducted and subjects withdrawn if the test was positive.

2.7. Withdrawal Criteria

Each subject was informed of their right to withdraw their consent at any time without giving a reason. Their decision to withdraw from the trial did not affect their future medical rights. The subject's reason for withdrawal was recorded. Subjects were notified that if they withdraw from the study, the research team would use their previously collected data for research purposes. Subjects were given a withdrawal letter and a letter was sent to their GP.

Also, the research team withdrew subjects if:

1. The subject was unable to adhere to the study protocol and study requirements;
2. There was a significant protocol deviation;

3. The subject became pregnant during the study;
4. Any new illness that affected their inclusion (exclusion criteria above);
5. Continuing the research would be harmful to the subject's health;
6. The subject was lost to follow up;
7. The trial was cancelled.

Any planned withdrawal was discussed with the participant beforehand to ensure that they were happy to be withdrawn.

2.8. Treatment allocation

Treatment allocation was carried out using an online software randomisation package called Sealed Envelope (<https://www.sealedenvelope.com/>). With standard randomisation there is still concern regarding unbalanced characteristics that might affect the primary outcome. Therefore to ensure a balance of the stratification factors (age, gender, ethnicity, diabetes duration) between intervention groups, the technique of minimisation was used. This is a deterministic procedure that allocates subjects to optimise balance in stratifying factors and has been shown to be better than both simple and stratified randomisation methods (Scott et al., 2002)

2.8.1. Blinding

The study was described as open label as neither the subjects nor the research team were blinded to the intervention arms, as blinding was not be possible with this type of nutritional research. Data analysis was however performed by a statistician not involved in the collection of the primary data to avoid bias (Karanicolas et al., 2010).

2.8.2. Sample Size Calculation

In order to achieve a clinically significant 10 kg weight loss between groups (SD 15 kg) (Christensen et al., 2011) at a 5% significance level with 80% power, a sample size of 74 participants was required. From the literature the mean attrition rates while following a very low energy diet and low energy diet are between 0 to 52% (Brown, 2015). Therefore,

following discussion within the investigator team we accounted for a drop out of 20%, thus giving a total of 90 participants for recruitment (45 per arm).

2.8.3. Statistical Analysis

Demographic factors and clinical characteristics were summarised with counts (percentages) for categorical variables, mean (standard deviation [SD]) for normally distributed continuous variables, or median (interquartile [IQR] or entire range) for other continuous variables.

The primary outcome was body weight loss at 12 months. Primary analysis used repeated measures analysis of covariance using a mixed model which takes account of the within-subject variability, using weight measurements at all post-randomisation time points and adjusting for baseline weight, randomisation factors (age, gender, ethnicity and diabetes duration), HbA1c and number of medications. The adjusted mean group differences for baseline and each time point with 95% confidence intervals (CI) were calculated. Both the crude unadjusted and adjusted estimates are presented, but the primary inference was based on the adjusted analysis. Area under the curve (AUC) and incremental area under the curve (iAUC) were calculated using the trapezoidal rule.

Secondary outcomes were analysed using similar methods. If an outcome was skewed, then appropriate transformations were performed. Both the primary outcome and secondary outcomes were analysed using the intention to treat principle. Findings were considered to be statistically significant at the 5% level. Statistical analyses were performed using Stata Special Edition Version 15.0 (StataCorp LP, College Station, TX) and SPSS v.25.0 (IBM Corp., Armonk, NY, USA).

The impact of non-response and missing data at 6 months follow-up were examined in a sensitivity analysis. In order to avoid a loss in efficiency missing values were imputed using multiple imputation by chained equations. In this, twenty imputed datasets are created by replacing missing values with simulated values from a set of imputation models built from all potential prognostic and the outcome variable (weight loss)

2.9. Anthropometric Measure

Weight and body composition were measured in the morning after a 10-12-hour overnight fast to avoid diurnal variation. Bioelectrical Impedance Analysis (BIA) was used to assess body composition changes using a MC-780MAP segmental body composition analyser comprising of 8 polar electrodes (Tanita UK Ltd, Middlesex, UK), using a high frequency current (50kHz, 500mA).

BIA has been shown to be a good predictor of Dual Energy X-Ray Absorptiometry (DEXA) derived fat free mass (FFM; $r=0.85-0.88$) (Roubenoff, 1996) and correlates well in assessing fat free mass (FFM) and body fat compared with DEXA (Pateyjohns et al., 2006).

Prior to the participants standing on the platform, the subject's, body type, gender, age and height were entered into the machine. Participants were asked to remove their shoes and socks and any objects in their pockets. Participants were asked to stand on the scales in bare feet without support and with their weight evenly distributed and then take hold of the hand sensors. The BIA then measured weight and impedance over the whole body and segmental areas. Weight was recorded to the nearest 0.1kg. Body fat and FFM were measured in both percentage and kilograms. To ensure accurate measurements participants were asked to:

- Abstain from eating or drinking within 4 hours of the assessment;
- Avoid moderate or vigorous activity within 12 hours of the assessment;
- Abstain from alcohol within 48 hours of the assessment;

Height was measured in the morning of the screening visit and then not repeated. Height was recorded using a standard stadiometer (Invicta Plastics Ltd., Leicester, UK). The subject was asked to stand with feet together and heel, buttocks and upper part of the back touching the scale. The head was placed in the Frankfort plane. The board is placed firmly down on the vertex, pressing the hair down as much as possible. Height was measured to nearest 0.1 cm.

Body mass Index (BMI) was calculated by dividing weight in kilograms by height in metres squared.

2.9.1. Additional anthropometric measures

Other anthropometric measurements can be used as a complement to weight, BMI and body composition to help identify potential health-related benefits. All anthropometric measurements were taken by a trained member of the research team to minimise variation in measurements.

Hip circumference was measured using a fibreglass measuring tape. Subjects were asked to stand in a relaxed position. The girth was taken at the level of the greatest posterior protuberance of the buttocks. The tape was passed around the hips from the side and adjusted to ensure no excessive indenting in the skin. Hip circumference was measure to the nearest 0.1cm.

Waist circumference was measured using a fibreglass measuring tape. Subjects were asked to stand in a relaxed position with their arms folded. The measurement was taken at the level of the narrowest point between the lower costal (10th rib) and the iliac crest. The tape was passed around the hips from the side and adjusted to ensure not excessive indenting in the skin and asked to put their arms down and relax. Waist circumference was measure to the nearest 0.1cm.

Waist to Hip Ratio was calculated by dividing the subject's waist by their hip measurement.

2.10. Study Visits

Subjects were seen a total of nine times over the duration of the study. All study visits were conducted either at the Imperial NIHR Clinical Research Facility, Hammersmith Hospital, London, United Kingdom or at the Diabetes Centre, Guy's Hospital, London, United Kingdom. There were three key research measurement visits, which occurred at baseline, 12, and 24 weeks. The other visits were review appointments focussing on the management of their diabetes, medication titration particularly insulin and long-term lifestyle modifications through using behaviour change techniques. This included goal setting, healthy eating, emotional and conscious eating, slip-ups and setbacks and maintenance of weight loss. In addition, telephone consultations aimed to help with insulin titration, help with behaviour

changes and provide support. Table 2.2 shows the assessments and study visit schedule throughout the study.

2.10.1. Screening and Baseline Measurement Visit (Visit 1)

Subjects who expressed an interest in participating in study were booked an appointment for a screening visit (Visit 1). A member of the research team reviewed the subject information sheet with the potential subject and informed consent was taken. Signed consent was obtained from subjects. A signed copy of informed consent form (ICF) was given to the subject for their records and a final copy was kept with the site master file. Each subject underwent the following measures to assess eligibility for the study.

These were performed by medically trained staff, where appropriate:

1. Clinical and medical history;
2. Clinical examination including electrocardiogram (ECG), heart rate and blood pressure;
3. Anthropometric measures and body composition;
4. Fasting blood sample;
5. Urinary pregnancy test (for women of child-bearing age).

At the end of the screening visit, subjects were provided with a 3-day food and blood-glucose monitoring diary to be completed if they were eligible for the study and asked to bring it to Visit 2.

If the potential subject did not fulfil the eligibility criteria during the screening visit, s/he was informed and the reasons explained at the screening visit. The fasting blood, ECG, and medical history were reviewed by the medical lead for each research site (Professor Dornhorst and Dr McGowan) to assess medical fitness for inclusion in the study. If blood results taken at screening were the reason for exclusion, the subject was contacted to discuss the findings and reasons for exclusion and blood samples sent to their GP.

Table 2. 2 Schedule of Enrolment, intervention, study visits and assessments for both groups

Visit:	V1 Screening	V2 Baseline	V3 W1	V4 W4	V5 W8	V6 W12	V7 W16	V8 W20	V9 W24
Measures									
Weight	x	x	x	x	x	x	x	x	x
Height	x								
Body Composition: Body Fat & FFM	x	x	x	x	x	x	x	x	x
BMI Calculation	x	x	x	x	x	x	x	x	x
Waist Circumference	x					x			x
Hip Circumference	x					x			x
Waist to Hip Ratio	x					x			x
Blood Samples: U+E, FBC, LFT, HbA1c, Fasting Blood Glucose, Fasting Lipid Profile, hsCRP	x	x				x			x
Mixed Meal Tolerance Test & Appetite Study		x				x			
Insulin Indices		x				x			x
Urine Sample: Albumin & Creatinine	x					x			x
Blood Pressure and Heart rate	x	x				x			x
3-Day Self-Monitoring Blood Glucose		x				x			x
3-Day Food Diary		x				x			x
Sleep Monitoring		x							x
Sleep and QoL Questionnaires: EQ5D		x				x			x
Clinical Interviews: Medication use change Diet Adherence Safety & Side effects	x	x	x	x	x	x	x	x	x
Physical Activity 7DayAccelerometry for Physical Activity and Sleep Study		x							x
Length of visit (hrs)	1.5	6	1	1	1	6	1	1	6

Abbreviations: W, week; FFM, fat free mass; BMI, body mass index, U+E, urine and electrolytes, FBC, full blood count, LFT, liver function test; HbA1c, glycated haemoglobin; hsCRP, high-sensitivity C-reactive protein; DEBQ, Dutch eating behaviour questionnaire; ESS, Epworth Sleepiness Scale; PSQI, Pittsburgh Sleep Quality Index.

2.10.2. Baseline Visit (Visit 2)

Subjects were asked to arrive after a 10-12 hour fast and were asked to avoid caffeine, alcohol and strenuous physical activity for 24 hours, for a 9am start time. Participants were asked to halve their night time insulin dose before attending and refrain from any diabetes medications on the day of the test. This was to avoid hypoglycaemia due to the extended fast

and avoid severe hyperglycaemia prior to the study day, which was expected with subjects treated with insulin. Weight and body composition were assessed by bioelectrical impedance analysis prior to the start of the study day (see previous section for detail, BIA, MC-780MAP Analyser, Tanita UK Ltd, Middlesex, UK). Subjects were asked to return the completed 3-day food and blood glucose monitoring diaries. Blood pressure (BP) was measured with an electronic blood pressure monitor, three times on the non-dominant arm in a sitting position and the average reading taken.

Participants underwent a Mixed Meal Tolerance Test (MMTT; Figure 2.2) for further details. The MMTT was used as this most closely represents the physiologic response during day-to-day life and allow the full incretin effect to be tested (Cersosimo et al., 2014). On arrival an intravenous cannula was placed in a suitable vein on the non-dominant arm by a trained member of the research team. The size of the cannula was determined by the member of staff according to accessibility of a suitable vein. This allowed for baseline blood samples to be taken and continued blood sampling to be undertaken. Two fasting blood samples were taken at -15 and 0 minutes. Participant received a standardised breakfast comprising of an Ensure Plus and asked to consume it within 10 minutes (please see Appendix for nutritional content) at 0 minutes. Due to the likelihood of significant insulin resistance, only one Ensure Plus was given to avoid significant hyperglycaemia and nausea. Further venous blood samples were collected throughout the study visit (15, 30, 45, 60, 90, 120, 150, 180, 210). Visual analogue scales (VAS) were completed by the participants after each blood samples to assess subjective appetite and hunger.

Subjects were randomised to the intervention or gold standard care group. Following randomisation subjects met with the research dietitian and clinician where they were given specific instructions related to the dietary changes and clinical management of their diabetes medications. There were 2 MMTT and appetite study days at study baseline and 12 weeks (Figure 2.2). The subjects attended the National Institute for Health Research Imperial Clinical Research Facility; all subjects received the same standard breakfast and lunch meals. The meals given to each group were identical in macronutrient composition and energy content.

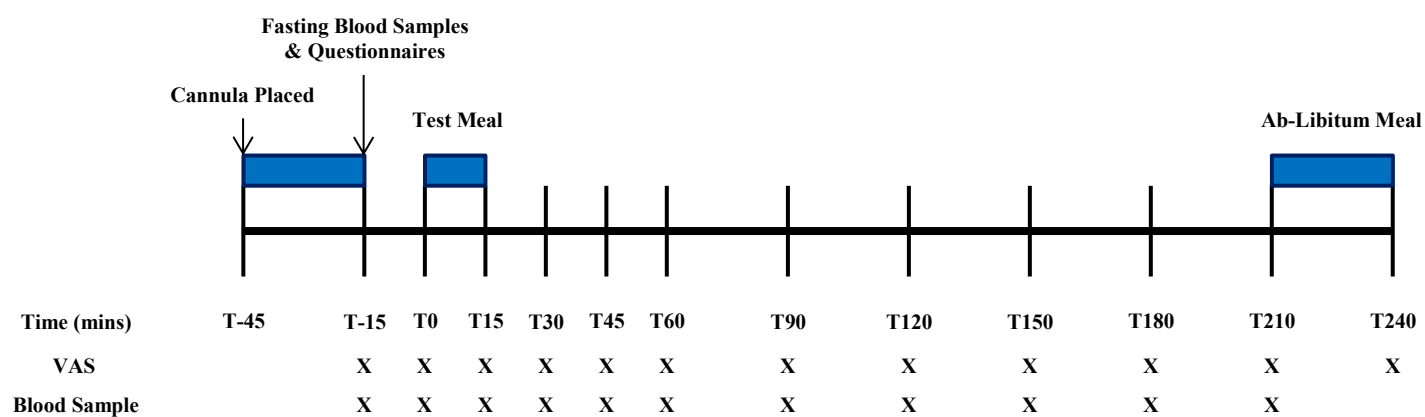


Figure 2. 2 Schematic representation of appetite study activities

This represented a study overview of the mixed meal tolerance test. Subjects were asked to arrive at 9am following an overnight fast. A cannula was placed 30 minutes before started the study visit. 2 fasting blood samples were taken prior to the subject consuming the test meal. VAS and blood samples were collected as indicated. Abbreviations VAS visual analogue scale

2.10.3. Insulin Titration

Insulin titration was done by a trained member of the research team and checked with the study medical team before advice was given. The following algorithm was used (Figure 2.3).

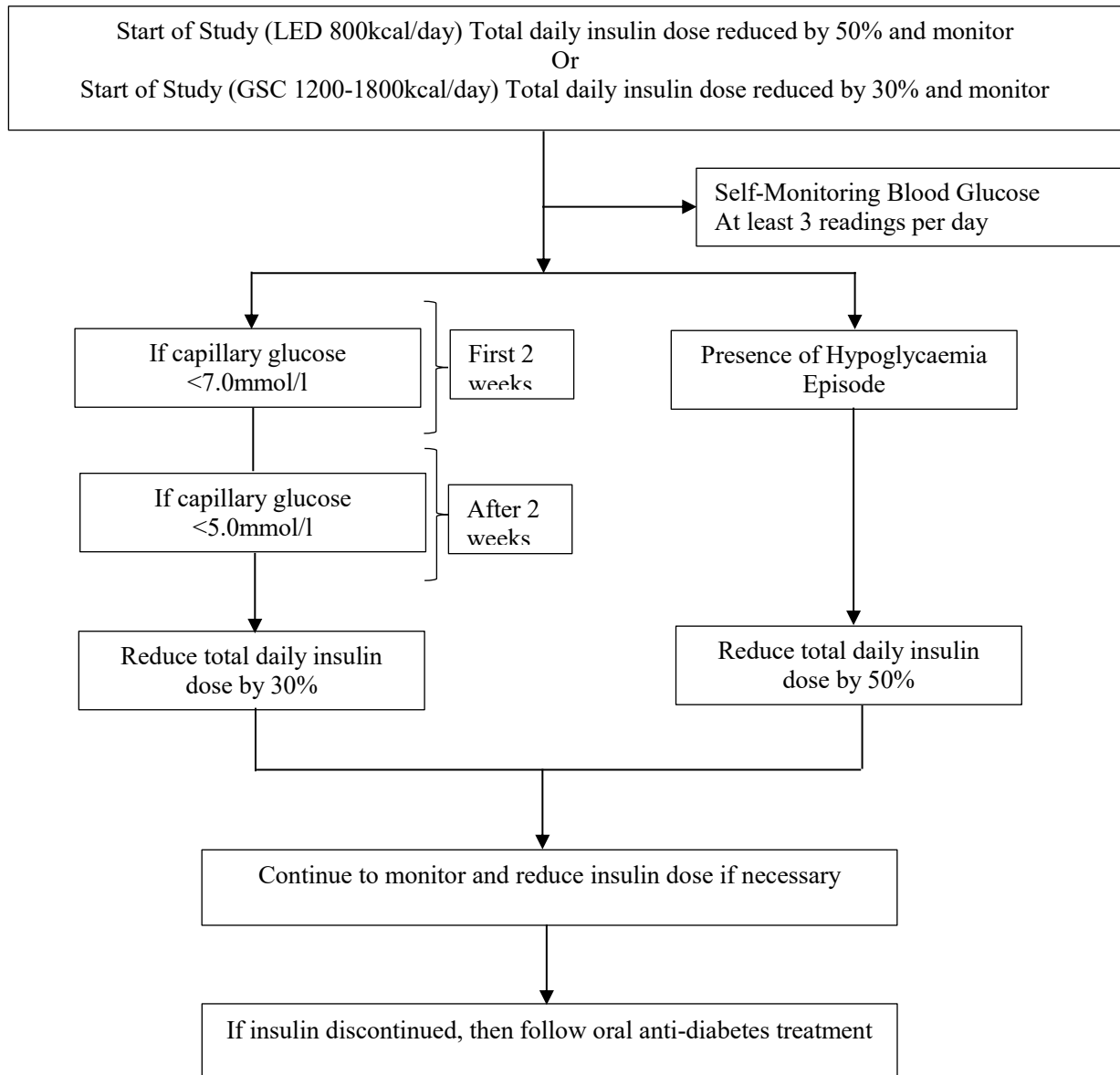


Figure 2. 3 Insulin Titration for LED and GSC Interventions

If fasting capillary glucose rises to ≥ 12 mmol/l on 3 consecutive days, then insulin was up-titrated as clinically indicated.

2.10.3.1. Up-titration of insulin

For basal bolus – if participants fasting blood glucose was greater than 7mmol/l then the basal insulin was increased by 2-4 units. If 2 hour postprandial glucose was greater than 12mmol/l then the pre-meal insulin was increased by 2-4 units.

For biphasic (twice a day) insulins– if the participant's pre-dinner blood glucose was greater than 12mmol/l then the morning insulin was increased by 2-4 units. If fasting blood glucose was greater than 7mmol/l then the evening insulin was increased by 2-4 units.

Following initial titration of insulin, further titration was according to individual blood glucose monitoring.

2.10.4. Subject Adherence

Adherence to the dietary advice is a significant concern within any nutritional intervention. Subjects were instructed to return all unused or part-used LED products at each visit. In addition, subjects were assessed how they adhered to the dietary and lifestyle changes using 3 different options either completely, almost or did not follow the advice.

In order to assess whether predicted weight losses were being achieved according the energy deficit advised a predictive weight loss calculation by Hall (2008) was used, incorporating baseline weight, body fat and physical activity, to give a predicted weight loss at 12, 18 and 24 weeks. This was the subtracted from actual weight loss to give the difference.

2.10.5. Telephone Consultations

Telephone consultations were scheduled during the week to allow for as much flexibility as possible to suit the needs of the subject. A member of the research team asked each subject to identify slots (days and times) that would be most suitable for them. Subjects had a clinical assessment, assessment of their adherence to the dietary changes, and queries to identify any issues with compliance or adverse events. Telephone consultations lasted up to 15 minutes and conducted every 2 weeks in between appointments a summary of the telephone consultation was made.

2.10.6. Subsequent Visits

There were a further 7 visits: after 1 week (Visit 3), 4 weeks (Visit 4), 8 weeks (Visit 5), 12 weeks (Visit 6), 16 weeks (Visit 7), 20 weeks (Visit 8), 24 weeks (Visit 9).

Table 2.2 shows the assessment and measures at each of these visits. Sessions were scheduled during the week with as much flexibility to suit the needs of the subject.

2.11. Visual Analogue Scales

Visual Analogue Scales (VAS) were used in investigation 1 to determine subjective sensations of appetite (Blundell et al., 2010). VAS are a 100mm long scales ranging from left extremity labelled “not at all” at 0 to the right extremity labelled “extremely” at 100. The participant was asked to mark using a vertical mark on the line how they felt at that particularly time point.

- Hunger (“how hungry do you feel right now?”)
- Nausea (“how sick do you feel right now?”)
- Desire to eat (“how pleasant would it be to eat right now?”)
- Prospective food intake (“how much do you think you could eat right now?”)
- Fullness (“how full do you feel right now?”)

A composite appetite score was calculated from the VAS using the following formula in a way to more easily represent the data collected (Woodend and Anderson, 2001):

$$\text{Appetite score} = [\text{Hunger} + (100 - \text{Fullness}) + \text{Desire to Eat} + \text{Appetite for Meal}] / 4$$

It is accepted that a mean score composed of key appetite scales can be used (Blundell et al., 2010).

2.12. *Ad Libitum* Meal Test

At the end of the mixed meal tolerance test (210 minutes), an ad libitum meal test was provided to provide a quantitative measure of prospective appetite. Participants were given a

tomato based pasta dish. This was provided to ensure homogeneity of the test meal. Participants were instructed to eat until they felt comfortably full. After this, the participants were asked to complete a final VAS (See Appendix 3 for energy and macronutrient content).

The meal was weighed before it was served excluding the bowl and the remainder was then weighed afterwards. The total meal consumed was calculated by subtracting these two figures. Energy intake at the ad libitum meal was calculated by multiplying total food intake (g) by the energy density of the meal in calories.

The test meal conditions were standardised. The room was kept free of strong odours and other distracting factors such as using their telephones, and reading or talking, which could potentially affect appetite and subsequent food intake (Fedoroff et al., 1997 Bellisle et al., 2009).

2.13. Questionnaires

Questionnaires were completed at baseline, 12 weeks and 24 weeks. These examined different aspect of the participant's life including general quality of life, sleep and eating behaviour. The questionnaires were introduced to the participants, explained how to answer them and clarifying any questions they had. This was repeated at each assessment (Appendix 6).

2.13.1. Quality of Life (EuroQol EQ5D)

EQ-5D is a standardized instrument for the measure of health-related quality of life (Herdman et al., 2011). It consists of five dimensions, mobility, self-care, usual activities, pain/discomfort and anxiety and depression. Questions are answered by ticking one of five answers of the following; 'not, slightly, moderate, severe or extreme. The EQ5D visual analogue scale allows a quantitative measure of health the subject's health on that day. The participant marks a line of the EQ5D VAS where 0 represents "worst imaginable health" and 100 represents "best imaginable health". Subjects completed the EQ5D by answering the response of their choice.

2.13.2. Epworth Sleepiness Scale

The Epworth Sleepiness Scale (ESS) is a validated screening questionnaire for sleepiness (Johns, 1991). This questionnaire assessed daytime sleepiness with the participants answering questions scoring each one, 0 – no change; 1 – slight change; 2 – moderate change or 3 – high chance of dozing in eight situations and their chance of dozing. Daytime sleepiness was defined as an ESS score of greater than 10 (Johns and Hocking, 1997).

2.13.3 Pittsburgh Sleep Quality Index

The Pittsburgh Sleep Quality Index (PSQI) is a validated self-reported questionnaire to assess sleep quality over the last month (Buysse et al., 1989). The measure consists of 19 questions that create 7 separate components that produce a global score on sleep quality. The components are subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication and daytime dysfunction. Poor sleep quality was defined as having a PSQI score equal or greater than 5 (Buysse et al., 1989).

2.14. Blood sample preparation

Venous blood (30 ml) was collected at the first fasted blood sample and aliquoted. A fasting blood sample was sent to the Department of Biochemistry at Hammersmith Hospital to assess fasting renal function, liver function, lipid profile, high-sensitive C-reactive protein (BD Serum Separating Tube Vacutainer) glycated haemoglobin and full blood count (Ethylenediaminetetraacetic acid (EDTA) Vacutainer). A 25ml specimen tube of urine (taken from the 24-hour urine collection) was also sent for assessment of urinary albumin to creatinine ratio. The remaining blood was separated in order to assess glucose (BD Fluoride/Oxalate Vacutainer) and serum insulin (BD Serum Separating Tube Vacutainer) measurements. At each following time point 12 ml blood was collected and aliquoted into 3 separate vacutainers for the assessment of glucose, serum insulin and plasma gut hormones (containing 100µl/ml whole blood Aprotinin pancreatic protease inhibitor, [Nordic Pharma UK Ltd, Reading, UK]) measurements.

NOTE: Samples for metabolomics and plasma gut hormones (BD Lithium Heparin Vacutainer for gut hormones containing 100µl/ml whole blood Aprotinin pancreatic protease inhibitor, [Nordic Pharma UK Ltd, Reading, UK]) were also taken for future analysis and are not included in this thesis.

Samples were centrifuged at 4000 revolutions per minute (RPM) at 4 centigrade (°C) for 10 minutes. Serum Separating Tubes were allowed to clot before centrifuging. The resulting plasma or serum was aliquoted into 2.0ml microtubes and immediately frozen at -80°C until analysis.

2.14.1. Glucose Analysis

Glucose was determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase with phenol and 4-aminophenazone to form a red-violet quinoneimine dye (Raba, 1995).

Glucose analysis was performed in house using Randox Glucose Assay Kit. Reagent (1000µl) was added to an Eppendorf and 10µl of sample was added and vortexed. The mixture was incubated for 10 minutes at 37°C. Next 200µl was pipetted into a 96 microwell plate and a spectrometry was used to assess the glucose concentrations of the sample. The machine was set up at 37°C with a wavelength of 540nm. Glucose analysis was carried out in separate assays according to subject with mean percentage coefficient of variance of 2.7%.

2.14.2. Radioimmunoassay

2.14.2.1. Principles of radioimmunoassay

Radioimmunoassays (RIA) were performed in the Department of Medicine at Imperial College London. The principle of RIA is based upon the competitive binding between radiolabelled antigen and unlabelled antigen for specific high-affinity antibody sites. A fixed concentration of radiolabelled tracer antigen is incubated with a constant dilution of antiserum. This limits the number of antigen binding sites on the antibody. When unlabelled antigen is added, there is a competition between the labelled tracer for the binding sites on the

antibody. With increasing concentrations of unlabelled antigen, the amount of radiolabelled antigen bound to the antibody site decreases.

After centrifugation, the bound (pellet) and unbound (supernatant) are separated and the amount of radioactivity is counted on a gamma counter.

As standard curve is set up with increasing concentrations of standard unlabelled antigen which allows for the quantification of the concentration of antigen in the unknown sample by interpolation. Insulin analysis was carried out in three parts with percentage coefficient of variance (% CV) of 4.7%, 2.6%, 8.8%

2.14.2.2. Insulin Analysis

The insulin RIA was carried out using a Millipore Human Insulin Specific RIA Kit (Millipore Corporation, Billerica, USA) according to the manufacturer's protocol.

The assay was incubated for 20-24 hours at room temperature before separation of the free from bound antibody labelled by secondary precipitating reagent. Tubes were drained and the radioactivity of the pellets was counted using a gamma scintillation counter (LB2111 Multi Crystal Gamma Counter, Berthold Technologies, Bad Wildbad, Germany) for 60 seconds in duplicate.

2.15. Estimation of insulin Sensitivity and Insulin Resistance

The gold standard measurement of whole body insulin sensitivity in vivo is the 'hyperinsulinemic euglycemic clamp' (DeFronzo et al., 1979). However, clamps are costly, not practical for large studies, time and labour intensive and require technical expertise to perform. As a result, surrogate measures of insulin sensitivity and resistance have been used instead and validated against the euglycemic clamp. These measures are based on fasting values. Evaluation of insulin sensitivity is important in insulin treated patients and therefore efforts were done to minimise the effect of IT by asking patients to half their night-time insulin dose before assessment visits and avoid diabetes medication on the morning.

2.15.1. HOMA-IR

The degree of basal insulin sensitivity was assessed using the Homeostatic model assessment (HOMA-IR). This is simply calculated using the following formula (Matthews et al., 1985):

$$\text{HOMA-IR} = (\text{fasting glucose} \times \text{fasting insulin}) / 22.5$$

Insulin concentration was measured in $\mu\text{U/L}$ and glucose in mmol/L , with 22.5 being a normalizing factor. According to the Oxford diabetes group, there is no absolute value for 'normal' vs. 'abnormal' HOMA-IR, as these vary depending on the assays used for glucose, insulin and C-peptide (Manley, 2007). However, an arbitrary figure for IR of 2.6 was used (Ascaso et al., 2003) with 1 representing normal IR (Wallace et al., 2004). It should be noted that HOMA-IR has potential problems when applied to patients treated with insulin as IT affects serum insulin concentrations and therefore the feedback system between the liver and pancreas (Okita et al., 2013), therefore the outcomes should take this into account, although it has been suggested that it can be used as long as glucose and insulin are in a steady state (Wallace et al., 2004). To minimize the effect of exogenous insulin, doses of insulin were halved the night before to enable a better assessment of insulin sensitivity.

2.15.2. Quantitative insulin sensitivity check index (QUICKI)

The QUICKI (Katz et al., 2000) remains one of the most used indices on insulin resistance and is inversely proportional to insulin sensitivity, with 0.31-0.44 indicating IR, although 0.33 will be used here and those ≤ 0.30 indicative of T2D. It has been shown to provide a reliable, reproducible and accurate index of insulin sensitivity (Mather et al., 2001)

$$\text{QUICKI} = 1 / (\log \text{Fasting glucose} + \log \text{fasting insulin})$$

2.15.3. Matsuda Index

The changes in whole body insulin sensitivity during the mixed meal tolerance test was assessed by the Matsuda Index (Matsuda and DeFronzo, 1999).

$$\text{Matsuda Index} = 10000 / (\text{square root} [\text{fasting glucose} \times \text{fasting insulin}] \times [\text{mean postprandial glucose} \times \text{postprandial insulin}])$$

Where glucose was measured in mmol/L and insulin is in $\mu\text{U/ml}$. Postprandial figures refer to the average concentration of either insulin or glucose from 15-210 minutes. With ≤ 2.5 being defined as insulin resistance (Kernan et al., 2003).

2.15.4. Insulinogenic Index

For the calculation of beta-cell function, the insulinogenic index (IGI) was calculated using the following formula (Seltzer et al., 1967, Phillips et al., 1994) and used as an assessment of early insulin secretion, as this is suppressed in T2D, and in part the reason for elevated postprandial glucose levels. The IGI is a ratio that represents the increase in insulin secretion that is matched to the corresponding release of glucose, where higher values indicate greater β -cell function.

$$\text{IGI} = (\text{increment in insulin 30-0 minutes}) / (\text{increment in glucose at 30-0 minutes})$$

Insulin was measured in pmol/L, with glucose is measured in mmol/L and increments are calculated from basal values. Basal values were considered to be the mean of -15 and 0 time points.

2.15.5. Disposition Index

β -cell function was estimated using the oral disposition index (DI) (Retnakaran et al., 2009, Utzschneider et al., 2009). This was calculated using the following formula:

$$\text{Disposition index} = \text{Insulinogenic index} \times (1/\text{fasting insulin})$$

Insulin was measured in pmol/L, with glucose is measured in mmol/L. A lower disposition index is indicative of inadequate β -cell compensation (Utzschneider et al., 2009).

2.16. Additional Assessments

2.16.1. Physical Activity

Physical activity was assessed through a 7-day accelerometer worn on the upper arm (SenseWear, BodyMedia Inc, Pittsburgh, USA). Subjects wore this on their non-dominant arm for the entire week including while they slept and only took it off to shower or if they went swimming.

2.16.2. Sleep Study

Sleep apnoea was assessed through an overnight non-invasive home monitoring device currently used in clinical practice (ApneaLink™ device, ResMed Corporation, Poway, USA). The Apnealink is a small device that measures nasal airflow and pulse oximetry. The sleep study was reviewed by members of the research team and a medical doctor and if there was an indication of sleep apnoea requiring treatment (apnoea-hypopnoea index $>5/h$ and Epworth Sleepiness Scale >15), the subject and the GP was informed so that appropriate referrals for assessment and treatment were made.

2.16.3. Cardiovascular Risk Assessment

Cardiovascular disease (CVD) and particularly, coronary heart disease (CHD) risk is elevated in those with T2D and specifically in those with more advanced T2D and medication burdens (Holden et al., 2015). The use of CVD risk assessments allows clinicians to identify those patients at greatest risk and adjust therapy accordingly. Furthermore, modelling is beneficial when trial data is not available and if the duration of the trial is less than lifelong (Adler, 2008)

The reduction of CVD and CHD related surrogate markers are not necessarily an indication that there will be an associated reduction in CVD morbidity or mortality. Therefore, two algorithms were chosen to assess if there was a reduction in predicted risk.

The QRISK CVD risk algorithm (QRISK2) was used (Hippisley-Cox et al., 2008) to assess 10-year CVD risk, while the Framingham hard coronary heart disease risk score was used to assess 10 year CHD mortality (Wilson et al., 1998, Expert Panel on Detection, 2001). These

two risk scores were chosen instead of other risk scores such as the UKPDS Risk Engine (Adler, 2008) as they best fit the requirement of the study. The QRISK2 is recommended by NICE and include the risk factors includes T2D, atrial fibrillation, ethnicity and chronic renal disease, which are major predictors of CVD mortality. In addition, the QRISK2 database is updated annually and calibrated to the latest version of the QResearch database (Hippisley-Cox et al., 2017), unlike the UKPDS and therefore is a better representation of the current UK population. As mortality was chosen as an outcome, the only score available was the Framingham hard coronary heart disease risk score and therefore this was chosen.

Chapter 3: The effect of a LED on weight loss, glucose homeostasis and insulin usage in obese T2D patients treated with insulin compared with gold standard clinical care

3.1. Introduction

Type 2 diabetes (T2D) and obesity are inextricably linked (Diabetes UK, 2005) with the epidemic of T2D being attributed primarily to the increased prevalence of obesity. In the UK, diabetes affects an estimated 3.7 million people (Diabetes, 2014), 90% of who have T2D and of whom up to 80% are overweight or obese (Diabetes UK, 2005). T2D is a chronic progressive condition with associated beta-cell decline, reduced insulin secretory capacity and is linked with increased dyslipidaemia, hypertension, risk of CVD and mortality (Buse et al., 2007). Insulin therapy in T2D management has now become routine. Despite insulin representing the most effective treatment for blood glucose control and subsequent metabolic improvements following its initiation, this is paralleled with weight gain. Studies have reported weight gains of between 3-9kg within the first year of insulin treatment (UKPDS, 1998, Dickinson PJ, 2002), with the majority of weight gain happening in the first year.

Intensive dietary and lifestyle interventions are the cornerstone for T2D management (Nield et al., 2008, SIGN, 2010b) and adjoined to this, weight loss has been identified key in achieving improvements in glycaemia and surrogate marker of CVD risk. Although weight losses of 5-10% show improvements in blood glucose, insulin sensitivity and CVD risk (National Collaborating Centre for Chronic, 2008), there is now suggestions that this is not a sufficient in those with a BMI>35kg/m² and obesity related complications including T2D (SIGN, 2010a).

Currently, bariatric surgery is the most effective treatment for both weight reduction and glycaemic control in T2D (Dixon et al., 2008, Buchwald et al., 2009) and has been shown to reverse the metabolic abnormalities characterising the disease (Guidone et al., 2006), particularly following Roux-en-y gastric bypass (RYGB). However, bariatric surgery is invasive, associated with substantial complications and also funding constraints mean that it is only available to a fraction of those eligible within the UK (Brown, 2015). The exact mechanism by which bariatric surgery brings about the improvement in T2D control remains a matter of contention. Although recently data have now linked the early improvements on glucose homeostasis seen with RYGB and also biliopancreatic diversion with the energy restriction seen after surgery (Lim et al., 2011, Lips et al., 2014, Steven et al., 2016b).

There is now evidence that acute energy restriction, using formula VLED has a role in removing ectopic fat from the pancreas and liver and therefore inducing reversal of two key features of T2DM, namely the lack of first phase insulin response and suppression of hepatic insulin sensitivity (Lim et al., 2011). In these early studies, several methodological weaknesses meant that although they served as a proof of concept of the twin cycle theory where fat accumulation in the liver and pancreas are key to the aetiology of T2D, they could not inform clinical practice. A recent RCT has now demonstrated that remission of T2D is possible within a primary care setting using a formula LED. In T2D patients with recently diagnosed T2D (less than 6 years), 46% of patients achieved remission compared to 4% in the control group (Lean et al., 2017). The degree of weight loss appeared to be crucial, with those losing more weight getting higher rates incidences of remission, in fact in individuals that lost 15kg, 86% achieved remission. However, the studies aforementioned have only focussed on patients with short diabetes duration and treated with oral diabetes medications and not looked at patients treated concurrently with insulin, who are often more difficult to manage and have a reduced likelihood of remission (Steven et al., 2016a).

At present, however there is very limited evidence for the use of formula VLED and LED in those people with T2D treated with insulin, meaning that their clinical application is difficult. From the sparse data that are available, there appears to be significant reductions in weight and improvements in glycaemic control, surrogate markers of CVD, pericardial fat and quality of life (Paisey et al., 2002, Dhindsa et al., 2003, Snel et al., 2011, Snel et al., 2012d, Snel et al., 2012a). Despite this there were methodological weakness including lack of controls, a comparator arm using VLED and small numbers where of all diabetes medications were ceased, which meant that definitive answers of the benefit could not be drawn. Of concern was the requirement for patients to have sufficient residual pancreatic function (measured by C-peptide) to allow stopping of all medications, especially among those with advanced stages of diabetes. It is therefore the intent of this study to examine the role of LED on body weight loss, glycaemic control and insulin usage, while addressing some of the weaknesses in the current literature, to help determine the utility of a LED as an obesity treatment in obese T2D patients treated with insulin.

3.2. Hypothesis

In obese type 2 diabetes patients treated with insulin, a 24-week dietary intervention with a formula low energy diet (LED) will result in greater body weight reduction leading to significant improvements in glycaemic control and a reduction in insulin usage when compared to gold standard clinical care (GSC).

3.2.1. Aims

This investigation was a randomised control trial which aimed to examine the effect of formula low energy diet on weight loss, glucose homeostasis and insulin usage compared to gold standard clinical care in obese T2D patients treated with insulin. Also, body composition and alteration in energy balance were examined.

With there being little consistency in the way that HbA1c is reported within the literature, both IFCC-standardised and DCCT-aligned measures have been used.

3.2.2. Outcomes

The primary outcome of this study was to examine body weight loss differences between the LED and GSC group.

Secondary outcome included

- Changes in glucose homeostasis and insulin usage
- Changes in body composition including fat mass, fat free mass and trunk fat
- Change in energy balance including changes in physical activity and energy intake

3.3. Methods

The methodology used within this investigation is summarised in detail within chapter 2 regarding recruitment, study design, intervention details and outcome measured. Please refer to this chapter for more details.

3.3.1. Recruitment data

For the purpose of this thesis, analysis was carried out on a sub-population of subjects who had completed 24 weeks of the study. The aim is to follow participants until 12 months; the work in this thesis focuses on recruitment and outcomes for subjects who have completed the interventions at 24 weeks. Of the 90 subjects randomised, 80 are included in this analysis comprising of an intention to treat population.

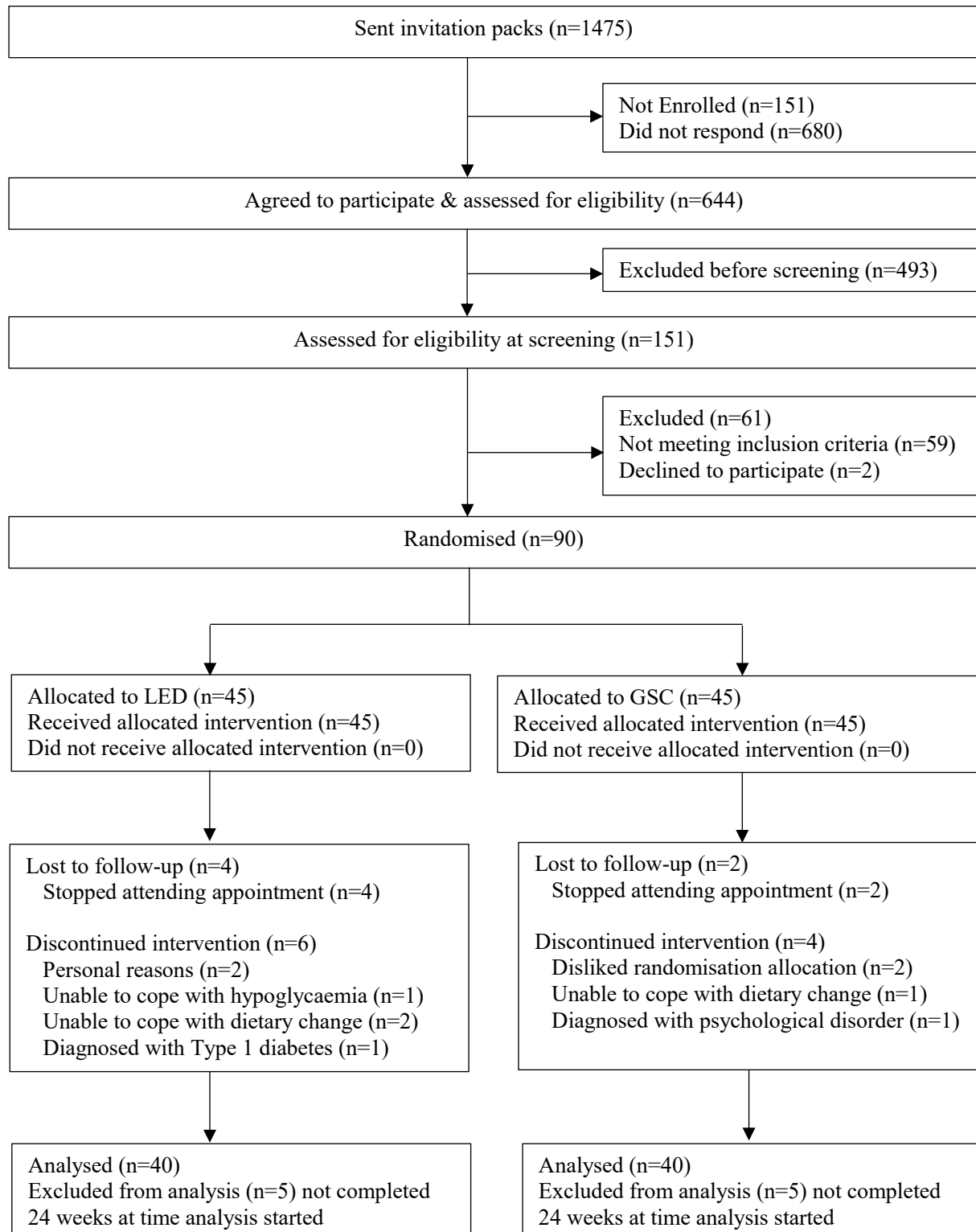
Between November 2015 and June 2017, a total of 1475 subjects were approached following identification (Figure 3.1) and sent an invitation pack during the 30-month recruitment period. The initial recruitment period had been 18-months however due to challenges recruiting this was extended and a secondary recruitment site added. The recruitment challenges were varied but included identifying eligible insulin patients, General Practitioners databases being incorrectly coded for insulin use, difficulty accessing clinics and patients not replying to invitations. Of these subjects, 644 agreed to participate and were pre-screened on the telephone to assess edibility, resulting in 151 potential subjects being invited to a screening visit. Of the subjects screened, 59 were ineligible according to the exclusion criteria (39%) and 2 declined to participate (0.02%), leaving 90 subjects to be randomised to one of the two treatment arms, low energy diet group (LED) or gold standard care group (GSC).

3.3.2. Attrition rates

In the first 12 weeks in total twelve subjects (15%) were either lost to follow-up or withdrew from the study, representing an adherence of 82.5% for the LED (seven subjects) and 87.5% for the GSC (five subjects). At 24 weeks, sixteen in total (20%) had been lost to follow-up or withdrew from the study with a total of ten from the LED group (25%) and six (15%) from the GSC group. The between group difference in adherence was non-significant at both 12 and 24 weeks ($p=0.264$, $p=0.531$, respectively).

Figure 3.1: CONSORT Flow diagram of the study

This follows the recruitment of participants from initial contact, screening, to randomisation and includes reason for withdrawal and exclusion (n=number of patients)



3.4. Results

3.4.1. Baseline characteristics of the subjects

The baseline characteristics are shown in Table 3.1 comparing LED and GSC groups. As a whole population (n=80), the majority of the subjects were women (60%) with a similar percentage split as in other similar studies using formula diets (Snel et al., 2012a, Lean et al., 2017). The median age was 57 years (IQR 49, 64), with approximately half being Caucasian (57.5%), a quarter black (26.3%), and the remaining subject either south Asian (13.8%) or mixed race (2.5%). This represents a more balanced population in terms of prevalence of T2D according to ethnic group compared with other UK studies (Lean et al., 2017).

The mean baseline weight was 102.8kg (standard deviation [SD] 19.5), which corresponded to a BMI of 36.6kg/m² (SD 5.1), a waist circumference of 120.4cm (SD 12.2) and hip circumference of 121.3cm (SD 12.2). Mean systolic blood pressure was 132mmHg (SD 17.1) with a diastolic of 74mmHg (SD 11.3). Hypertension was diagnosed in the 77.5% of subjects with CVD and high cholesterol being reported in 22.5% and 76.3%, respectively.

Subjects all had long standing diabetes with the median duration being 13 years (IQR 8, 20). The mean HbA1c was elevated at 9.1% (SD 1.8) or 75.7mmol/mol (SD 19.4) with a mean insulin dose of 73Units (SD 49) or 0.64U/kg (IQR 0.38, 0.92). The mean number of diabetes medications was 2.6 (SD 0.86), with the majority of patients being on dual (37.3%) or triple (39.8%) therapy; 12% were taking over 4 diabetes medications. The majority of subjects were on metformin (87.5%) and insulin, other medications included sulphonylureas (28.8%), GLP-1 (20%) and SGLT-2 inhibitors (11.3%) The baseline characteristic table (Table 3.1) outlines the medications between the groups. There were no significant differences between the two intervention groups in any of the baseline characteristics.

Table 3.1 Baseline characteristics of subjects comparing low energy diet and gold standard care groups

Characteristics	Gold Standard Care (n=40)	Low Energy Diet (n=40)
Age at randomization, median (IQR)	56.7 (49.7, 63.8)	57.6 (50.3, 63.6)
Sex, n (%)		
Male	15 (37.5)	17 (42.5)
Female	25 (62.5)	23 (57.5)
Ethnicity, n (%)		
Caucasian	23 (57.5)	23 (57.5)
Mixed	1 (2.5)	1 (2.5)
Asian	7 (17.5)	4 (10.0)
Black	9 (22.5)	12 (30.0)
Weight (kg), mean (SD)	100.9 (17.7)	104.6 (21.2)
Body mass index (kg/m ²), mean (SD)	36.4 (5.1)	36.7 (5.2)
Waist circumference (cm), mean (SD)	120.1 (11.4)	120.7 (13.1)
Hip circumference (cm), mean (SD)	121.3 (12.2)	121.4 (12.4)
Waist to hip ratio, mean (SD)	0.99 (0.06)	1.00 (0.07)
Body fat (%), mean (SD)	40.6 (7.5)	41.0 (7.9)
HbA1c (%), mean (SD)	9.3 (1.7)	8.8 (1.8)
HbA1c (mmol/mol)	78.5 (18.7)	73.0 (19.7)
Fasting glucose (mmol/L)	10.2 (2.9)	10.1 (3.7)
Insulin (U), mean (SD)	75.5 (55.5)	71.1 (41.1)
Insulin (U/kg), median (IQR)	0.64 (0.39, 0.91)	0.65 (0.34, 0.93)
Other medications, n (%)		
Metformin	37 (92.5)	33 (82.5)
SU	14 (35.0)	9 (22.5)
GLP-1	3 (7.5)	13 (32.5)
Gliptins	6 (15.0)	4 (10.0)
SGLT2 inhibitors	3 (7.5)	6 (15.0)
Glitazones	1 (2.5)	0 (0)
Duration of Diabetes), median (IQR)	12.5 (6.5, 18.0)	13.0 (9.0, 20.0)
Blood pressure (mmHg), (SD)		
-Systolic	133.3 (18.2)	131.9 (16.2)
-Diastolic	74.5 (13.0)	73.5 (9.4)
Total Cholesterol (mmol/L)	4.21 (1.38)	3.97 (1.04)
HDL cholesterol (mmol/L)	1.15 (0.36)	1.08 (0.31)
Triglycerides (mmol/L)	1.83 (1.48)	1.88 (0.94)
Hypertension, n (%)	30 (75.0)	32 (80.0)
CHD, n (%)	9 (22.5)	9 (22.5)
Smoking, n (%)	7 (17.5)	8 (20.0)
Statins, n (%)	36 (90.0)	37 (92.5)
Retinopathy, n (%)	13 (32.5)	17 (42.5)
Nephropathy, n (%)	11 (27.5)	5 (12.5)
Neuropathy, n (%)	16 (40.0)	10 (25.0)
Microalbuminuria, n (%)**	16 (43.2)	10 (25.6)
Estimated glomerular filtration rate***	76.6 (22.2)	75.8 (17.8)

Data presented as mean (SD), n (%) or median (IQR) unless otherwise specified. No significant difference between groups in any characteristics. Abbreviations: HbA1c – glycated haemoglobin. SU, sulphonylureas; GLP-1 glucagon like peptide-1 agonists, SGLT-2 sodium glucose co-transport-2; mmHg, 102illimetre of mercury; Nephropathy defined as have eGFR ≤60; * Metabolic syndrome defined using International Diabetes Federation criteria; **Albumin to creatinine ratio of Microalbuminuria defined as >2.5mg/mmol men and >3.5 women mg/mmol; *** (ml/min per 1.73m²).

3.4.2. Body composition

3.4.2.1. Changes in primary outcome – Weight Loss

The primary outcome variable of this study was weight loss. Figure 3.2 shows the mean change in body weight from baseline to 24 weeks.

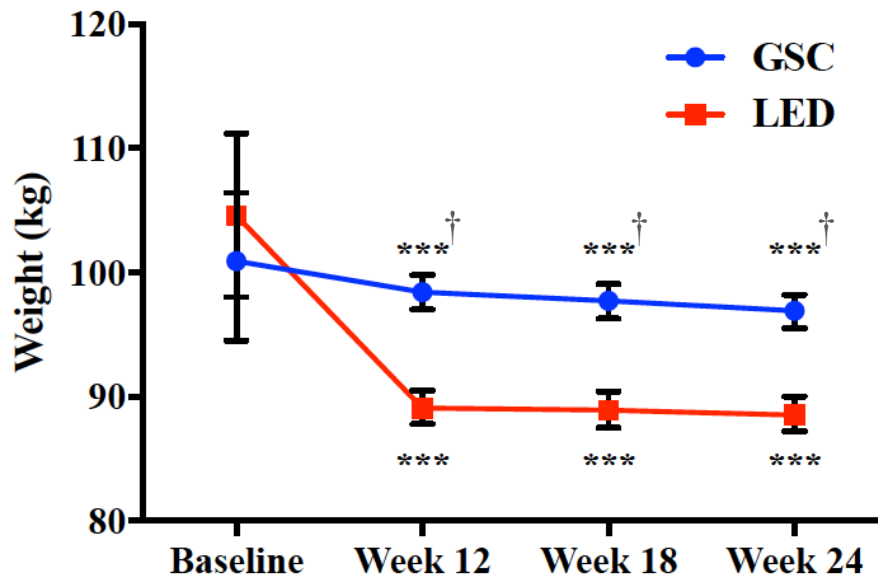


Figure 3. 2 Body weight change comparing LED and GSC at 12, 18 and 24 weeks.

Line graph represents mean body weight with standard errors. P values calculated using adjusted mixed linear modelling *** $p < 0.001$ †: adjusted between group comparison. Subjects lost significantly more weight with LED than GSC. Abbreviations: GSC, gold standard clinical care; LED, low energy diet; kg, kilogram

Mean bodyweight fell by 14.0kg in the LED group and by 5.7kg in the control group at 24 weeks. There was a significant difference in weight loss between groups at 24 weeks in favour of the LED (<0.0001). The adjusted difference was -8.3kg (95% CI -11.0 to -5.6, $p < 0.0001$).

For those in the LED group their weight dropped significantly during the total diet replacement (TDR) phase by 13.4kg (SD 4.7), following the food reintroduction phase they continued to lose weight (-0.6kg; Figure 3.2). Similar patterns were found for BMI. This highlights the importance of acute caloric restriction within the initial phases of weight loss with 99.6% of weight being lost in the initial TDR phase. This was not seen within the GSC, where there was a more gradual reduction in weight.

Figure 3.3 shows weight loss achieved at 24 weeks separated off into weight loss categories. At 24 weeks, 70% of LED subjects lost ≥ 10 kg weight loss compared with 17% in the GSC group. Impressively 46.7% achieved ≥ 15 kg weight loss at 24 weeks compared with 5.9% in the GSC group. This data taken as a whole demonstrates that the use of a formula LED over a 24-week period is significantly more effective at weight reduction than gold standard clinical care.

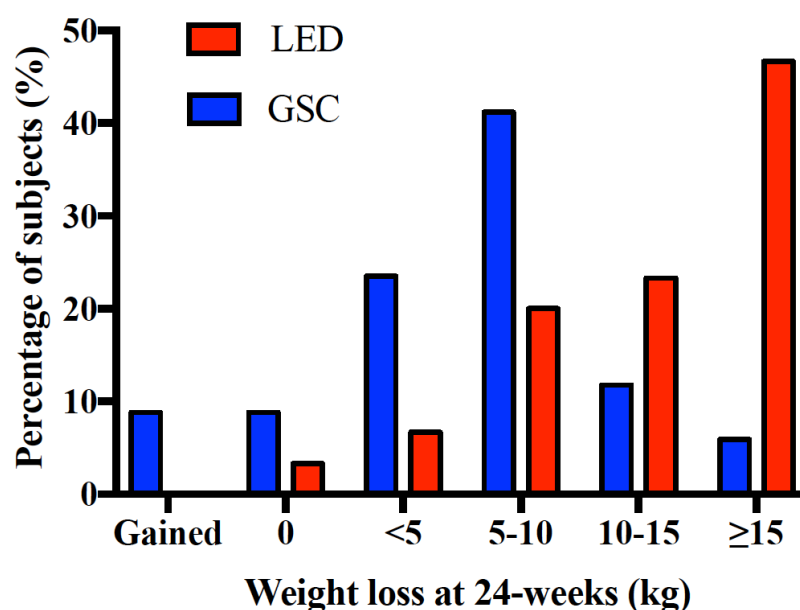


Figure 3. 3: Percentage of participants for GSC and LED achieving different categorical weight loss (e.g. maintainer, $<5\%$, $\geq 10\%$ etc.).

The weight losses are cumulative. Weight gainers are participants gaining ≥ 0.5 kg in weight. Abbreviations: GSC, gold standard clinical care; LED, low energy diet

3.4.2.2. Waist and Hip Circumference

Changes in waist and hip circumferences are shown in Figure 3.4.

3.4.2.2.1. Waist circumference

There was a significant difference in waist circumference (WC) between groups at 24 weeks. There was a significant adjusted difference between groups at 24 weeks of -7.4 cm (95% CI - 10.1 to -4.8 , $p < 0.0001$, Figure 3.4). Both groups had within group reduction in their WC (Table 3.3)

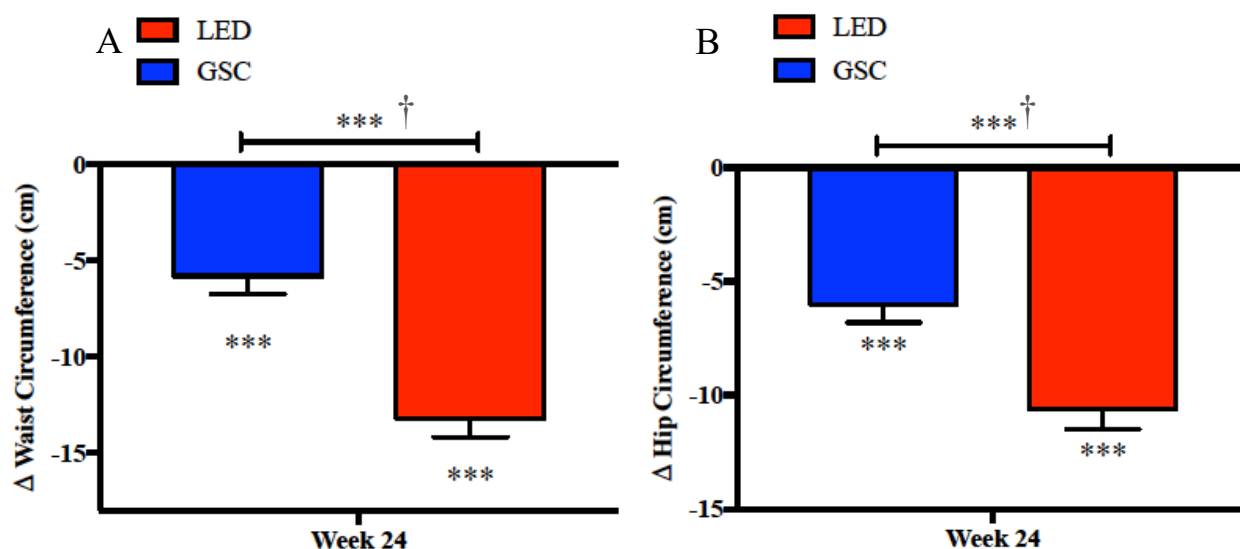


Figure 3. 4 Comparison of change in waist & hip circumference between groups at 24 weeks.

Bar charts represent mean waist and hip circumference with standard errors. (A) waist circumference (B) hip circumference. P values calculated using adjusted mixed linear modelling *** $p < 0.001$ †: adjusted between group comparison. Abbreviations: GSC, gold standard clinical care; LED, low energy diet; Δ , change

3.4.2.2.2. Hip circumference

There was a significant adjusted difference in hip circumference (HC) between groups at 24 weeks. The adjusted difference at 24 weeks was -4.6cm (95% CI -7.0 to -2.2, $p < 0.0001$). Similar to WC, both groups had a significant reduction in HC at 24 weeks compared to baseline (Table 3.3)

3.4.2.2.3. Waist to Hip Ratio

Waist to hip ratio fell significantly with the LED at 24 weeks (-0.03 (SD 0.05, $p < 0.001$). No change was seen in the GSC group. This resulted in an adjusted difference between the groups of -0.03 at 24 weeks in favour of the LED (95% CI -0.05 to -0.01; $p = 0.004$).

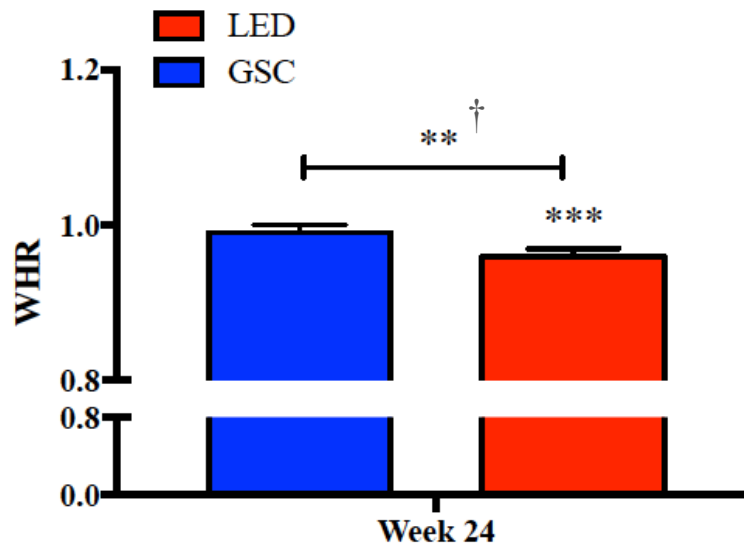


Figure 3. 5 Comparison of change in waist to hip ratio between groups at 24 weeks.

Bar charts represent WHR with standard errors. P values calculated using adjusted mixed linear modelling * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ †: adjusted between group comparison. Abbreviations: GSC, gold standard clinical care; LED, low energy diet; WHR, waist to hip ratio

3.4.2.3. Fat Mass and Fat Free Mass Body composition

Changes in fat mass and fat free mass are shown in Figure 3.6. There was not a significant difference between the groups at baseline in body composition

3.4.2.3.1. Fat mass

There was a significant difference in fat mass (FM) between groups at 24 weeks, with the adjusted difference being -3.8% (95% CI -5.8 to -1.9, $p < 0.0001$), in favour of the LED group (Figure 3.6). Within the LED this represented an actually fat mass loss of -10.7kg (SD 4.6, $p < 0.001$) at 24 weeks. In the GSC, fat mass reduced by -4.37kg (SD 4.53, $p < 0.001$) at 24 weeks (Table 3.3).

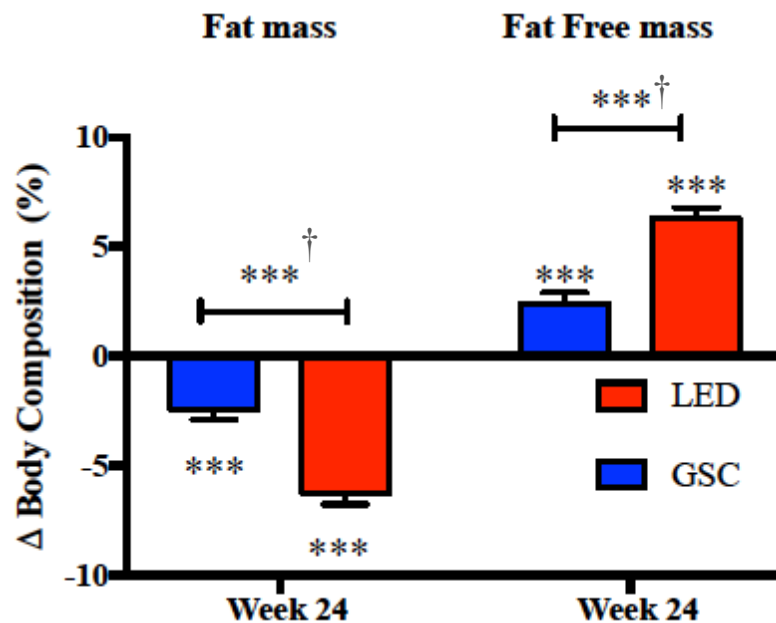


Figure 3. 6 Comparison of change in body composition between LED and GSC at 24 weeks.

Bar charts represent mean body composition changes with standard errors. P values calculated using adjusted mixed linear modelling ***p<0.001 †: adjusted between group comparison. Abbreviations: GSC, gold standard clinical care; LED, low energy diet; Δ, change

3.4.2.3.2. Fat free mass

There was a significant difference between groups in regard to percentage FFM loss, with the percentage of FFM increased in both groups as FM percentage reduced. There was a significant difference between groups, at 24 weeks the adjusted difference was 3.9% (95% CI 2.5 to 5.3), in favour of the LED group (Table 3.3).

In terms of absolute FFM loss, there was a significant adjusted difference between groups at 24 weeks of -1.47kg (95% CI -2.8 to -0.1, p=0.03). However, following food reintroduction the LED gained FFM (1.3kg) suggesting that FFM was attenuated despite continued overall weight loss in this period (Figure 3.6).

Over the period of 24 weeks the majority of weight loss seen within both groups was from fat mass suggesting a positive change in body composition from baseline.

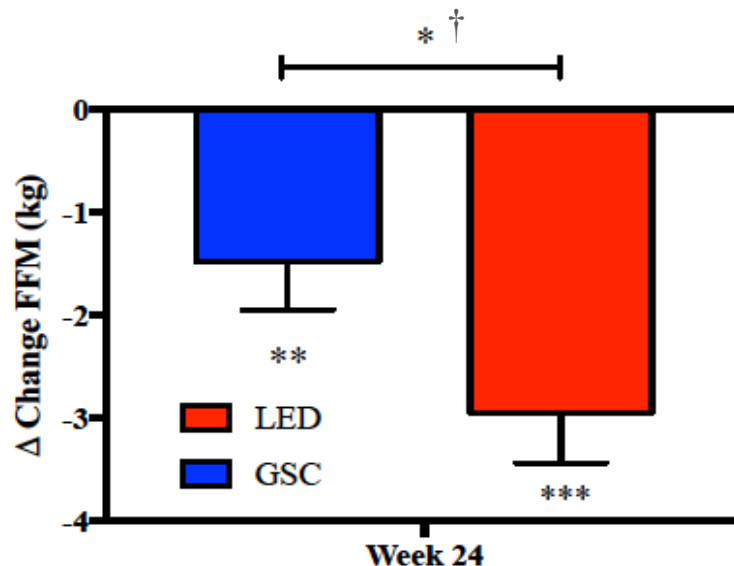


Figure 3. 7 Comparison of change in FFM between LED and GSC at 24 weeks.

Bar charts represent mean body composition changes with standard errors. P values calculated using adjusted mixed linear modelling * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ †: adjusted between group comparison. Abbreviations: GSC, gold standard clinical care; LED, low energy diet; Δ , change; FFM, fat free mass

Comparison of fat and fat free mass loss between groups as percentage of body weight lost

When represented as absolute losses relative to total weight loss, the losses in FFM within the LED group at 24 weeks the LED had a relatively smaller loss coming from FFM, with 21% presenting FFM from the total weight loss compared with 25.7% in the GSC group.

3.4.2.3.3. Trunk fat

Trunk fat changes over 24 weeks for the LED and GSC are shown in Figure 3.8.

Trunk fat was assessed using Tanita segmental analysis, this was unavailable in all subjects therefore a sub-set analysis was done on the data available.

There was a significant difference in percentage trunk fat mass (FM) between groups at 24 weeks. The adjusted difference was -4.7% (95% CI -6.5 to -2.8, $p < 0.0001$) (Figure 3.8). Both groups had a significant reduction in trunk fat over the 24-week period (Figure 3.8; Table 3.3).

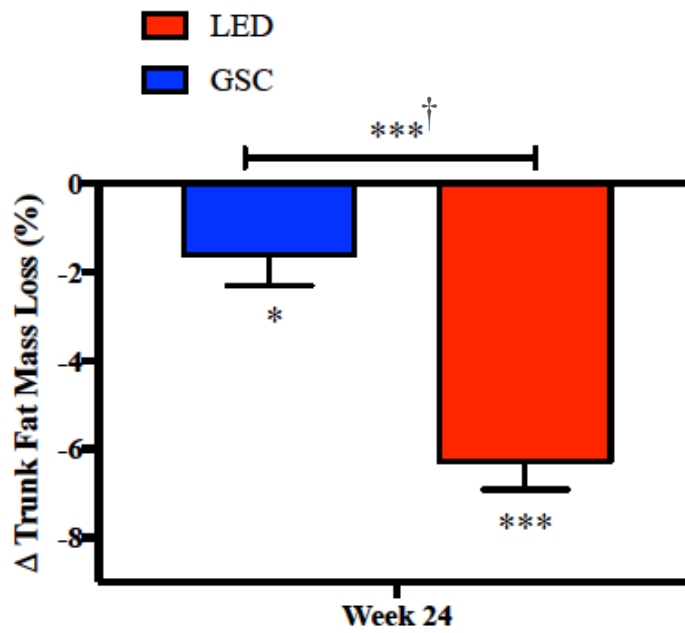


Figure 3.8 Comparison of change in Trunk Fat between LED and GSC at 24 weeks.

Bar charts represent mean trunk fat percentage changes with standard errors. P values calculated using adjusted mixed linear modelling * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ †: adjusted between group comparison. Abbreviations: GSC, gold standard clinical care; LED, low energy diet; Δ, change

Table 3. 2 Primary Outcome Weight Change comparing LED and GSC at 12 and 24 weeks

Mean (SD)						Intervention effect		
	Baseline	Week 12	Change	Week 24	Change	Estimate (95% CI)		P value
Weight (kg)								
LED	104.6 (21.2)	89.0 (18.1)	-13.4 (4.7) ***	88.4 (18.6)	-14.0 (4.9) ***	0-12	-9.2 (-11.9 to -6.6)	<0.0001
GSC	100.9 (17.7)	97.9 (19.3)	-4.2 (4.7) ***	96.1 (20.4)	-5.7 (4.7) ***	12-24	-8.3 (-11.0 to -5.6)	<0.0001

Data represents mean plus standard deviations p values calculated using adjusted mixed linear modelling ***p<0.001 within group from baseline. Intervention effects reported as estimated mean differences (LED minus GSC); Abbreviations kg, kilogram, LED Low energy diet, GSC Gold standard care

Table 3. 3 Anthropometric Outcome comparing LED and GSC at 12 and 24 weeks

Mean (SD)					Intervention effect			
	Baseline	Week 12	Change	Week 24	Change	Estimate (95% CI)		P value
BMI (kg/m ²)								
LED	36.7 (5.2)	31.6 (4.8)	-4.7 (1.6) ***	31.3 (4.8)	-4.9 (1.7) ***	0-12	-3.2 (-4.1 to -2.3)	<0.0001
GSC	36.4 (5.1)	35.2 (5.7)	-1.5 (1.6) ***	34.7 (6.2)	-2.0 (1.6) ***	12-24	-2.9 (-3.8 to -2.0)	<0.0001
Waist C (cm)								
LED	120.7 (13.1)	108.8 (13.5)	-11.3 (6.1) ***	106.4 (13.2)	-13.2 (6.2) ***	0-12	-7.1 (-9.7 to -4.5)	<0.0001
GSC	120.1 (11.4)	117.3 (11.0)	-4.2 (5.9) ***	115.8 (12.3)	-5.8 (5.9) ***	12-24	-7.4 (-10.1 to -4.8)	<0.0001
Hip C (cm)								
LED	121.4 (12.4)	111.7 (11.3)	-8.6 (5.5) ***	109.7 (10.5)	-10.6 (5.7) ***	0-12	-4.1 (-6.5 to -1.8)	<0.001
GSC	121.3 (12.2)	117.7 (10.3)	-4.4 (5.2) ***	116.4 (13.0)	-6.0 (5.2) ***	12-24	-4.6 (-7.0 to -2.2)	<0.0001
WHR								
LED	1.00 (0.07)	0.97 (0.06)	-0.02 (0.04) ***	0.96 (0.06)	-0.03 (0.05) ***	0-12	-0.03 (-0.05 to -0.01)	0.010
GSC	0.99 (0.06)	1.0 (0.07)	0.00 (0.04)	0.99 (0.07)	0.00 (0.04)	12-24	-0.03 (-0.05 to -0.01)	0.004
Fat Mass (%)								
LED	41.0 (7.9)	35.1 (9.7)	-5.3 (3.2)	33.9 (10.2)	-6.2 (3.3)	0-12	-3.5 (-4.9 to -2.1)	<0.0001
GSC	40.6 (7.5)	39.1 (8.1)	-1.8 (3.1)	38.9 (8.8)	-2.4 (3.2)	12-24	-3.8 (-5.2 to -2.4)	<0.0001
Fat Free Mass (%)								
LED	59 (7.9)	64.9 (9.7)	5.3 (3.2)	66.1 (10.3)	6.3 (3.3)	0-12	3.5 (2.1 to 4.8)	<0.0001
GSC	59.4 (7.5)	60.9 (8.1)	1.9 (3.1)	61.1 (8.7)	2.4 (3.2)	12-24	3.9 (2.5 to 5.3)	<0.0001
Trunk Fat (%)								
LED	39.8 (4.6)	34.4 (6.0)	-4.8 (4.0) ***	31.9 (6.3)	-6.3 (4.0) ***	0-12	-3.2 (-4.9 to -1.4)	<0.0001
GSC	37.8 (4.67)	37.0 (5.9)	-1.6 (4.3) *	36.8 (5.2)	-1.6 (4.2) *	12-24	-4.7 (-6.5 to -2.8)	<0.0001

Data represents mean plus standard deviations p values calculated using adjusted mixed linear modelling *p<0.05, ***p<0.001 within group from baseline. Intervention effects reported as estimated mean differences (LED minus GSC). Abbreviations C, circumference, % percentage, WHR, waist hip ratio, LED Low energy diet, GSC Gold standard care

3.4.3. Glucose Homeostasis

3.4.3.1. HbA1c

Mean changes in HbA1c at 24 weeks are shown in Figure 3.9. Both the IFCC-standardised and DCCT-aligned measures are reported.

Mean HbA1c fell by 1.4% (SD 1.31, $p<0.001$; 15.8mmol/mol) in the LED group and by -0.67% (SD 1.28, $p<0.001$; 7.3mmol/mol) at 24 weeks, an adjusted difference of -0.77% (95% CI -1.35 to -0.18, $p<0.01$) or -8.5mmol/mol (-14.9. to -2.2, $p=0.009$).

In terms of actual HbA1c reduction, this was reflecting in a drop from a baseline HbA1c of 9.3% (SD 1.7) to 8.2% (SD 1.7) or 78.5mmol/mol (SD 18.7) to 66.1mmol/mol (SD 18.9) at 24 weeks within the GSC group, while the LED had a reduction from 8.8% (SD 1.8) to 7.0% (SD 1.3) or 73mmol/mol (SD 19.7) to 53.1mmol/mol (SD 14.7).

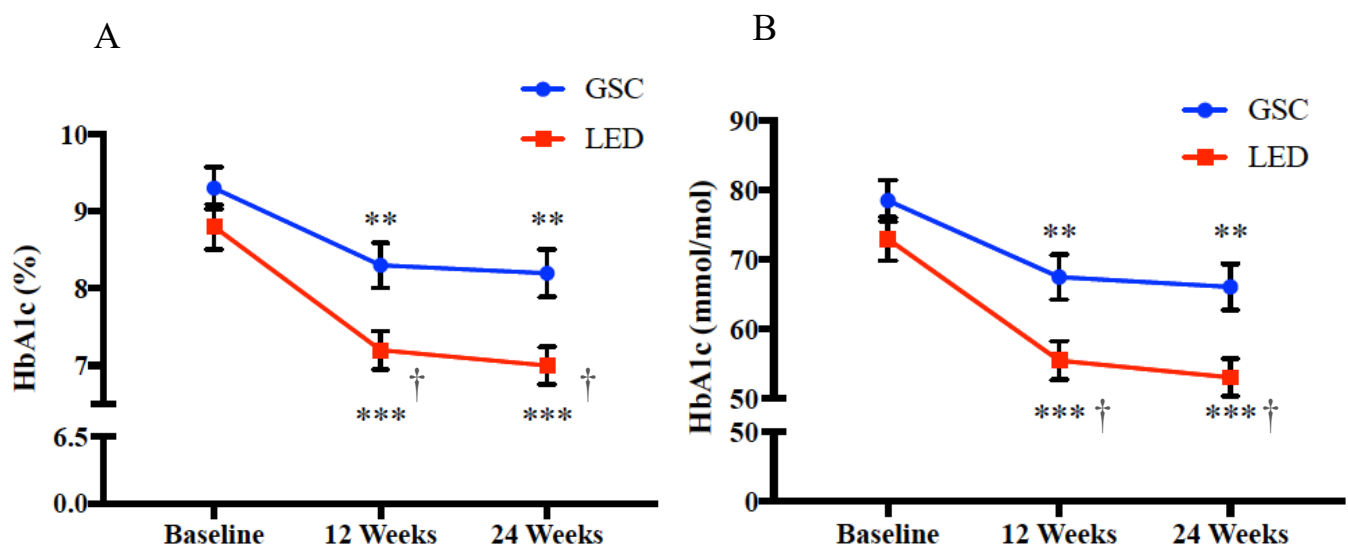


Figure 3. 9 Comparison of change in HbA1C between LED and GSC at 12 and 24 weeks.

Bar charts represent insulin usage changes with standard errors. (A) DCCT-aligned measure (B) IFCC-standardised measure. P values calculated using adjusted mixed linear modelling * $p<0.05$, ** $p<0.01$, *** $p<0.001$ †: adjusted between group comparison. Abbreviations: GSC, gold standard clinical care; LED, low energy diet; Δ, change; HbA1c, glycated haemoglobin.

3.4.3.2. Cessation of insulin treatment

Figure 3.10 compares changes in insulin cessation between LED and GSC at both 12 and 24 weeks.

The number of patients that ceased insulin over the period of the 12 and 24-week was significantly greater in the LED group compared with the GSC group. At 12 weeks no subjects had stopped insulin in the GSC group compared to 12 subjects (36.4%, $p<0.001$) in the LED group. Over the subsequent 12 weeks, 3 subjects ceased insulin in the GSC, with an additional subject ceasing in the LED group, making a total of 13 subjects (43.3%, $p<0.002$).

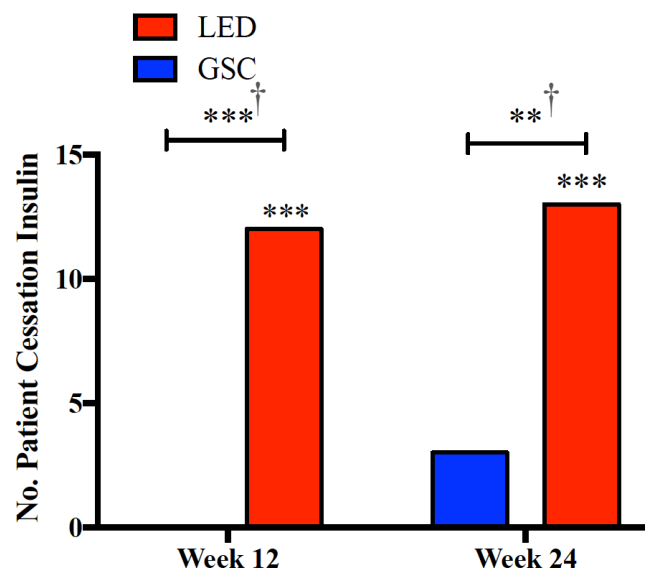


Figure 3. 10 Comparison of change in Insulin cessation between LED and GSC at 12 and 24 weeks.

Bar charts represent number of patients that ceased insulin. P values calculated using adjusted mixed linear modelling ** $p<0.01$, *** $p<0.001$ †: adjusted between group comparison. Abbreviations: GSC, gold standard clinical care; LED, low energy diet

3.4.3.3. Insulin dose

Insulin usage will be presented in two ways, firstly as overall unit taken per day (U) and secondly relative to the subject's body weight, reported in unit per kilogram body weight (U/kg), this allowed more direct comparison between subjects in term of daily insulin requirements.

There was a significant difference in insulin usage between groups at 24 weeks. The between group adjusted difference was -15.1U (CI -26.4 to -3.8, $p=0.009$) greater reduction in the LED group at 24 weeks.

This reflected a reduction at 24 weeks from 75.4U (SD 54.9) to 39.6U (SD 32.2) in the GSC group, while the LED group were taking a mean insulin dose of 21.3U (SD 26.3) down from 71.1 (SD 40.7).

In terms of change in units per kg, the adjusted difference was -0.14U/kg (CI -0.25 to -0.03, $p=0.01$, Figure 3.11b) at 24 weeks in favour of the LED group. This reflected at 24 weeks a reduction from 0.69 U/kg (SD 0.41) to 0.25 U/kg (SD 0.25) in the LED group and 0.74U/kg (SD 0.49) to 0.41U/kg in the GSC group (SD 0.31).

Combining this data together there appears a clear benefit to the use of a LED in terms of weight reduction, insulin usage and improvement of overall glycaemic control.

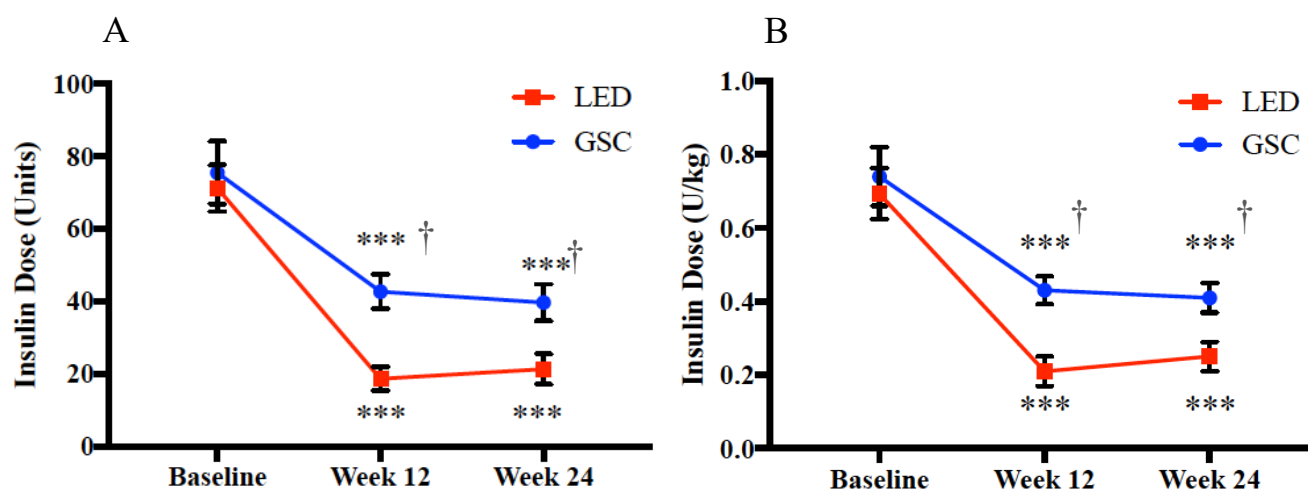


Figure 3. 11 Comparison of change in Insulin dose reduction between LED and GSC at 12 and 24 weeks.

Bar charts represent mean insulin dose in units and U/kg with standard errors. P values calculated using adjusted mixed linear modelling * $p<0.05$, *** $p<0.001$ †: adjusted between group comparison. (A) presents insulin usage as units. (B) presents insulin usage as units per kilogram. Abbreviations: GSC, gold standard clinical care; LED, low energy diet.

3.4.3.4. Fasting Plasma Glucose

Data on the change in fasting plasma glucose from baseline to 24 weeks is shown in table 3.4 and figure 3.12a.

Fasting plasma glucose (FPG) reduced from 10.2mmol/L to 7.1mmol/L in the LED group and from 10.2mmol/L to 9.6mmol/L in the GSC at 24 weeks. This represented a near normalisation in fasting blood glucose in the LED group.

There was a significant difference in FPG between groups at 24 weeks. At 24 weeks the adjusted difference was -2.48mmol/L (95% CI -4.13 to -0.84, $p=0.003$). At 24 weeks the LED group had a significant reduction in FPG (-2.59mmol/l, SD 3.86, $p<0.001$), while the GSC group saw no significant reduction.

3.4.3.5. Fasting serum insulin

Figure 3.12b and Table 3.4 shows fasting serum insulin (FSI) levels from baseline to 24 weeks between groups.

There was a significant difference in FSI between groups at 24 weeks. The adjusted difference between the two groups was -78.8pmol (95% CI -153.1 to -4.4, $p=0.04$). Despite a significant reduction in insulin, both groups were still hyperinsulinemic, with substantially higher FSI than T2D patients not treated with insulin (Lim et al., 2011).

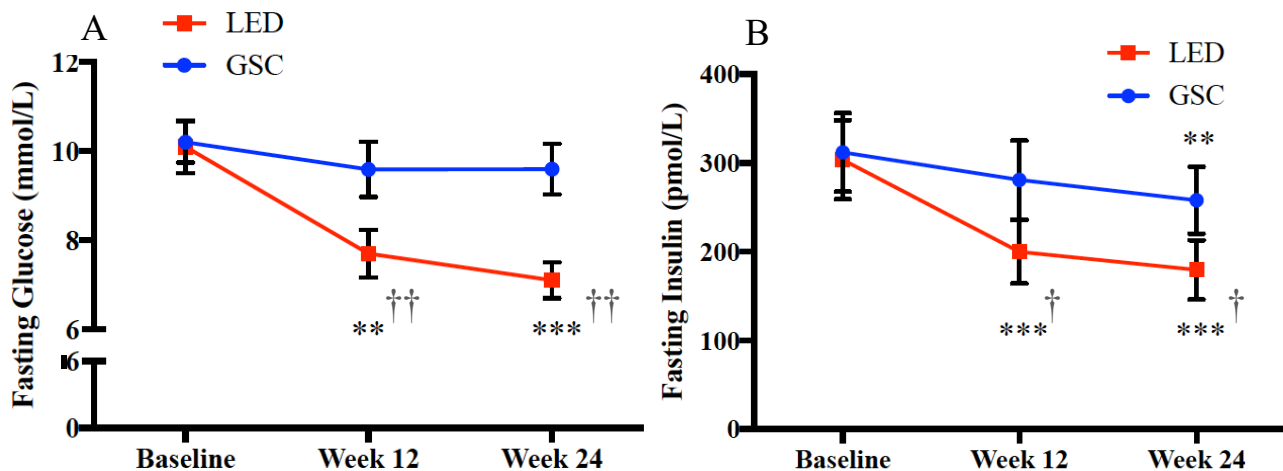


Figure 3.12 Comparison of change in fasting glucose and insulin between LED and GSC at 12 and 24 weeks.

Line graph represents mean fasting glucose and insulin with standard errors. (A) fasting blood glucose (B) fasting serum insulin. P values calculated using adjusted mixed linear modelling ** $p < 0.01$, *** $p < 0.001$ compared from baseline; †: $p < 0.01$, ††: $p < 0.001$ adjusted between group comparison. Abbreviations: GSC, gold standard clinical care; LED, low energy diet;

3.4.3.6. Plasma glucose concentrations during MMTT

Time course comparing baseline and AUC₀₋₂₁₀ data for plasma glucose concentrations are shown in Figure 3.13 and Figure 3.14, respectively.

There was a significant difference in postprandial plasma glucose concentrations between groups at 30, 60, 90, 120, 150, 180 and 210 minutes, with a significant effect on glucose levels in the second latter stages of the MMTT. Plasma glucose gradually increased over the first 90minutes where it peaked in both groups following ingestion of the test meal after which it decreased.

The postprandial glucose response to the test meal at 12 weeks was significantly better following the TDR phase of the LED (Figure 3.13). It would appear from the time course data that blood glucose was handled better by subject following the LED. This was also reflected in the AUC₀₋₂₁₀ for glucose with a significant adjusted difference between groups of -2.3mmol/L per minute (95% CI -4.1 to -0.5, $p = 0.014$) (Figure 3.14).

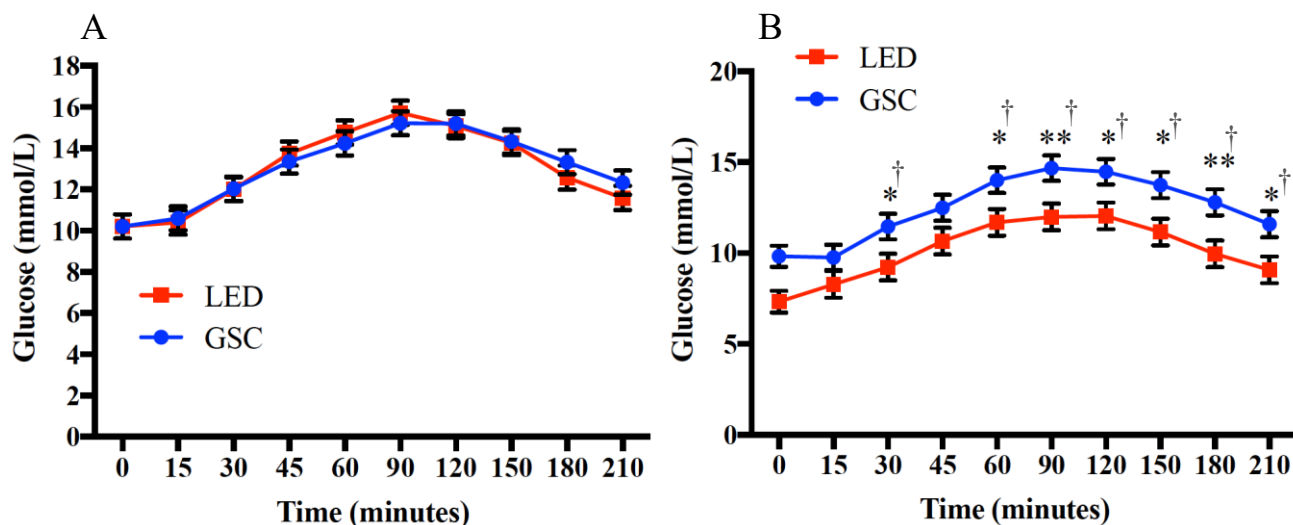


Figure 3. 13 Plasma glucose concentrations comparing GSC and LED during a MMTT.

Data presents (A) mean plasma glucose at baseline and (B) mean plasma glucose at 12 weeks following GSC and LED. P values calculated using adjusted mixed linear modelling † $p < 0.05$, †** $p < 0.01$ for adjusted between group comparison. Abbreviations GSC, gold standard clinical care; LED, low energy diet

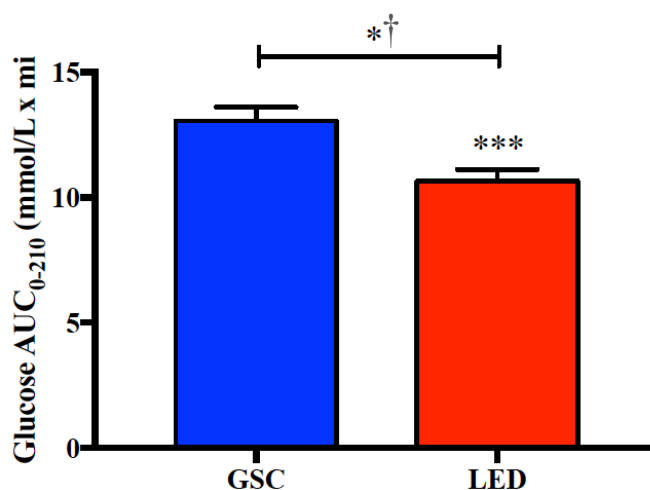


Figure 3. 14 Plasma AUC₀₋₂₁₀ glucose concentrations comparing GSC and LED.

Data presents mean plasma AUC₀₋₂₁₀ glucose with standard errors. P values calculated using adjusted mixed linear modelling * $p < 0.05$, *** $p < 0.001$ †: adjusted between group comparison. Abbreviations AUC, area under the curve; GSC, gold standard clinical care; LED, low energy diet

3.4.3.7. Serum insulin concentrations during MMTT

Time course comparing baseline and AUC₀₋₂₁₀ data for serum insulin concentrations are shown in Figure 3.15 and Figure 3.16, respectively.

Analysing the time course data shows that there was no difference in postprandial serum insulin concentrations following a test meal.

Peak insulin release was during the MMTT was lower at 12 weeks within the LED group, falling from 571.8pmol to 495.2pmol. In comparison there was an increase in peak insulin release in the GSC, going from 453.8pmol to 555.6pmol at 12 weeks. Although it did not reach significance there was a trend following peak insulin for the LED to have lower insulin concentrations.

The AUC₀₋₂₁₀ for insulin did show a difference with an adjusted difference between groups - 106.5pmol/L per minute (95% CI -178.2 to 34.8, p=0.004).

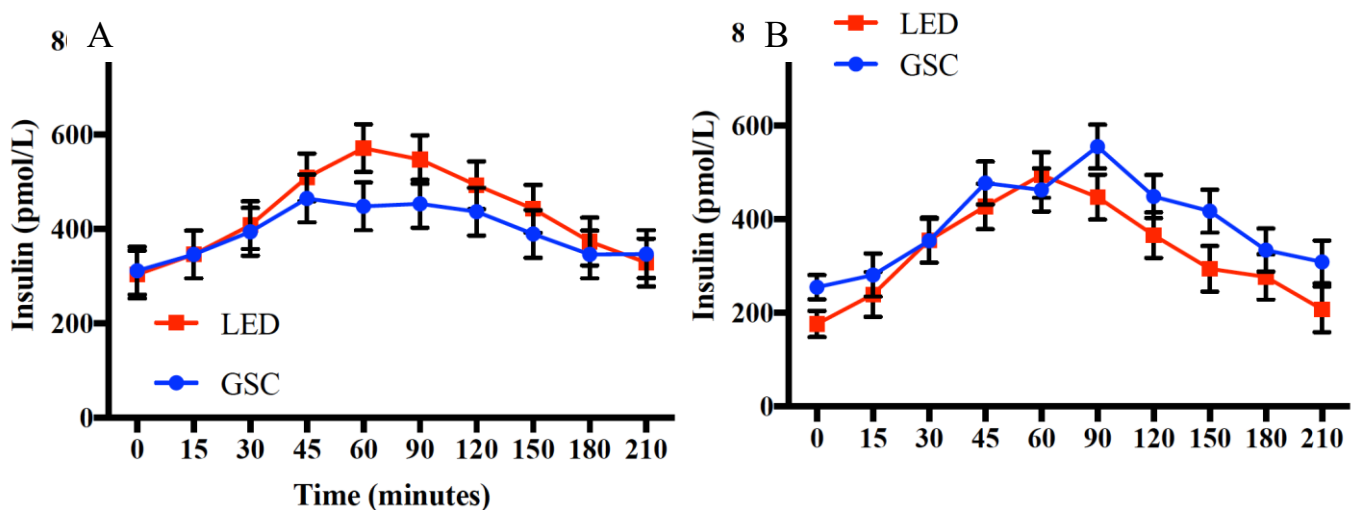


Figure 3. 15 Serum insulin concentrations comparing GSC and LED during a MMTT.

Data presents (A) mean plasma insulin at baseline and (B) mean plasma insulin at 12 weeks following GSC and LED. Abbreviations GSC, gold standard clinical care; LED, low energy diet; Δ, change

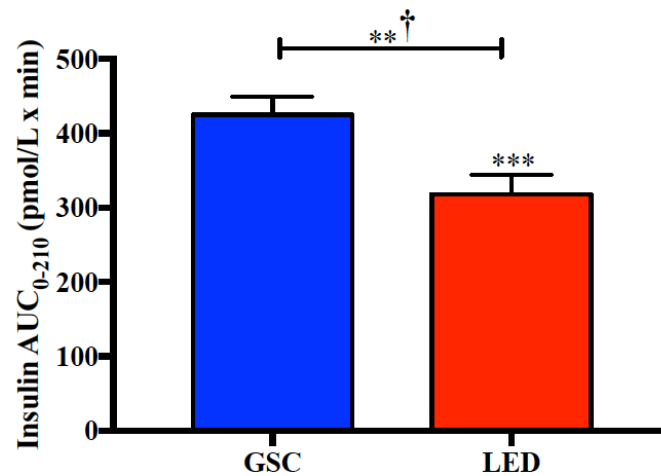


Figure 3. 16 Serum AUC₀₋₂₁₀ insulin concentrations comparing GSC and LED.

Data presents mean plasma AUC₀₋₂₁₀ insulin with standard errors. GSC and LED. P values calculated using adjusted mixed linear modelling **p<0.01, ***p<0.001 †: adjusted between group comparison. Abbreviations: AUC, area under the curve; GSC, gold standard clinical care; LED, low energy diet;

3.4.3.8. Insulin Resistance Insulin Indices

Insulin indices data for the HOMA-IR, QUICKI, Matsuda index, Insulinogenic Index and Oral Disposition index are shown in Figure 3.17.

3.4.3.8.1. HOMA-IR

HOMA-IR was calculated using the HOMA-IR calculation from the Oxford Diabetes Trial Unit (Matthews et al. 1985). At baseline both groups were extremely insulin resistant with the mean HOMA-IR being 22.4 (SD 21.5) in the GSC group and 23.7 (SD 29.6) in the LED group.

At 24 weeks, HOMA-IR reduced by -13.5 (SD 16.1) in the LED group and by -4.6 (SD 15.0) in the GSC group, with an adjusted difference of -8.9 (95% CI -15.72 to -2.11, p<0.010). This suggests that there was an improvement in insulin resistance within both groups, which in part could go to explain the improvement in glucose homeostasis.

3.4.3.8.2. QUICKI

At baseline the QUICKI was 0.26 (SD 0.02) within the GSC group and 0.26 (SD 0.02) within the LED group indicating that insulin sensitivity was suppressed.

There was a significant difference in QUICKI between the groups at 24 weeks in favour of the LED with an adjusted difference between the groups was 0.02 (95% CI 0.009 to 0.030, $p < 0.0001$)

3.4.3.9. Whole-body insulin sensitivity and beta-cell function

Whole-body insulin sensitivity and beta-cell function was assessed using glucose and insulin values from the mixed meal tolerance test, as a result data related to this is only available for comparison at 12 weeks and not at 24 weeks. As time course data was only available at week 12, the Matsuda index, insulinogenic index and oral disposition index was only calculated at that time point.

3.4.3.9.1. Matsuda Index

At baseline the Matsuda index (MI) was 1.24 (SD 0.70) within the GSC group and 1.19 (SD 0.66) in the LED group. There was a significant difference in MI between groups at 12 weeks. MI increased by 1.19 (SD 1.19, $p < 0.001$) in the LED group and 0.27 (SD 0.83, $p = 0.113$) in the GSC group (adjusted difference 0.93, 95% CI 0.44 to 1.42, $p = 0.001$). This indicates that there was an improvement in whole body insulin resistance, with the LED close to the predefined cut of ≤ 2.5 for IR (Kernan et al., 2003).

3.4.3.9.2. Insulinogenic index

There was no significant difference in insulinogenic index (IGI) between the groups. There was also no significant difference between baseline and week 12 in either group, despite an apparent increase within the LED group (22.2, SD 102.2), showing that there was not an improvement in early phase insulin response to glucose (Kosaka et al., 1996, Del Prato, 2003).

3.4.3.9.3. Oral Disposition Index

There was no difference in Oral disposition index (DI_o) between the groups at 12 weeks. DI_o increased significantly by 0.49 (SD 0.93, $p=0.009$) within the LED group and by 0.22 but this did not reach between groups significance (SD 0.56, $p=0.08$).

There was however a significant increase in DI_o in the LED group suggesting that there was possibly an improvement in β -cell function to compensate for the IR.

Indices of Insulin sensitivity and beta-cell function

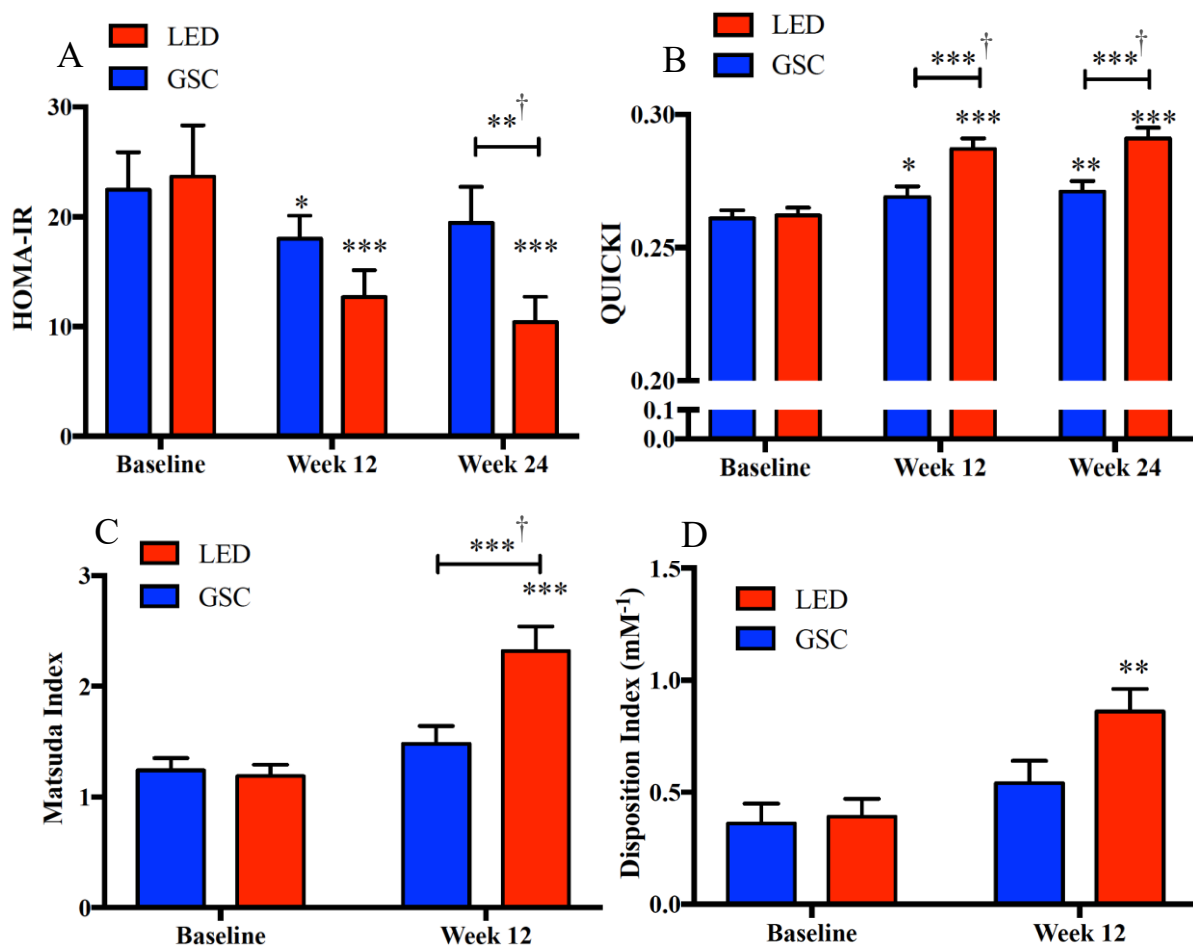


Figure 3. 17 Comparison of effect of LED on changes in different indices of insulin sensitivity and beta-cell function at 12 and 24 weeks.

Data presented as mean with standard error. P values calculated using adjusted mixed linear modelling * $p<0.05$, ** $p<0.01$, *** $p<0.001$ †: adjusted between group comparison. Abbreviations; HOMA-IR, homeostasis model assessment-insulin resistance; QUICKI, Quantitative Insulin Sensitivity Check Index: Abbreviations: GSC, gold standard clinical care; LED, low energy diet.

Table 3. 4 Glycaemic outcomes comparing LED and GSC at 12 and 24 weeks

	Mean (SD)						Intervention effect	
	Baseline	Week 12	Change	Week 24	Change		Estimate (95% CI)	P value
HbA1c (%)								
LED	8.8 (1.8)	7.2 (1.4)	-1.43 (1.3) ***	7.0 (1.3)	-1.44 (1.3) ***	0-12	-0.79 (-1.4 to -0.2)	0.007
GSC	9.3 (1.7)	8.3 (1.7)	-0.65 (1.3) **	8.2 (1.7)	-0.67 (1.3) **	12-24	-0.77 (-1.4 to -0.2)	0.010
HbA1c (mmol/mol)								
LED	73 (19.7)	55.5 (15.7)	-15.7 (14.4) ***	53.1 (14.7)	-15.8 (14.7) ***	0-12	-8.73 (-14.9 to -2.49)	0.006
GSC	78.5 (18.7)	67.5 (19.8)	-6.9 (14.0) **	66.1 (18.9)	-7.3 (14.2) **	12-24	-8.54 (-14.9 to -2.18)	0.009
Glucose (mmol/L)								
LED	10.1 (3.7)	7.7 (3.4)	-2.30 (3.8) ***	7.1 (2.6)	-2.59 (3.9) ***	0-12	-1.97 (-3.6 to -0.34)	0.017
GSC	10.2 (2.9)	9.6 (3.9)	-0.32 (3.6)-	9.6 (3.6)	-0.10 (3.6)	12-24	-2.48 (-4.13 to -0.84)	0.003
Insulin (pmol/L)								
LED	303.4 (280.8)	199.9 (202.2)	-122.7 (168.2) ***	179.5(178.7)	-152.0 (176.2) ***	0-12	-77.0 (-149.1 to -5.0)	0.04
GSC	311 (280.1)	280.7 (263.0)	-45.7 (160.8)	257.9 (237.6)	-73.2 (163.0) **	12-24	-78.8 (-153.1 to -4.4)	0.04
Insulin Dose (U)								
LED	71.1 (40.7)	18.6 (20.6)	-53.9 (25.5) ***	21.3 (26.3)	-50.9 (26.3) ***	0-12	-21.5 (-32.6 to -10.4)	<0.0001
GSC	75.4 (54.9)	42.6 (29.8)	-32.4 (24.9) ***	39.6 (32.2)	-35.8 (25.2) ***	12-24	-15.1 (-26.4 to -3.8)	0.009
Insulin Dose (U/kg)								
LED	0.69 (0.41)	0.21 (0.24)	-0.50 (0.25) ***	0.25 (0.32)	-0.46 (0.25) ***	0-12	-0.20 (-0.31 to -0.97)	<0.0001
GSC	0.74 (0.49)	0.43 (0.27)	-0.30 (0.24) ***	0.41 (0.31)	-0.32 (0.24) ***	12-24	-0.14 (-0.25 to -0.03)	0.01

Data represents mean plus standard deviations p values calculated using adjusted mixed linear modelling **p<0.01, ***p<0.001 within group from baseline. Intervention effects reported as estimated mean differences (LED minus GSC). Abbreviations: HbA1c, glycated haemoglobin; LED Low energy diet; GSC Gold standard care; U, units, U/kg units per kilogram

Table 3. 5 Indices of insulin sensitivity and beta-cell function comparing LED and GSC at 12 and 24 weeks

	Mean (SD)					Intervention effect		
	Baseline	Week 12	Change	Week 24	Change	Estimate (95% CI)		P value
HOMA-IR								
LED	23.7 (29.6)	12.7 (15.4)	-11.5 (15.5) ***	10.4 (14.7)	-13.5 (16.1) ***	0-12	-5.8 (-12.4 to 0.87)	0.088
GSC	22.4 (21.5)	18.0 (13.4)	-5.7 (14.8) *	19.4 (20.7)	-4.6 (15.0)	12-24	-8.9 (-15.7 to -2.1)	0.010
QUICKI								
LED	0.26 (0.02)	0.29 (0.03)	0.03 (0.02) ***	0.29 (0.03)	0.03 (0.03) ***	0-12	0.02 (0.01 to 0.03)	0.001
GSC	0.26 (0.02)	0.27 (0.03)	0.01 (0.02) *	0.27 (0.03)	0.01 (0.02) **	12-24	0.02 (0.01 to 0.03)	<0.001
MI								
LED	1.19 (0.66)	2.32 (1.4)	1.19 (1.19) ***	-	-	0-12	0.93 (0.44 to 1.42)	0.001
GSC	1.24 (0.70)	1.48 (1.04)	0.27 (0.83)	-	-	12-24	-	-
IGI								
LED	79.4 (94.3)	100.1 (75.6)	15.5 (105.9)	-	-	0-12	22.8 (-19.2 to 64.9)	0.287
GSC	71.7 (65.9)	74.1 (62.8)	4.15 (75.1)	-	-	12-24	-	-
DI ₀								
LED	0.39 (0.41)	0.86 (0.66)	0.48 (0.69) **	-	-	0-12	-0.28 (-0.09 to 0.67)	0.143
GSC	0.36 (0.32)	0.54 (0.63)	0.22 (0.50)	-	-	12-24	-	-

Data represents mean plus standard deviations p values calculated using adjusted mixed linear modelling *p<0.05, **p<0.01, ***p<0.001 within group from baseline. Intervention effects reported as estimated mean differences (LED minus GSC). Abbreviations: HOMA-IR Homeostatic model assessment, IGI, Insulinogenic index, QUICKI Quantitative insulin sensitivity check index, DI Disposition Index, LED Low energy diet, GSC Gold standard care

3.4.4. Adverse effects of LED & GSC groups

The side effects of the subjects within the LED & GSC groups over the whole 24-week period are reported in Table 3.6. and agree with what other studies that have reported the side effects following a formula LED.

Table 3. 6 Self-reported adverse events among the subject in the LED & GSC group at 24 weeks.

Side Effect	Frequency of side effect in LED (n, %)	Frequency of side effect in GSC (n, %)
Constipation	21 (52.5)	13 (32.5)
Sensitivity to cold	21 (52.5)	1 (2.5)
Flatulence	18 (45)	2 (5)
Dizziness	16 (40)	7 (17.5)
Diarrhoea	16 (40)	5 (12.5)
Bad breath	14 (35)	0 (0)
Dry Skin	12 (30)	0 (0)
Mood changes	12 (30)	12 (30)
Sleeplessness	11 (27.5)	9 (22.5)
Fatigue	11 (27.5)	15 (37.5)
Skin Irritation	8 (20)	4 (10)
Headaches	10 (25)	6 (15)
Back Pain	7 (17.5)	6 (15)
Depressive tendencies	7 (17.5)	11 (27.5)
Anxiety	7 (17.5)	11 (27.5)
Joint Pain	7 (17.5)	10 (25)
Toothache	7 (17.5)	3 (7.5)
Hair loss	6 (15)	1 (2.5)
Nausea	6 (15)	6 (15)
Perianal Itching	5 (12.5)	0 (0)
Abdominal Pain	5 (12.5)	5 (12.5)
Cramps	4 (10)	2 (5)
Allergic Rash	4 (10)	1 (2.5)
Sciatic Pain	4 (10)	4 (10)
Heart Burn	3 (7.5)	2 (5)
Eczema	3 (7.5)	1 (2.5)
Swollen Joints	2 (5)	5 (12.5.)
Hives	2 (5)	0 (0)
Redness	2 (5)	4 (10)
Epigastric pain	1 (2.5)	5 (12.5)
Taste alterations	1 (2.5)	0 (0)
Influenza	3 (7.5)	2 (5)

Data are presented as reported cases no. (%)

3.4.4.1. Self-reported hypoglycaemic episodes

Data on the self-reported hypoglycaemia is shown in Figure 3.18.

As hypoglycaemic episodes are expected within T2D patients treated with insulin these were investigated. If a hypoglycaemic episode required additional assistance to treat, then this would have been recorded as a serious adverse event. At each review appointment whether telephone or face-to-face, subjects were asked to report how many hypoglycaemic episodes they experienced over the proceeding 2-week period.

At 12 and 24-week there was a non-significant adjusted incidence rate ratio (IRR) of having a hypoglycaemic episode between the two groups.

At 12-week this denoted of a 3% reduction in hypoglycaemic episodes in the LED compared with the GSC (IRR 0.97, 95% CI 0.54 to 1.73, $p=0.908$). While at 24-week the risk of hypoglycaemic episodes reduced to 38% compared with the GSC group (IRR 0.62, 95% CI 0.36 to 1.07, $p=0.087$).

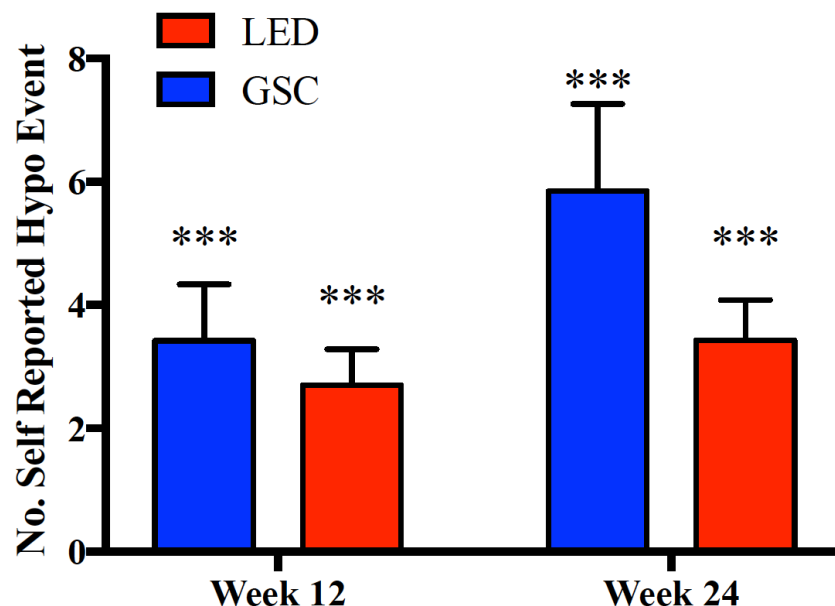


Figure 3. 18 Comparison of effect of LED to GSC on self-reported hypoglycaemic episodes at 12 & 24 weeks.

Bar graph represents mean hypoglycaemic episodes with standard errors. P values calculated using adjusted incidence ratios *** $p<0.001$. Abbreviations: GSC, gold standard clinical care; LED, low energy diet.

3.4.4.2. Serious Adverse events

Fourteen serious adverse events (SAE) were reported, 9 within the GSC group and 5 within the LED group. Due to the nature of the subjects recruited to the study the events reported were not un-expected as these subjects had advanced T2D and were often on multiple therapies for other obesity related co-morbidities. Only one SAE was deemed potentially related to the intervention and that was an episode of postural hypotension resulting in fainting. Two subjects were diagnosed with having cancer. One subject in the LED group who completed 12 months was diagnosed with prostate cancer and the other was in the GSC group who was diagnosed with squamous cell carcinoma in the ear canal. This was successfully removed and the subject was discharged. Two serious adverse events were reported in the same subject, this was due to a fractured tibia and fibula in the ankle joint. Two subjects reported worsening retinopathy one in the LED and one in the GSC group. Other SAEs involved an abscess, two suspected transient ischemic attacks but were not confirmed as such an episode of shortness of breath. No subjects experienced a hypoglycaemic episode that required assistance. No mortality was observed in the study.

3.4.5. Assessment of compliance to dietary recommendations

3.4.5.1. Predictive weight losses

Figure 3.19 shows the difference between predictive weight loss and actual weight loss over the 24 weeks between each group.

The difference between predicted weight loss and actual weight loss is important to assess adherence to dietary change and track subject's progress. Using a predictive weight loss calculation by Hall (2008) using baseline weight, body fat and physical activity, this gave a predicted weight loss at 12, 18 and 24 weeks. This was then subtracted from actual weight loss to give the difference.

There was a non-significant adjusted difference between groups at 12 weeks (-1.12kg, 95% CI -5.32 to 3.09, $p=0.602$), however as time progress this difference increased and at 24 weeks was -4.84kg (95% CI -9.07 to -0.61, $p=0.03$). Mean predicted weight loss was significantly greater in both groups than that achieved as part of the intervention with those in

the LED predicted to lose an additional 15.57kg (SD 9.86, $p<0.0001$) and the GSC a further - 9.38kg (SD 9.48, $p<0.0001$). This suggests that there may have been compliance issues with subjects following the diet particularly in the food reintroduction phase.

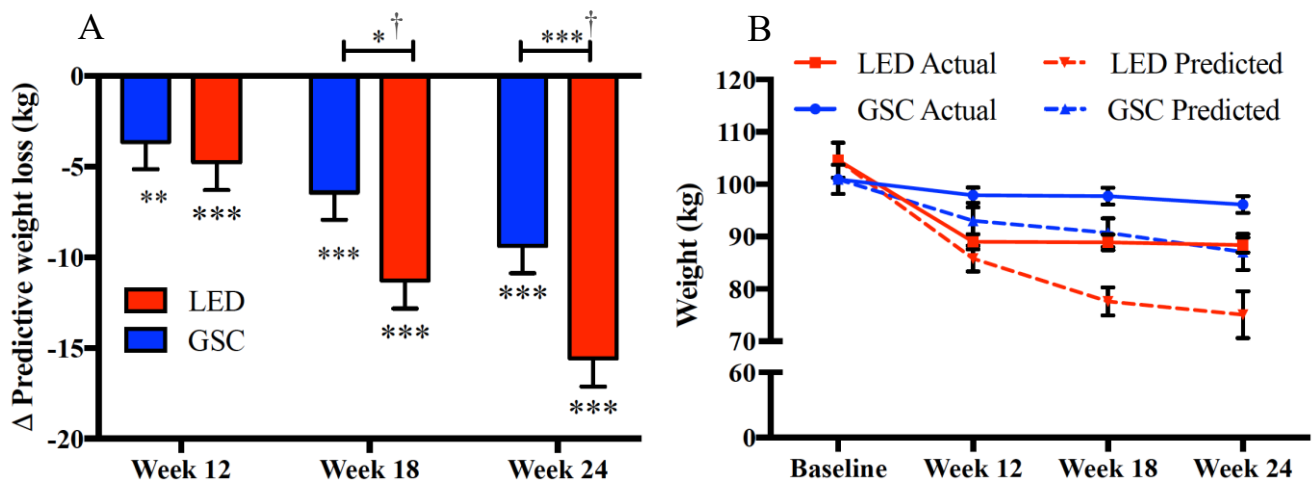


Figure 3. 19 Comparison of observed weight loss versus predicted weight loss following the total diet replacement, low energy diet and GSC at 12, 18 & 24 weeks.

(A) predicted weight losses from Hall equation for LED and GSC (B) graph comparing actual body weight loss with predicted for both groups. P values calculated using adjusted mixed linear modelling * $p<0.05$, *** $p<0.001$ †: adjusted between group comparison. Abbreviations: GSC, gold standard clinical care; LED, low energy diet; Δ , change;

3.4.6. Quality of Life

Quality of life (QoL) was assessed using the EuroQoL 5 Dimensions (EQ-5D) visual analogue scale that recorded the subjects self-rated health status on the assessment visits. Baseline figures and changes are seen in Table 3.7 and the change at 12 and 24 weeks can be seen in Figure 3.20.

Table 3. 7 Quality of Life score from visual analogue scale comparing LED and GSC at 12 and 24 weeks

	GSC	LED	IE (95% CI)	P value
Baseline	63.4 (19.2)	68.7 (18.1)	-	-
12 weeks	70.0 (21.8)	78.1 (14.0) ***	6.2 (-1.6 to 13.9)	0.119
24 weeks	68.0 (32.1)	82.3 (14.2) ***	11.9 (4.1 to 19.7)	0.003

Data represents mean QoL score with standard deviations. Abbreviations LED, low energy diet; GSC, gold standard clinical care; Kcal, kilocalories; LED, low energy diet; GSC, gold standard clinical care, QoL quality of life; IE intervention effect p values calculated using adjusted mixed linear modelling ***p<0.001 within group from baseline. Intervention effects reported as estimated mean differences (LED minus GSC). 95% CI, confidence intervals

EQ-5D improved by 12.94 points (SD 18.2, p<0.001) in the LED group and further increased marginally by 1.1 points (SD 17.5, p=0.263) at 24 weeks. This was associated with an adjusted between group difference of 11.9 points (95% CI 4.06 to 19.70, p=0.003). At 12-week despite a significant increase in QoL in the LED group of 9.2 points (p=0.001), there was no difference between groups (adjusted difference 6.2 points, 95% CI -1.58 to 13.87, p=0.119).

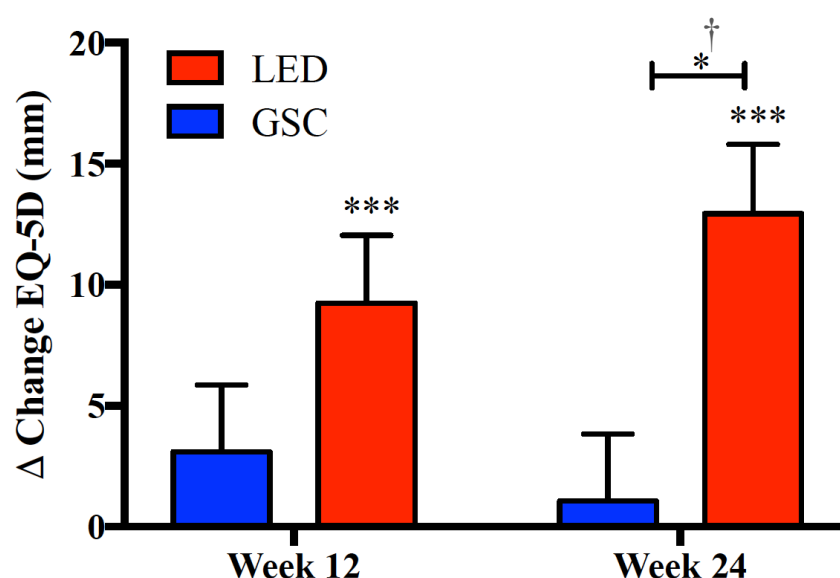


Figure 3. 20 Quality of life changes following LED and GSC at 12 & 24 weeks.

Bar graph represents mean percentage energy reduction with standard errors. P values calculated using adjusted mixed linear modelling *p<0.05, ***p<0.001 †: adjusted between group comparison. Abbreviations LED, low energy diet; GSC, gold standard clinical care; Δ, change; EQ5D, EuroQoL 5 dimensions

3.4.7. Energy intake and appetite

3.4.7.1. Ad-libitum energy intake

Data on the ad-libitum energy intake is shown in table 3.8 and Figure 3.21.

Table 3. 8 Energy intake at *ad libitum* following the mixed meal tolerance test for LED and GSC

	GSC	LED	p Value
Baseline EI (kcal)	365.1 (146.4)	352.5 (155.7)	0.709
Week 12 EI (kcal)	344 .7 (127.6)	311.8 (133.5)	0.260

Data represents mean energy intakes with standard deviations. Abbreviations LED, low energy diet; GSC, gold standard clinical care; Kcal, kilocalories; LED, low energy diet; GSC, gold standard clinical care; EI energy intake

There was a no difference in percentage energy intake between groups at 12-week with an adjusted difference of -32.9kcal (95% CI -90.2 to 24.4, $p=0.260$). When calculated as a percentage difference in energy intake this remained non-significant (adjusted difference - 5.9%, 95% CI -25.1 to 13.3, $p=0.547$, Figure 3.21).

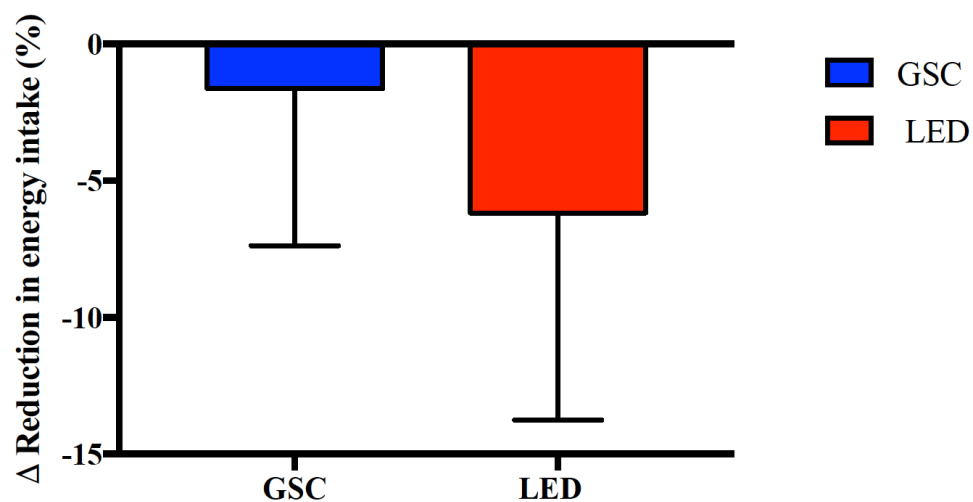


Figure 3. 21 Reduction in energy intake at *Ad-Libitum* meal

Bar graph represents mean percentage energy reduction with standard errors compared with baseline. Abbreviations LED, low energy diet; GSC, gold standard clinical care, Δ , change, %, percentage

3.4.7.2. Appetite during Mixed Meal Tolerance Test – Visual Analogue Scales

3.4.7.2.1. Composite Appetite score

A composite appetite score was calculated as in the methods section 2. Time course and area under the curve AUC₀₋₂₁₀ data for composite appetite score (CAS) are shown in Figure 3.22.

There was no difference in composite appetite score between groups at baseline and there was not a difference between groups for CAS AUC₀₋₂₁₀ ($p=0.056$). Over the time course post hoc analysis identified that composite appetite was significantly less at 60 minutes with an adjusted between group difference of -12.0 (95% CI -23.1 to -0.88, $p=0.034$). Although other time points did not reach significance, there was a trend towards reduced CAS over the initial 90 minutes of the MMTT. Analysis of the AUC showed that overall CAS significantly reduced within the LED group compared with the GSC (-7.3 mm/min, 95% CI -13.4 to -1.4, $p=0.016$).

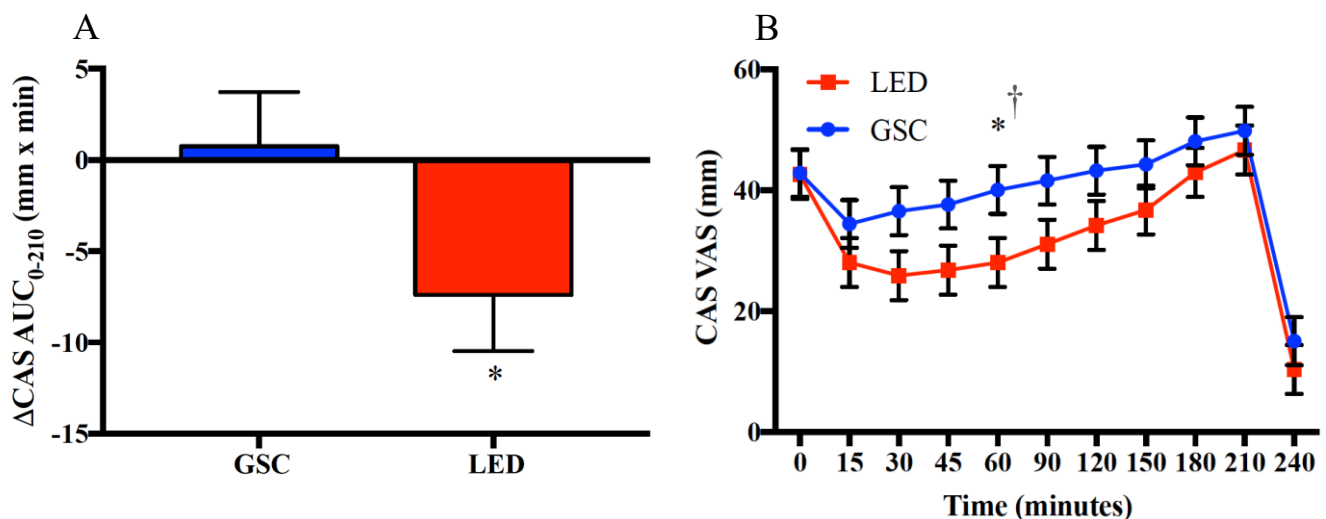


Figure 3. 22 Composite appetite score visual analogue scales following LED and GSC at 12 weeks.

Data represents mean plus standard error (A) Δ AUC₀₋₂₁₀ of the composite appetite score over the course of the mixed meal test (min x min) (B) composite appetite score over time. P values calculated using adjusted mixed linear modelling * $p<0.05$ AUC comparison from baseline; *† $p<0.05$ adjusted between group comparison. Abbreviations: AUC = area under the curve; CAS, composite appetite score; VAS, visual appetite score; Δ , change; LED, low energy diet; GSC, gold standard clinical care

3.4.7.2.2. Hunger

Time course and AUC₀₋₂₁₀ data for hunger are shown in Figure 3.23.

There was no difference in AUC₀₋₂₁₀ hunger between groups (-7.4 mm/min, 95% CI -16.2 to 1.4, $p=0.10$), which corresponded with no difference being found between fasting hunger scores at either baseline ($p=0.978$) or at week 12 ($p=0.870$) between the groups.

In the time course data after consumption of the test meal, hunger reduced in both the LED and the GSC until 30 minutes, after which there was a gradual increase over the remainder of the test. There was a difference in hunger at 45 and 60 minutes (Figure 3.23) following the test meal, and a trend similar to that of the CAS of reduction in hunger over the initial 90 minutes.

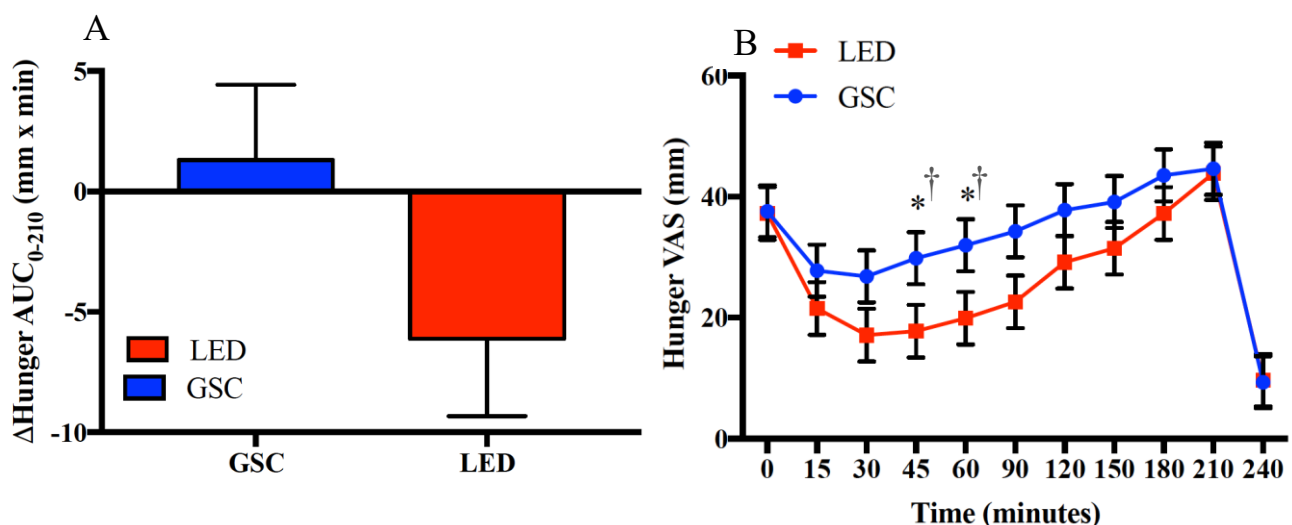


Figure 3. 23 Hunger visual analogue scales following LED and GSC at 12 weeks.

Data represents mean plus standard error. (A) Δ AUC₀₋₂₁₀ of the hunger over the course of the mixed meal test (min x min) (B) hunger over time. P values calculated using adjusted mixed linear modelling *† $p<0.05$ adjusted between group comparison. Abbreviations: AUC = area under the curve; CAS = composite appetite score; VAS, visual appetite score; Δ , change; LED, low energy diet; GSC, gold standard clinical care

3.4.7.2.3. Fullness

Time course and AUC₀₋₂₁₀ data for fullness are shown in Figure 3.24.

There was a significant difference in the AUC₀₋₂₁₀ fullness data between groups at 12 weeks with an adjusted difference of 11.5 (95% CI 1.6 to 21.4, $p=0.023$), with the LED having significantly greater overall fullness, with a mean increase of 10.9mm/min (SD 3.6, $p=0.003$).

In addition, this was confirmed by the time course data showing that subjects on the LED had greater fullness at 30, 45, 60 and 120 minutes following the start of the MMTT (Figure 3.24). However, there was no difference at baseline ($p=0.956$) or 12-week ($p=0.899$) in regard to fasting fullness.

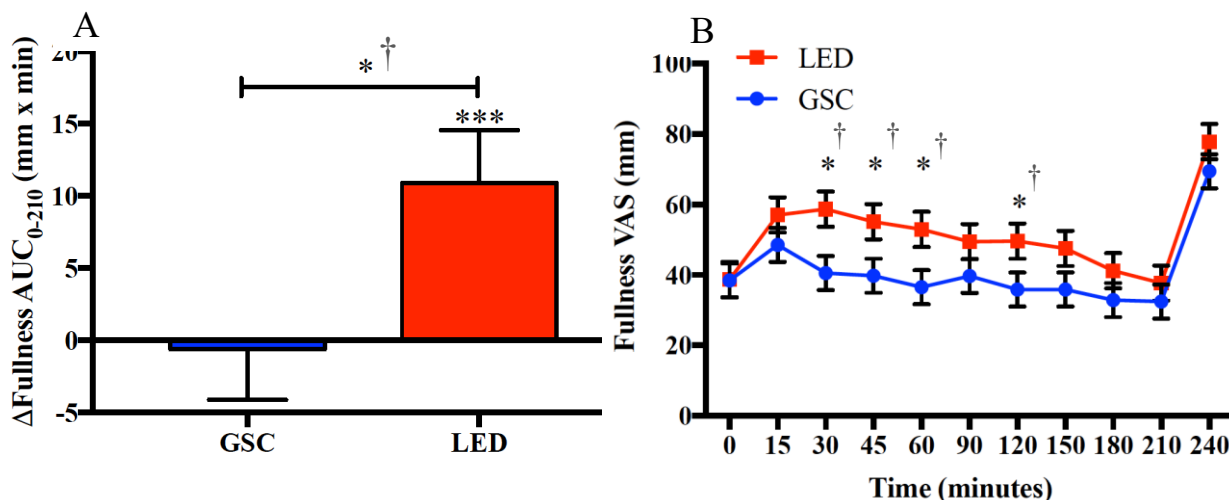


Figure 3. 24 Fullness visual analogue scales following LED and GSC at 12 weeks.

Data represents mean plus standard error. (A) Δ AUC₀₋₂₁₀ of the fullness over the course of the mixed meal test (min x min) (B) fullness over time. P values calculated using adjusted mixed linear modelling * $p<0.05$, *** $p<0.001$ AUC comparison from baseline; *† $p<0.05$ adjusted between group comparison. Abbreviations: AUC, area under the curve; VAS, visual appetite score; Δ , change; LED, low energy diet; GSC, gold standard clinical care

3.4.7.2.4. Desire for a meal

There was no difference in desire for a meal between groups within the time course data or in the desire for a meal AUC_{0-210} (-5.8 mm/min, 95% CI -14.6 to 3.1, $p=0.202$). Also, no difference was found between fasting desire for a meal scores at baseline ($p=0.867$) or at week 12 ($p=0.599$). Despite there being no difference there was a trend towards a reduced desire for a meal in both the time course and AUC data (Figure 3.25).

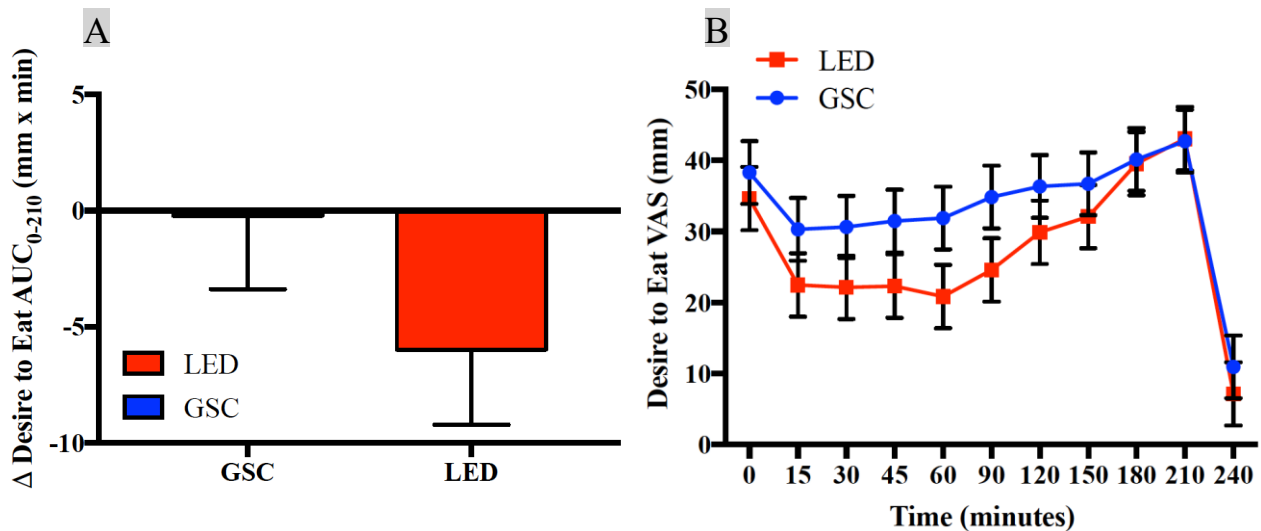


Figure 3. 25 Desire for a meal visual analogue scales following LED and GSC at 12 weeks.

Data represents mean plus standard error. (A) ΔAUC_{0-210} of the desire for a meal over the course of the mixed meal test (min x min) (B) desire for a meal over time. Abbreviations: AUC, area under the curve; VAS, visual appetite score; Δ , change; LED, low energy diet; GSC, gold standard clinical care

3.4.7.2.5. Appetite for a meal

There was no difference in appetite for a meal between groups within the time course data or in the appetite for a meal AUC_{0-210} (-6.3 mm/min, 95% CI -15.5 to 2.8, $p=0.176$). Also, no difference was found between fasting appetite for a meal scores at baseline ($p=0.392$) or at week 12 ($p=0.550$). Despite there being no difference there was a trend towards a reduced appetite for a meal in both the time courses and AUC data (Figure 3.26).

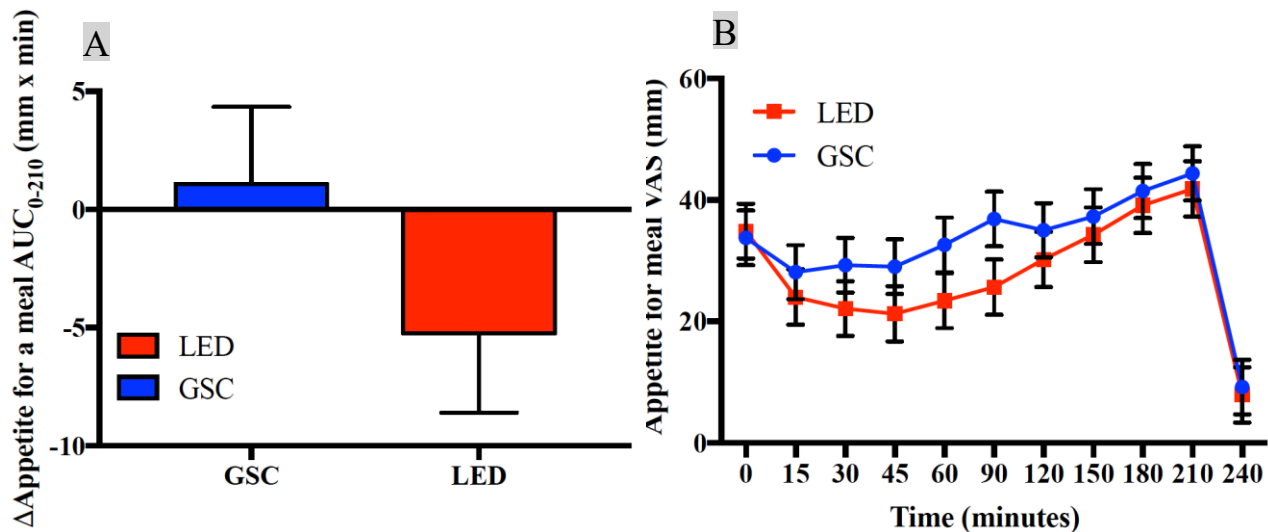


Figure 3. 26 Appetite for a meal visual analogue scales following LED and GSC at 12 weeks.

Data represents mean plus standard error. (A) ΔAUC_{0-210} of the appetite for a meal over the course of the mixed meal test (min x min) (B) appetite for a meal over time. Abbreviations: AUC, area under the curve; VAS, visual appetite score; Δ , change; LED, low energy diet; GSC, gold standard clinical care

Assimilating the data it would appear that there is an effect on overall appetite in subjects using the formula LED, particularly fullness, although this does not appear to influence *ad-libitum* food intake.

3.4.7.3. Energy intake under free-living conditions

The energy and macronutrient intake of the subjects within the LED and GSC are summarised and reported in Table 3.9 and shown in Figure 3.27.

Energy and macronutrient intake was measured using a 3-day food diary to assess if there were dietary changes that might associate with the changes seen with weight and glycaemic

control. At baseline there were no difference between groups in any dietary parameter. The baseline energy intake were estimated to be 1661kcal/day for the LED and 1747kcal/day, which were less than the mean total energy expenditures (LED 2388kcal/day; GSC 2315kcal/day), which would suggest that subjects should be losing weight.

There was a significant adjusted between group difference at 24 weeks with adjusted differences being only found in energy (-205.4kcal, 95% CI -398.7 to -12.2, $p=0.037$) and fat (-16.0g, 95% CI -26.7 to -5.4, $p=0.003$), with protein and carbohydrate intakes not differing (1.2g and -24.8g, respectively). Fibre intake represents an important dietary factor regarding the management of glycaemic control and therefore was also measured. Fibre intake at 24 weeks difference between groups with an adjusted between group difference of -2.5g, 95% CI -4.4 to -0.7, $p=0.007$ in favour of the GSC group.

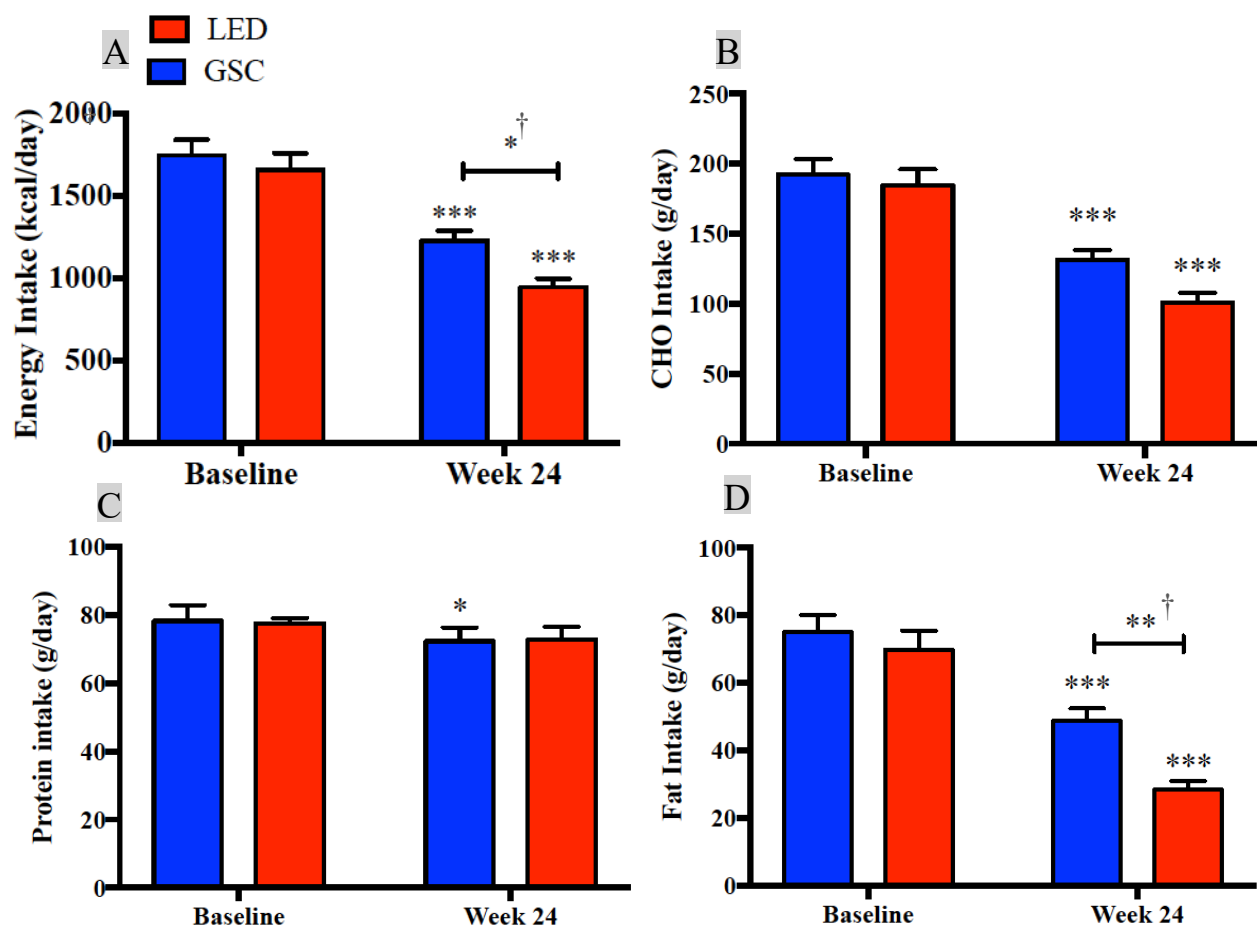


Figure 3. 27 Energy, carbohydrate, protein and fat intakes following LED and GSC at 24 weeks.

Data represents mean plus standard error. (A) Energy (B) carbohydrate (C) protein (D) fat p values calculated using adjusted mixed linear modelling *p<0.05, ***p<0.001 †: adjusted between group comparison. Abbreviations: LED, low energy diet; GSC, gold standard clinical care; g, grams; kcal, kilocalories, CHO, carbohydrate

Table 3. 9 Macronutrient and Energy Intake Outcomes comparing LED and GSC at 12 and 24 weeks

	Mean (SD)					Intervention effect		
	Baseline	Week 12	Change	Week 24	Change	Estimate (95% CI)		P value
EI (kcal)								
LED	1661.2 (631.1)	866.9 (403.4)	-839.2 (428.1) ***	947.6 (324.3)	-717.9 (449.6) ***	0-12	-474.7 (-663.0 to -286.4)	<0.0001
GSC	1746.9 (603.9)	1317.9 (420.0)	-364.6 (430.9) ***	1228.7 (380.2)	-512.5 (431.3) ***	12-24	-205.4 (-398.7 to -12.2)	0.037
CHO (g)								
LED	184.5 (71.7)	124.9 (59.8)	-61.7 (55.9) ***	100.9 (42.7)	-81.9 (59.3) ***	0-12	-23.8 (-48.5 to 0.82)	0.058
GSC	192 (69.5)	148.4 (60.1)	-37.9 (56.5) ***	131.4 (41.4)	-57.2 (56.6) ***	12-24	-24.8 (-50.2 to 0.69)	0.057
Protein (g)								
LED	77.7 (29.3)	53.1 (9.9)	-26.5 (22.3) ***	72.8 (25.4)	-6.5 (23.9)	0-12	-18.3 (-28.2 to -8.4)	<0.0001
GSC	78.3 (29.1)	70.2 (21.7)	-8.2 (22.7) *	72.4 (25.3)	-7.7 (22.7) *	12-24	1.2 (-9.0 to 11.4)	0.819
Fat (g)								
LED	69.9 (35.4)	18.2 (18.2)	-54.8 (23.5) ***	28.4 (16.2)	-42.8 (23.9) ***	0-12	-34.8 (-45.2 to -24.5)	<0.0001
GSC	74.9 (32.4)	51.9 (23.7)	-20.0 (23.7) ***	48.6 (23.8)	-26.8 (23.8) ***	12-24	-16.0 (-26.7 to -5.4)	0.003
Fibre (g)								
LED	12.8 (5.6)	7.7 (3.0)	-5.5 (4.1) ***	7.8 (2.8)	-5.2 (4.3) ***	0-12	-4.2 (-6.07 to -2.49)	<0.0001
GSC	13.0 (5.0)	11.7 (4.4)	-1.2 (4.1)	10.4 (3.8)	-2.6 (4.1) ***	12-24	-2.5 (-4.4 to -0.68)	0.007

Data represents mean plus standard deviations. P values calculated using adjusted mixed linear modelling * $p < 0.05$, *** $p < 0.001$ within group from baseline. Intervention effects reported as estimated mean differences (LED minus GSC). Abbreviations: EI Energy intake, CHO, carbohydrate; LED Low energy diet, GSC Gold Standard care, g grams, kcal kilocalories

3.4.8. Physical Activity

Physical activity was measured using an accelerometer that was worn for 7 days and the average measurements were calculated.

3.4.8.1. Steps

The daily number of steps were compared between groups and shown in Figure 3.28.

There was a no difference in the number of steps between groups at 24 weeks (1330.9 steps, 95% CI -5.32 to 3.09, $p=0.119$). Despite this, there was a significant increase in the number of steps with the LED group suggested that the intervention group became more active over the 24-week period (difference 1495 steps, $p=0.018$). This data was unable to assess other forms of physical activity include resistance workouts, therefore it was only possible to assess aerobic physical activity.

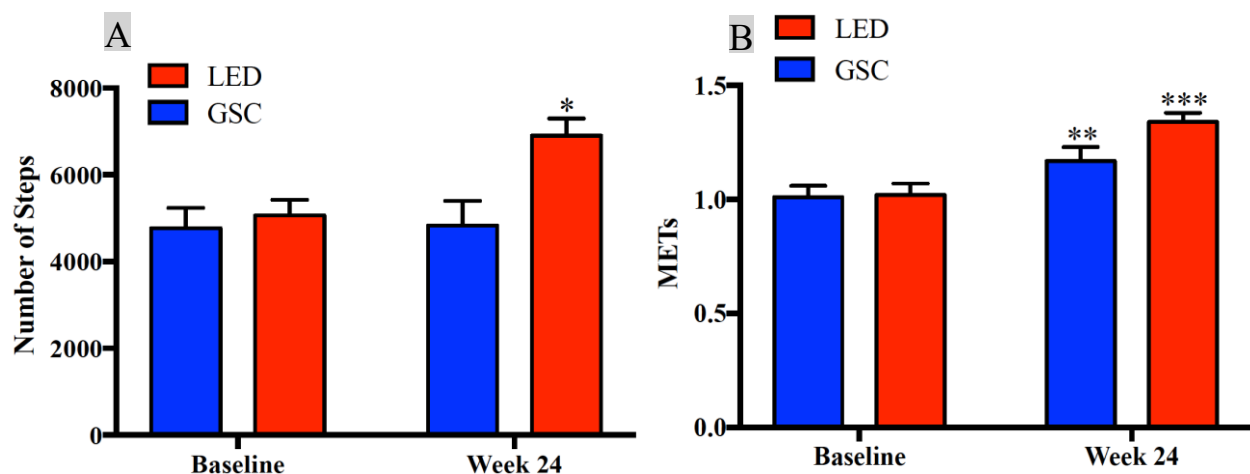


Figure 3. 28 Number of Steps and metabolic equivalents following LED and GSC at 24 weeks.

Data represents mean number of steps per day (A) and MET per day (B) plus standard error. P values calculated using adjusted mixed linear modelling * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Abbreviations: LED, low energy diet; GSC, gold standard clinical care; MET metabolic equivalents

3.4.8.2. Metabolic equivalents

There was a no difference in metabolic equivalents (MET) between groups at 24 weeks (0.13, 95% CI -0.04 to 0.30, $p=0.135$), despite this there was a significant increase in METs in both groups (GSC $p=0.017$, LED $p<0.0001$).

3.5. Discussion

3.5.1. Summary of findings

- Body weight loss was significantly greater at 24 weeks after using an LED compared to GSC in insulin treated T2D subjects.
- 70% of subjects on the LED achieved a 10kg weight loss at 24 weeks, with 47% losing over 15kg.
- Total insulin usage was significantly reduced in both groups, although there was a greater reduction in the LED group.
- Thirteen subjects in the LED group discontinued insulin over the 24-week period
- There was a significantly greater improvement in whole body insulin resistance with the LED intervention.
- Fasting glucose and insulin were significantly less at 24 weeks in the LED group compared to baseline and compared to the GSC group.
- Body composition was significantly improved in both groups with greater improvements in the LED group. Interestingly, fat free mass losses were minimal following the LED.
- This is the first time that an LED has been shown to reduce weight, insulin usage and glycaemic control compared to gold standard clinical care in obese T2D subjects treated with insulin.

3.5.2 Detailed Discussion

The aim of the present study was to examine the effect of a formula LED on weight loss, insulin usage and glycaemic control compared with GSC in obese patients with T2D concurrently treated with insulin. Data have previously shown that using a formula LED is an effective tool at inducing significant weight loss and improving glycaemia in recently diagnosed T2D patients (Lean et al., 2017). This had not been previously confirmed in those concurrently treated with insulin and compared with a control group within a pragmatic setting.

The results from this study are unique in that they start to answer the questions whether formula LED can be used within a clinical setting to treat obese T2D patients concurrently

treated within insulin. A summary of all the current strategies to aid weight loss, glycaemic control, insulin reduction and other metabolic outcome can be found in the Appendix 6.

Weight Loss

Data from this study shows that the use of an LED is an effective weight loss strategy compared to gold standard clinical care. The data show that a weight loss of -13.4kg and -14.0kg are achievable in obese T2D patients treated with insulin using an LED at 12 and 24 weeks. Using an LED resulted in significantly greater body weight loss compared with GSC at both 12 and 24 weeks, with an adjusted between group difference of -9.2kg and -8.3kg, respectively.

When compared with other studies within T2D patients, the weight losses at both 12 and 24 weeks are comparable (Rehackova et al., 2017). In a recent systematic review, at 6 months, in comparison to standard care, there was a -8.48kg difference between the groups, which is almost identical to our adjusted difference of 8.3kg. It should, however, be noted that there was significant heterogeneity amongst studies in the systematic review, and studies did not focus specifically on insulin treated patients.

In comparison with previous studies using traditional dietary treatments in T2D subjects concurrently using insulin, the weight loss presented here are significantly higher. In a large RCT comparing intensive lifestyle management (ILI) with diabetes education (DSE) in patients with T2D, 15.5% of whom were taking insulin, it was observed that even those on insulin therapy lost an average 7.6% (SD 7.0) of initial weight (Pi-Sunyer et al., 2007). Despite this impressive weight loss, those not on insulin lost more weight (8.6%) while also having greater remission of their diabetes (Pi-Sunyer et al., 2007, Gregg et al., 2012).

In the largest randomised control study to date comparing bariatric surgery with intensive medical management (IMM) in T2D patients, laparoscopic Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG) were found to be superior in achieving greater reductions in HbA1c, CVD risk, and medications usage, while also improving quality of life (Schauer et al., 2012, Schauer et al., 2014, Schauer et al., 2017b). With half the patients in the IMM being treated with insulin, this presents one of the only studies that have included patients concurrently treated with insulin. In direct comparison, the weight loss achieved

within the IMM at 12-months was -5.4kg (SD 8.0), with maximal weight loss being achieved at 9 months, suggesting that the weight loss achieved was significantly less than the LED arm of the current study population who achieved 14kg at 24-weeks. From the point of view of other outcomes, in comparison to the LED, improvements in glycaemic control (-1.4%), and inflammatory markers (C-reactive -2.2mg/L) were similar. However, the reductions in systolic blood pressure of (-1.3mmHg) and the effect on blood lipids, particularly HDL and LDL cholesterol, were significantly less than the LED group. This apparent difference in weight loss and particularly in the CVD risk outcomes may have been due to the focus of the IMM being on pharmacotherapy management and less focus on lifestyle and weight loss. Nevertheless, the results were similar to those from our GSC group. It is apparent that using an LED approach is more effective to the glucocentric approach in the IMM group in terms of weight loss, blood pressure & blood lipids.

Interestingly, weight loss was maintained from week 12 to week 24 within the LED group which is important in clinical practice, as often there is a weight rebound seen following acute weight loss. Firstly, this is possibly related to the significant reduction in insulin dose seen within both the LED and GSC group. In addition, this may have been due to the continued contact that the subjects received over this time. Data from Sumithran and colleagues (Sumithran et al., 2011) showed that in obese subject who lost weight with VLED for 10 weeks, loss of contact was associated with weight regain, with the majority regaining the weight lost during the energy restriction phase at one year follow-up. It is planned to assess the 12-month outcomes of the current study; this will allow a more direct comparison with previous studies.

Studies have shown that weight loss and maintenance in those with T2D is more challenging, particularly those treated with insulin (Guare et al., 1995, Looker et al., 2001, Pi-Sunyer et al., 2007). This is particularly evident within the Look AHEAD trial one year data, that showed that despite both being in the intensive arm, those on insulin lost approximately 1.1% less than insulin non-users ($p=0.002$) (Pi-Sunyer et al., 2007). Furthermore, it was highlighted that they were less likely to achieve 10% weight loss. Impressively compared to their control arm that lost 0.3%, ours lost 6.0%, showing, that given the right input, insulin treated patients are able to lose clinically significant weight.

When comparing the GSC with other similar weight loss interventions, the GSC results in either similar or better outcomes. In a systematic review of a calorie restricted diet (600kcal deficit) in T2D patients the mean pooled weight loss was -5.85kg at 12 months (Avenell et al., 2004). In another review, looking specifically at T2D patients, weight losses at 6 months with dietary changes similar to the GSC, a pooled weight loss of 4.5kg was reported, with the 12 month pooled means of all dietary intervention ranging from 2.4 to 8.5kg (Franz et al., 2015). It should be mentioned that insulin treated patients were not reported on; therefore, direct comparisons with the GSC intervention in the current study is difficult.

The behaviour techniques themselves appeared to have been of benefit with the fact that the GSC group lost -5.7kg (SD 4.7) at 24 weeks, equivalent to 6.0% weight loss, which is greater than the 4.5kg reported previously (Franz et al., 2015). There are several potential elements that may have played a role within this progress including the reduction insulin doses itself, motivation to lose weight, not wishing to see an increase in HbA1c or increase insulin dosage, although here, the key elements are believed to be related to the behavioural change elements within the intervention combined with a slower reintroduction of conventional food. The benefit of the behavioural element appears not just to be important within the GSC group, with a systematic review comparing VLED alone with VLED plus behaviour change showing a between group difference of -3.9kg (95% CI -6.7 to -1.1) with VLED, but a mean weight loss -10.3kg with when a behavioural programme was added to the VLED intervention (Parretti et al., 2016).

The list of behaviour skills used in the current study are summarised in Table 2.1. The use of self-monitoring of eating, activity and weight has been shown to benefit body weight loss maintenance both following traditional weight loss methods and with formula VLED and LED (Wing and Hill, 2001, Christensen et al., 2017a). In a recent study, reviewing psychosocial determinants for maintaining weight loss after an LED, it was identified that those who maintained body weight reported a structured meal pattern, no comfort eating, and less psychological stress compared to weight regainers (Christensen et al., 2017a). Also, Christensen et al. identified a concept they called ‘instrumentalization of eating behaviour’. This involved making eating behaviour an instrument to maintain weight loss and involved 3 elements, calorie counting, choosing healthy food options to aid weight maintenance, and self-monitoring. Interestingly, the identification that current psychological stress played an important role with weight maintenance (Christensen et al., 2017a) shows that helping

patients to manage these challenging times is an important part of any weight management intervention.

The above factors almost mirror those identified within the US National Weight Control Registry (NWCR) (Hill et al., 2005), which aimed to characterise those who succeeded in long-term weight maintenance. Those who maintained their weight loss reported eating breakfast almost every day, having a diet relatively low in dietary fat, using self-monitoring and engaging in regular physical activity. It should be highlighted though, the time expended in physical activity in NWCR participants was significant (approximately 60 minutes of moderate physical activity per day). Whether this level of physical activity is sustainable or depends on levels of macronutrient intake is unclear. All these elements played key roles within the behaviour techniques used within the current study and could in part explain the benefits seen with both weight loss and weight maintenance at 24 weeks.

Future qualitative work is necessary to study what lifestyle and behavioural factors were related to successful body weight loss and glycaemic outcomes in both the LED and GSC groups. This will allow further refinement of the intervention, focusing on approaches that are most successful.

Hunger is a key factor in weight control (Wing et al., 2008) and with a biased appetite regulatory system in favour of energy storage, particularly in the obese (Murphy and Bloom, 2004) and with the role of hedonic hunger (Lowe and Butryn, 2007), there is a need to help patients identify firstly 'true' hunger and secondly 'satiety'. One of the key techniques used within this study was the hunger scale, which aims to help individuals identify when they are physically satisfied, and to help control hedonic hunger and reduce to consumption of high calorie, palatable foods. This technique is used within clinical practice and has been shown to be helpful for patients to become more aware of their relationship around food (Brown et al., 2015).

It can be seen that if patients are left unsupported, with minimal input, following weight loss that they will regain weight, whether using traditional weight loss dietary methods or a formula VLED (Sumithran et al., 2011, Purcell et al., 2014). It is believed that gradual reintroduction of food helps with the weight maintenance seen in the long-term (Leeds, 2014, Brown, 2015). This was shown in a study in obese patients, having lost $\geq 10\%$ body weight,

that compared the refeeding phase duration of 1 or 6 weeks. This longer duration of food reintroduction resulted in a significantly less weight gain at 52 weeks (8.2kg vs. 3.9kg, respectively) (Gripeteg et al., 2010). Reasons for this included lower food intakes during the refeeding phase and the subjects being able to manage their eating behaviour. This was assessed through the Three Factor Eating Questionnaire that showed increased dietary restraint and less external eating, which may help to explain the weight maintenance seen within our patient group. Also, the fear of reintroducing conventional food and that this will result in weight regain, which was also expressed by the subjects in this study. Therefore, the use of a partial formula diet, enabled subjects to feel more in control of their food intake, and that they were able to reintroduce food without weight regain. This area needs further research and evaluation but is believed to be key in this process.

Finally, the role of physical activity has been highlighted as an important element in weight maintenance (Donnelly et al., 2009a, Swift et al., 2014). However, the quality of evidence demonstrating this is limited to observational and retrospective analysis. The actual volume of recommended physical activity of 150minutes/week of moderate activity has been shown to improve both blood glucose and CVD risk (Boule et al., 2001). This level of activity is difficult to achieve and maintain for many subjects. Increasing levels of activity may be difficult in individuals with longstanding T2D treated with insulin who have additional co-morbidities such as hypertension. These individuals will be at risk of hypoglycaemia, which may prevent them pursuing physical activity. Reductions in insulin dosage and insulin cessation may allow greater ability to undertake physical activity and help in weight loss maintenance in the long-term. This needs to be tested in future studies.

In terms of physical activity, and despite insulin dose reduction, there was a no difference in physical activity at 24 weeks between the two groups and there appeared a very minimal effect on overall measured activity at 24 weeks. Although there appeared to be a significant increase in metabolic equivalents, the clinical relevance is questionable. With 1 metabolic equivalent being equal to a person at rest, the increase of 0.3 in the LED group and 0.1 in the GSC per day, is unlikely to influence weight homeostasis. The addition of an extra 1000 plus steps within the LED group may be more relevant. Although there may not have been an influence on weight loss *per se*, there might be some metabolic benefits, particularly in older patients (Tudor-Locke et al., 2011a, Tudor-Locke et al., 2011b). Physical activity was not a focus of the current study, which may explain the lack of significant changes.

Based on current evidence approximately 7 hours/week of moderate activity is required for successful weight loss (Donnelly et al., 2009a). Clearly, this did not occur in the current study. Therefore, with the available data, it can therefore be hypothesised that the main driver of the significant weight loss in both groups was energy restriction rather than an increase in energy expenditure. It must be added that the result from this analysis may not be representative. Using the accelerometer as an assessment tool for physical activity during 7 random days represents only a snapshot and might not reflect the habitual changes to physical activity. It would therefore have been beneficial to assess physical activity through the use of an accelerometer worn throughout the study. This should be possible with the available technology and should be incorporated in clinical trials of weight loss.

The decision not to include a rescue plan for weight regain was intentional, as although there was a risk of weight regain and worsening glycaemia, this would better reflect the use of these dietary methods in usual clinical practice. In daily clinical practice, there would likely be an intensive period of contact followed by returning to usual practice. It is very clear from the Look AHEAD study data that attendance to appointments makes a significant impact on outcome (Wadden et al., 2011); therefore, aiming to make the intervention as pragmatic as possible was intentional, and enabled for a clearer distinction as to whether the LED itself was in fact beneficial. However, in a recent study that did use rescue plans it appears that these plans help maintain weight loss following the LED phase (Lean et al., 2017); in retrospect, this may have helped to maintain the benefits that we have seen at 24 weeks.

The direct impact of the above elements on weight maintenance is difficult to ascertain without formal assessment, which is difficult in a quantitative format. Data from the Dutch Eating Behaviour Questionnaire (van Strien et al., 1986) was collected from the subject, but will not be reported in this thesis. This should help to identify the role of emotional and external eating in successful weight loss and maintenance, and whether the LED impacted on these following the TDR phase.

Percentage Categories

Even more impressive was the percentage of subjects achieving a clinically significant degree of weight loss. In the LED group, 70% of subjects lost $\geq 10\text{kg}$ over 24 weeks and 46.7%

losing $\geq 15\text{kg}$, while in the GSC, 17.6% lost $\geq 10\text{kg}$ and only 5.9%, $\geq 15\text{kg}$. Within a recent study, that used an LED in T2D patients off all oral hypoglycaemic medications (OHA) the reported percentage of patients achieving 15kg weight loss was 24%, almost half that reported in our study (Lean et al., 2017). It will be of interest to see if the observed significant weight losses are maintained over 12 months. Also of note is the significant weight reduction seen within the GSC group, which is typically not seen within control groups in other studies, (-1.0kg) (Lean et al., 2017). This data was not expected and demonstrates in part that the right combination of dietary advice, behaviour change, and physical activity used within this study were highly effective. It is obviously difficult to identify the exact mechanism by which this occurred and further analysis of the data is warranted.

These impressive weight losses show that even within patients with advanced T2D and concurrently treated within insulin, clinically significant weight loss is possible with an LED.

Predicted Weight Loss

The weight loss during the total diet replacement phase (TDR) of the LED was equivalent to 1.1kg per week. With the weight loss estimates per week ranging from 1.2-2.5kg within the literature (Saris, 2001, Lean et al., 2017), this might suggest that weight loss within this patient group was slower than expected. In fact, in obese T2D patients who had their insulin stopped at the start of the formula VLED, weight losses were 1.48kg per week over a 16-week period was observed (Snel et al., 2012a), suggesting that the weight loss seen may have been affected by concurrent insulin treatment. Although this is true, as mentioned, traditionally obese T2D patients concurrently treated with insulin have found it more difficult to lose weight; therefore, the results from this study are encouraging.

Weight loss trajectories were further explored by using the use of predictive weight loss equations (Hall and Jordan, 2008). Within nutrition research, one of the problems is the adherence of patients to the prescribed dietary plan and whether in a free-living environment they continue to follow this. Unfortunately, this is rarely seen and therefore the use of predictive weight loss enables clinicians and researchers to see if patients are doing what they say they are. As can be seen from Figure 3.19, there was a significant discrepancy between the weight loss that was predicted and that achieved; at all time points, the actual weight loss

was significantly less than that predicted, and greater in the LED group. In a review looking at this apparent disparity, Heymsfield and colleagues (Heymsfield et al., 2007) observed the same outcome as our study, in that there was a significant disparity between predicted and actual weight loss, with individuals achieving <25% that of the predicted value. Several mechanisms might be to explain discrepancies between actual and predicted weight loss. Firstly, it could be that subjects simply did not adhere to diet as instructed. Dietary intakes were assessed using a 3-day food diary and *ad libitum* test meal. The use of self-reported food intake, particularly in the obese population has its limitations particularly regarding underreporting (Johnson, 2002). With studies showing obese subjects, underreporting energy intake by between 30-70% compared with lean subjects (Prentice et al., 1986, Lichtman et al., 1992). In the current study, there also appeared to be substantial underreporting. At baseline, when the difference between measured total energy expenditure and self-reported energy intake were analysed, on average, subjects were underreporting energy intakes by between 568 to 647kcal per day, equivalent to between 33-43%. This amount of energy deficit would have encouraged weight loss in the majority of patients at the commencement of the study. However, many subjects reported weight stabilisation or slight weight gain at the start of the study, suggesting that substantive underreporting of food intake was happening. Despite predictive weight loss indicating that there appears to be significant non-adherence, particularly in the food reintroduction phase, the reliability of these results can be compared with previous studies in obese T2D patients. Although a direct comparison is not possible due to heterogeneity in study design, it is seen that obese T2D patients on diet therapy alone lose approximately 14.5kg in the TDR phase and 13.0kg at 20 weeks (Lean et al., 2017), demonstrating that this is a problem within this type of research.

Other mechanisms might have been the increased risk of hypoglycaemia, hypothalamic insulin resistance effecting appetite regulation, the anabolic effect of insulin or the influence of exogenous insulin administration (Figure 1.1) (Brown et al. 2017). One key hypothesis is compensatory eating due to hypoglycaemic episode resulting in the LED group and GSC group breaking the diet. However, with lower hypoglycaemic episodes in the LED group compared to the GSC group per person over the 12-week period (3.4 vs. 5.9, respectively) counters this, as the GSC lost less weight. Furthermore, there was no correlation between weight loss and hypoglycaemic episodes at 24 weeks with a weak Pearson correlation coefficient of $r=0.153$ ($p=0.395$). Data to support this within T2D insulin treated patients is lacking, but data from the Diabetes Control and Complication Trial (DCCT) of those with

type 1 diabetes shows clearly that non-severe hypoglycaemia is associated with weight gain (Bumbu et al., 2018).

Body Composition

The alteration in body composition is of particular interest here, due to the LED group losing more weight, the absolute reductions in fat and fat free mass are significantly greater than those in the GSC and would seem to infer that there was a detrimental effect on body composition; however, this does not paint the entire picture as will be discussed.

From previous work, the degree of fat-free mass (FFM) loss following VELD and LED varied between 14-30% of weight loss depending on exercise (Webster et al., 1984, Chaston et al., 2007), with the degree of FFM loss increasing with the degree of energy deficit (Chaston et al., 2007). In a recent study assessing the use of VLED prior to bariatric surgery in those with a higher BMI (46kg/m², SD 4.4) found elevated FFM losses of 41.6% (Nielsen et al., 2016), suggesting that caution should be taken in individuals with an initial body weight of greater than 135kg. As the average weight of the subjects in the LED group was 104.6kg (SD 21.2), this might explain the apparent disparity in the data where excessive FFM loss was not seen. Furthermore, in the only subject with a weight over 135kg at baseline, FFM loss was not elevated at either 12 week or 24-weeks with percentage FFM losses of 26.5% and 28.8% of weight loss. That said, there might be benefit from monitoring FFM in those individuals with higher baseline body weights, although further research is required.

In addition to this, between weeks 12 and 24, FFM continued to increase in the LED group, while in the GSC group, there was a continued reduction. This means that when presented as a percentage of the total weight lost at 24 weeks, the LED group lost less FFM than the GSC group (21% vs. 25.7%). This might be explained by several factors. One explanation is that the GSC lost more weight over this period, which could have resulted in a catabolic state and reduction in FFM, while the LED group had relative weight stabilisation. Another explanation is the high protein content of the formula diet in the partial meal replacement phase, which may have supported maintenance of FFM (Piatti et al., 1994).

Physical activity, in addition to the use of LED, has consistently been shown to reduce FFM loss (Janssen and Ross, 1999, Rice et al., 1999, Janssen et al., 2002), with exercise

programmes achieving <22% of percentage FFM loss (Chaston et al., 2007) which would be comparable to the losses seen within the LED group. Interestingly, women were found to have less FFM loss than men, although this may have in part been due to men losing more overall weight, which is consistently seen within the literature. Thus, the improved FFM loss seen within the LED group may well have been due to them following the physical activity advice in the methods section 2.3.1.4 (Haskell et al., 2007, Donnelly et al., 2009a, O'Donovan et al., 2010, NICE, 2014b, NICE, 2015). Indeed, subjects reported undertaking resistance exercise during the study period, in combination with the relative increase in dietary protein in the LED group from 12 to 24 weeks (53.1kg to 72.8g); there may therefore have been muscle preservation. The accelerometer data, however, suggests that only relatively small changes in activity occurred. Therefore, the protein content of the formula diet as well as greater levels of micronutrients is the most likely explanation. What is clear is that no matter how people lose weight, there is always going to be a loss of FFM and therefore it is important to minimise this loss.

Glycated Haemoglobin

Following the LED, there was a significant overall improvement in glycaemic control compared to GSC. With significant differences found with HbA1c, fasting glucose, fasting insulin, HOMA-IR, QUICKI and Matsuda index all on the background of a reduction of insulin usage of 74%, demonstrating the robust impact the LED intervention had on overall glycaemic control.

An HbA1c reduction of 1.4% or 15.7mmol/mol represents both an impressive and clinically significant reduction in glycaemic control. This is comparable to many of the currently available diabetes medications (Chaudhury et al., 2017). Data from the UKPDS demonstrated that for every 1% reduction in HbA1c there was an associated relative risk reduction of for microvascular complications (37%), diabetes related death (21%) and myocardial infarction (14%) (UKPDS Study Group, 1998, Stratton et al., 2000). Therefore, if the observed improvement with LED is maintained, this can translate to significant reductions in diabetes related complications. In a systematic review comparing VLED alone and the addition of behaviour change reported a mean change of 1.23% (-3.4 to 1.0) and suggested that the intervention effect was not consistent throughout studies. When looking at the mean HbA1c

changes these were between -1.2 to 1.27%, with fasting blood glucose reducing by between -0.22 to -2.54 (Parretti et al., 2016).

To date, bariatric surgery remains the most successful treatment for both weight reduction and glycaemic control in those with T2D (Leong, 2012) and impressively the reductions in HbA1c are similar to those seen with bariatric surgery (Brown et al., 2017). In studies using VLED, significant reductions in HbA1C have also been seen, in advanced patient with T2D which have ranged from 0.3 to 1.5%; this variability is due to differing methodologies and study periods, although in one study that reviewed patients at 18 months, HbA1c had returned to baseline (Jazet et al., 2007, Hammer et al., 2008, Snel et al., 2011, Snel et al., 2012a, Snel et al., 2012b).

With intensification of treatment comes an increased risk of hypoglycaemia, which has been suggested to impact on mortality risk (Currie et al., 2010). However, in this study, the improvement was not related to pharmacotherapy intensification and rather weight loss with an associated lower number of hypoglycaemic episodes. Furthermore, it has previously been highlighted that both high and low HbA1c levels may influence risk of all-cause mortality (Currie et al., 2010), and that ideally HbA1c should aim to be between 6-8%, with the lowest mortality being associated with an HbA1c of approximately 7.5% (Currie et al., 2010, Colayco et al., 2011). At both 12 and 24 weeks the subject in the LED group met this target.

Insulin usage

Over the 24-week study, there was a sustained reduction in insulin usage in both the LED and GSC groups. There was a greater reduction in the LED group with a between group adjusted difference of -21.5unit and -15.1units at 12 and 24 weeks, respectively. It is currently very difficult to compare the findings with previous studies as a very limited number of studies have used VLED exclusively in insulin treated patients (Paisey et al., 2002, Dhindsa et al., 2003) and so often the results are confounded. In one of the only studies that reported data on insulin doses reductions at one year (Dhindsa et al., 2003) there was a reduction of 61units/day, which was comparable to the 51units reported in our study. In those ceasing all insulin at the start of TDR, 10 subjects needed to restart insulin therapy, showing that the practice of ceasing all medication is not appropriate for all (Dhindsa et al., 2003).

Over the period of food reintroduction, there was a small increase in the insulin usage within LED group. This likely represented returning to a more traditional diet and having greater intake of carbohydrate than during the TDR phase. This was, however, not reflected in the food diaries, which suggested that there was a continued reduction in carbohydrate intake of approximately 24g; this is however potentially biased by misreporting and underreporting.

Insulin treatment cessation

Weight loss using intensive lifestyle interventions have been shown to dramatically reduce insulin doses (Reaven, 1985). Thirteen subjects at the end of 24 weeks had come off insulin completely, with an average pre-insulin usage of 45.6units (SD 6.5); this represents a significant impact on not just the patient but also the healthcare system. In studies in advanced T2D with residual pancreatic function allowing them to stop insulin at the start of the VLED, despite significant weight loss there appeared to be a gradual decline over time of glycaemic control and slow reintroduction of insulin at the end of the study (Dhindsa et al., 2003, Snel et al., 2011).

Similar to diabetes remission, the cessation of insulin therapy has been observed to be greater in those treated with bariatric surgery compared with intensive lifestyle management (Schauer et al., 2017b) with long term cessation being reported (Jimenez et al., 2012). Although this may be true, insulin cessation varies between surgical procedures, with greater numbers ceasing IT at 1 year in RYGB (62%) compared with laparoscopic adjustable gastric band (LAGB) (Ardestani et al., 2015). Independent predictors following bariatric surgery of insulin therapy cessation were age, preoperative BMI, not taking basal bolus IT, bariatric procedure and percentage excess body weight loss (%EBWL) (Ardestani et al., 2015, Ghio, 2017).

This was reflected in the data from this study. When simple analysis was undertaken in those who stopped insulin had a lower insulin dose at baseline and 24 weeks ($p=0.002$; $p<0.0001$, respectively), lower HbA1c at baseline and week 24 ($p=0.02$, $p=0.004$), greater weight loss ($p=0.002$), had greater reduction in waist circumference ($p=0.03$) and lower total body insulin resistance ($p=0.04$).

Possible remission

Remission rates have been reported to be as high as 58-95%, particularly following biliopancreatic diversion (BPD) or Roux-en-Y gastric bypass (RYGB) (Buchwald et al., 2009). In spite of high remission rates being reported in those T2D patients not treated with insulin, the relative rates of remission in those patients on IT are lower with figures ranging from 19.3 to 72.2% (Jimenez et al., 2012, Ardestani et al., 2015, Ghio, 2017).

No patients achieved complete remission of their T2D at 24-weeks, based on ADA definitions, precluding that patients need to have an HbA1c of $\leq 6.5\%$ (48mmol/mol) and off all diabetes medication for at least 12-months (Buse et al., 2009). At 24-weeks there were 2 patient who were off all medications and had an HbA1c less than 6.5% (48mmol/mol), both of whom had been on the LED. In the only study to have its primary outcome as diabetes remission, 46% of T2D patients achieved this at 12 months; also, of interest was the association between remission and degree of weight loss. (Lean et al., 2017). This is an intriguing possibility that there may be obese T2D patients treated with insulin who may have the opportunity to be defined as not having T2D after weight loss despite long duration of diabetes.

Furthermore, at least for some patients although β -cell function continues to decline over time (UKPDS Study Group, 1998) it may be that this can be slowed even in those with longstanding T2D, necessitating insulin therapy. It will be interesting at the end of the study (12 months) to see if any of these patients have managed to achieve actual remission at this time.

Fasting Glucose and Insulin

Fasting glucose is largely determined by the relative concentrations of insulin and glucagon in the fasted state (Roden et al., 1996). With fasting insulin being a key regulator of glucose homeostasis. Fasting plasma glucose levels are determined by the combination of β -cell function and the presence of insulin resistance, where T2D occurs in the presence of insulin resistance and corresponding deficiency in β -cell function to counter the IR.

The ability to quantify insulin sensitivity is important to understand the pathophysiology of T2D. Initially it is important to look at fasting insulin and glucose levels. Fasting plasma glucose improved significantly following the LED after both the TDR and food reintroduction phases; this, however, was not seen in the GSC group. Blood glucose reduced to 7.1mmol/L (SD 2.6) at 24 weeks. This corresponded with a significant reduction in fasting insulin at both 12 and 24 weeks following the LED, with a adjusted between group difference of -77pmol/L (95% CI -149.1 to -5.0, $p=0.04$) and -79pmol/L (95% CI -153.1 to -4.4 $p=0.04$) respectively. In a series of studies by the same research group on advanced obese T2D patients, there appears to be a similar reduction in both fasting plasma glucose and insulin, of between 1.5-4.7mmol/l for glucose and by 174pmol/L for fasting insulin (Jazet et al., 2007, Hammer et al., 2008, Snel et al., 2011, Snel et al., 2012a, Snel et al., 2012b).

Energy restriction lowers plasma glucose and insulin concentrations and hepatic glucose production, thereby improving hepatic insulin resistance (Lim et al., 2011). Taken alone, this would suggest that there was an improvement in hepatic and possibly peripheral insulin sensitivity, resulting in better disposal of plasma glucose in the fasted state, an associated reduction in endogenous glucose production, and resulting in a lower insulin secretion.

The measurement of fasting glucose and insulin provides important although limited information regarding β -cell function, as they only represent the fasted state and do not reflect the ability of the β -cell to respond to a glucose stimulus (Cobelli et al., 2007). Therefore, using a mixed meal tolerance tests enables a more dynamic view of insulin and glucose response to a meal. Furthermore, a mixed meal allows the assessment of the effect of the incretin response (Elrick et al., 1964, Triplitt and Chiquette, 2006).

This meal tolerance data showed that following LED there was a greater postprandial ability to effectively manage the glucose load, particularly after the first 60 minutes, where all glucose readings were significantly lower. This was also seen by a significantly lower total glucose AUC (Figure 3.13).

This improvement was not, however reflected, in insulin secretory response when looking at the time course data (Figure 3.14b). Despite an initially lower fasting insulin level, the acute response to the glucose stimulus produced an almost identical increase in insulin and a lower

associated peak. There was, however, a trend after 60 minutes of lower insulin concentrations, corresponding with the lower glucose concentrations.

The reduction in insulin AUC might suggest that with the improved insulin sensitivity resulting in better glucose disposal leading to an overall lower release of insulin over the test period.

From this data, it would appear that the changes are associated with improved insulin sensitivity, decreased fasting insulin resistance but not an associated improvement in beta-cell output. This would suggest that the changes seen are possibly hepatic related and not due to increased insulin secretion. In studies that have directly measured the effect of a VLED using hyperinsulinemic-euglycemic clamps, it has been observed after VLED that plasma glucose, insulin and HbA1c all improve, alongside an increased clearance of insulin during the clamp, a significant reduction in endogenous glucose production, improved insulin sensitivity and improved suppression of lipolysis (Jazet et al., 2008, Snel et al., 2012a). Also, there was increased insulin receptor expression after the diet in skeletal muscle. Altogether, this confirms that diet induced weight loss improves glycaemic control through ameliorating both hepatic and peripheral insulin resistance (Jazet et al., 2008, Snel et al., 2012a).

All three of the measures for insulin resistance, the Homeostatic model assessment (HOMA-IR), Quantitative insulin sensitivity check index (QUICKI) and Matsuda Index showed that whole body insulin resistance was significantly improved after the LED. This improvement in HOMA-IR (-13.5) following LED in this study has also been seen in insulin resistant patients, with reductions of 9.6-10.7 being reported and associated with similar reduction in other glycaemic parameters (Jazet et al., 2005c, Snel et al., 2011).

In attempting to assess if there was a change in first phase insulin response and secretion, the insulinogenic index (IGI) and oral disposition index (DI_o) were calculated. However, as expected from the mixed meal tolerance test (MMTT) data, there was no change in first phase insulin response or oral disposition index (DI_o) suggesting that insulin secretion was not affected, particularly in the first phase.

This lack of change seen in the IGI and DI_o may be been due to the advanced stages of diabetes within this subject population. The data from the UKPDS (UKPDS Study Group,

1998) demonstrated that even before diagnosis β -cell decline had already occurred, with a reduction of 50% by the time of diagnosis which is gradually reduced further over time. The effect of β -cell decline can be seen within bariatric surgery data, in which those T2D patients treated within insulin having a significantly lower rates of diabetes remission and likelihood of relapse (Ardestani et al., 2015, Jimenez et al., 2012, Ghio, 2017). Even with bariatric surgery there is still a significant relapse of T2D over a 5 year period with reports of this occurring in between 41.7-50% of patients (Schauer et al., 2017b).

This would suggest that despite the ability for a LED to improve IR in advanced T2D that for the majority this will not result in returning of pancreatic function.

Mechanisms of Glucose Homeostasis

The mechanisms by which the LED brings about the significant improvements in glycaemic control are difficult to allude to with the data currently available for this thesis; however, future work will hopefully gain insight into some of this.

From the available literature that has previously used VLED in obese patients with T2D there are some possible suggested pathways. In human studies a low energy diet has been shown to cause reversal of fasting hyperglycaemia, reduced rates of hepatic glucose output and improved hepatic insulin sensitivity (Petersen et al., 2005), where the authors associated this improvement with a resolution in fatty liver.

The prevailing theory at the present time is that with significant weight loss following a VLED or LED that there is a reduction of pancreatic and hepatic ectopic fat and intramyocellular lipids, resulting in resolution of hepatic insulin resistance and increased first phase insulin response (Lim et al., 2011). It is now recognised that it is not the IMCL *per se* rather the increase in the derivatives such as diacylglycerol (DAG) and Long-Chain fatty acid Co-A which causes the IRS-1 to be unable to activate PI-3K through an increased activation of protein kinase C (Jazet et al., 2005a, Morino et al., 2006)

Focusing specifically at the liver, recent rodent data have shown some possible pathways to how VLED may improve glycaemic control in the short term (Perry et al., 2018). Of interest was that with the reduction in hepatic triglycerides (TG) and hepatic diacylglycerol (DAG)

there was a 2.5-fold increase in insulin-mediated suppression of hepatic glucose production (HGP) and an increase in Akt2 phosphorylation. In addition, using new techniques, allowed the measurement of HGP in awake rats, showing that a VLED reduces both net hepatic glycogenolysis and pyruvate kinase flux, resulting in reduced HGP. Furthermore, VLED helped to reduce hepatic acetyl-CoA content through reduction in intra-hepatic lipolysis and decreased β -oxidation, resulting in reduced hepatic gluconeogenesis.

From the current data, we might be able to suggest that the improved IR, as evident from the glucose data and improvement in the insulin indices, combined with a significant reduction in fat and waist circumference, is driven by a reduction in liver fat.

Glucose homeostasis is complex and involves multiple hormones apart from insulin. It is important to examine other hormones in particular glucagon which mobilises hepatic glucose. Other key meal responsive hormones include the incretin hormones, particularly glucagon-like peptide-1 (GLP-1). In addition to stimulating insulin secretion, GLP-1 has been shown to exert a variety of other metabolic actions including promotion of satiety and reduced food intake, slowing of gastric emptying, protection of β -cell apoptosis and suppression and glucagon release (Meier, 2012). Previous studies have suggested a diet-induced weight loss reduction in GLP-1 concentrations (Sumithran et al., 2011), which might preclude its role within the improvements seen in glucose homeostasis; however further analysis of the data is required to see if this is true. Future work should examine alterations in key hormones regulating glucose homeostasis in response to the LED intervention.

Energy intake and appetite

Appetite can be assessed using both objective measures such as the *ad libitum* test meal and proxy measures such as Visual Analogue Scales (VAS). While in this study there was no effect on *ad libitum* energy intake following either the LED or GSC, there was an effect on both VAS AUC₀₋₂₁₀ and time course data following the LED.

The data presented is of particular significance as it challenges the commonly held belief that subjective appetite and hunger increase significantly after weight loss (Sumithran et al., 2011).

Ad Libitum Meal

Satiation was measured through the measurement of *ad libitum* test meal food consumption under experimental conditions. There was no difference in the percentage change in energy intake following the *ad libitum* test meal, with an adjusted difference of -5.9% (95% CI -25.1 to 13.3, $p=0.547$, Figure 3.21). This lack of difference in energy intake appears to be reflected in the data from the VAS.

The use of self-reported VAS to assess hunger, fullness, satiety and desire to eat has been highly recommended as it aligns in time with corresponding physiological processes of food intake. Hunger, appetite and desire to eat, however, are complex and multifaceted and are difficult to assess outside of controlled experimental design (Blundell et al., 2010). While findings from VAS may not reflect behaviour outside the controlled setting, it provides a surrogate estimate.

The key finding from the VAS measures employed is that subjects following a LED were significantly fuller after the TDR phase, despite having lost 13.4kg (Figure 3.24). There was a between group difference on 10.9mm/min over the course of the MMTT, while within from 30 to 60 minutes there was a significantly greater fullness. Although there were no other between group differences found, there were trends towards significance within the composite appetite score, hunger and desire to eat, seen in both the AUC_{0-210} and time course data.

Unfortunately for this thesis we were not able to analyse the effect of the LED on gut appetite hormones that would have helped to identify a mechanistic action to the apparent subjective reduction in appetite scores. In place of not having this, previous data from the literature can be reviewed to examine potential mechanisms. The effect of hunger and compensatory eating after weight loss is often one of the key reasons that patients struggle with weight maintenance and high rates of attrition (Gilbert et al., 2009, Gibson et al., 2015). The compensatory changes that occur following diet-induced weight loss include reduction of energy expenditure, increased in orexigenic hormone ghrelin, reductions in anorexigenic hormones including CCK, GLP-1, PYY and circulating leptin (Leibel and Hirsch, 1984, Leibel et al., 1995, Geldszus et al., 1996, Cummings et al., 2002, Chearskul et al., 2008); all designed to encourage weight regain and increased appetite.

Sumithran and colleagues gave participants to a 10-week VLED (Sumithran et al., 2011). After this, they followed the subjects one year later. It was observed that levels of hunger were increased which was associated with higher ghrelin and lower PYY levels. They concluded that these hormonal changes are responsible for unsuccessful weight loss maintenance. The observations using LED in this study counters these previous findings. The key difference appears that food was gradually introduced with addition support, while no further input was provided in the Sumithran study after the initial weight loss. This suggests that gradual food reintroduction is likely to be important in weight loss maintenance following a LED.

One of the key feature of VLED and low carbohydrate ketogenic diets is the apparent suppression of appetite reported by patients despite the highly restricted energy intake (Astrup et al., 2004). One of the key hypotheses is that ketosis is responsible for this apparent reduction in hunger (Howard, 1989, Astrup et al., 2004). During this study fasting urinary ketone were assessed to see if dietary adherence was occurring, as previously it had been suggested that urinary ketones might be an indicator of dietary adherence. Unlike previous studies, urinary ketones were found to be negative, even during the TDR phase. The explanation for this is due to the level of dietary carbohydrate restriction being insufficient (120g/day) to induce enough production of ketone bodies, particularly acetone to be excreted from the urine. This does not mean that patients were not in ketosis and future research will aim to assess both urine and plasma levels of β HB to see if our data is in support of others.

Quality of Life

Patients with T2D have a poorer quality of life (QoL) than those with no chronic disease (Rubin and Peyrot, 1999), with the addition of obesity and insulin therapy, both of which have also been to influence QoL (Kolotkin et al., 2001, Pouwer and Hermanns, 2009). Studies directly assessing insulin therapy on QoL have found that regime complexity, fear of injections, number of daily injections, weight gain, and social worries contribute to the lower QoL (Bretzel et al., 2008, Pouwer and Hermanns, 2009, Peyrot et al., 2010, Brown et al., 2017, Russell-Jones et al., 2018). Furthermore, insulin treatment is associated with an adverse impact on medication adherence (Doggrell and Chan, 2014) and reduced quality of life (Snel et al., 2012d).

QoL as assessed by the VAS from the EQ-5D was significantly improved following the use of the LED with a consistent increase from 68.7 at baseline to 82.3 at 24 weeks (adjusted difference 11.9, 4.1 to 19.7, $p=0.003$). This represents a clinically significant improvement in self-reported QoL. No change was seen within the GSC group.

In line with current findings, two previous studies using formula VLED and LED have highlighted an improvement in QoL within both recently diagnosed patients and those with advanced T2D who before the study had been treated with insulin (Snel et al., 2012d, Lean et al., 2017). Indeed, QoL improved more within our patient population more than a recent study in recently diagnosed T2D, using the same EQ-5D. Lean and Colleagues showed an improvement of 7.2 points (SD 21.3) compared with their control who had a decrease in QoL by 2.9 points (SD 15.5) (Lean et al., 2017). In another study factors related to QoL improvement included weight loss, discontinuation of insulin therapy and OHA, and reduced side effects (Snel et al., 2012d). Interestingly, although within the short-term exercise did not influence QoL its role with maintaining weight loss and glucose control (Donnelly et al., 2009a) suggests that it has a role to play.

Side effects

In general, the diet was well tolerated by the majority of the subjects and the observed side effects mirrored those seen in similar studies (Table 3.6) (Christensen et al., 2011, Lean et al., 2017).

Adherence to the study

As mentioned previously, predicted weight loss was used to assess if subjects were adhering to the diet. The equation used by Hall (Hall and Jordan, 2008) is superior to the traditional assumption that an energy deficit of 3500kcal equates to a body weight loss of 1 pound, as it includes body composition and energy expenditure change over time. The authors suggested that if predicted weight losses are not achieved, this is related to either non-adherence or reduced energy expenditure. This might in part explain why there was a difference, and that lack of dietary adherence may not have been the only cause.

Self-reported adherence for the study was good, although no formal assessment was completed, as with any nutritional research, without subjects being admitted into a hospital setting, it is difficult to assess if they are following advice.

One key feature that appeared to be reinforced by subjects was the fact that they had ‘tried everything’ before joining the study. Often subjects had heard of the diet from the media and were interested if they could stop taking insulin. This, like diabetes remission, is a very powerful motivator for patients who are often injecting insulin multiple times a day and felt that this significantly affected their quality of life.

Although no formal assessment was completed of the behaviour change programme that helped subjects to make lifestyle changes as high quality validated tool are sparse. In the future, qualitative assessment of these patients would help to yield how some were successful at adhering to the dietary changes and other were not.

In two recent studies assessing the patient’s views of formula VLED and LED, there has been evidence on how best to help patients through this journey of change. One study has already been mentioned in which instrumentalization of eating behaviour was seen to be the key, alongside a structured meal pattern, no comfort eating and less psychological stress (Christensen et al., 2017a). The second is a qualitative study of subjects from the initial Counterbalance study (Rehackova et al., 2017), before and after an 8-week VLED which highlighted behaviour-regulation strategies that aided adherence. The key factors were removing food from the environment, planning ahead, avoidance of tempting situations or places and self-distraction techniques. In addition, social support, the rapid changes seen with the VLED in terms of weight loss and blood glucose and the simplicity of the formula product also appeared to be important (Rehackova et al., 2017).

Chapter 4: The effect of a LED on short-term surrogate markers of complications associated with T2D and obesity in obese T2D patients treated with insulin compared with gold standard clinical care

4.1. Introduction

In a recent report from the office of National Statistics cardiovascular diseases remained the leading cause of premature death in England and Scotland, accounting for 42% and 45% of all deaths, respectively (Statistics, 2018). With both T2D and obesity having been shown to increase the risk of cardiovascular disease (CVD) (Kannel and McGee, 1979, Hubert et al., 1983, Haffner and Cassells, 2003, Pi-Sunyer, 2009, Diabetes UK, 2012) it is essential that effective treatments aiming to stem these CVD risks are developed.

Intensive glycaemic control using insulin intensification was traditionally thought to be beneficial for both microvascular and macrovascular complications associated with T2D (UKPDS Study Group, 1998, Stratton et al., 2000). However, evidence now suggests that there is not benefit to intensive glycaemic control with respect to all-cause and CVD mortality (Gerstein et al., 2008, Hemmingsen et al., 2011, Hemmingsen et al., 2013). In those with T2D, there appears to be a U-shaped relationship with HbA1c and mortality, in which an HbA1c less than 6% or greater than 8% results in greater mortality, with an HbA1c of approximately 7.5% being associated with lowest all cause-mortality (Currie et al., 2010, Colayco et al., 2011).

As HbA1c increases there is also a progressive increase in the risk of CHD, CVD and mortality, with those on insulin treatment presenting with the highest risk (Eeg-Olofsson et al., 2010). With intensification of insulin therapy, episodes of hypoglycaemia increase, which has in part, been suggested to contribute to the increased risk of mortality seen in insulin-treated patients (Currie et al., 2010). Indeed, data have suggested that there is a dose-response relationship between insulin and risk of death in subject with T2D (Gamble et al., 2010, Holden SE, 2013), with each one unit increase of insulin being associated with an increased hazard ratio of all-cause mortality of 1.54 (Holden et al., 2015).

Insulin therapy has been associated with weight gain, with studies suggesting that gains of between 3 to 9kg within the first year (UKPDS Study Group, 1998, Larger et al., 2001, Brown et al., 2017). The majority of this weight gain has been suggested to be fat, particularly visceral fat (Sinha et al., 1996, Rigalleau et al., 1999) which has been linked to CVD and insulin resistance (IR) (Turkoglu et al., 2003, Yusuf et al., 2004). Interestingly, however, the relative impact of insulin associated weight gain (IAWG) on CVD risk remains

unclear. Though IAWG has been shown to negatively affect surrogate markers of CVD risk, lipid profile, increased systolic blood pressure and renal glomerular hyperfiltration (Tomaszewski et al., 2007, Jansen et al., 2010), there has been no associated observation of increased CVD mortality (Aas et al., 2009), which may be in part due to the improved glycaemia seen following insulin initiation.

Obstructive sleep apnoea (OSA) and diabetes-related kidney disease (DKD) are two commonly coexisting conditions with T2D and are both linked with increased CVD risk and mortality (Valmadrid et al., 2000, So et al., 2006, Somers et al., 2008, Marshall et al., 2008). OSA is associated with multiple intermittent hypoxic episodes secondary to partial or complete obstruction of the airway during sleep (McNicholas, 2008). OSA is associated with hypertension and increased risk of arrhythmias and is closely linked with T2D and obesity (Shepard, 1992). DKD is the biggest cause of end stage renal failure (Ritz et al., 1999, Gross et al., 2005, Tuttle et al., 2014), with T2D, obesity and hypertension being key to its aetiology.

Management of weight in obese patients with T2D has been highlighted as the primary nutritional management strategy (Diabetes UK, 2018) with 5-10% of body weight loss being shown to improve HbA1c, blood lipids, blood pressure, insulin sensitivity and CVD risk (Wing et al., 2011, Franz et al., 2015). Larger weight losses of 10-15% are however more beneficial, not just from a CVD risk point of view but also maintenance (Wing et al., 2011, Wadden et al., 2011). Despite this, there remain very few dietary treatments currently available that are able to consistently produce this degree of weight loss in obese patients and particularly in those obese T2D patients treated with insulin.

The use of formula very low energy diets (VLED) has been shown to improve cardiac function and decrease pericardial fat, and has been suggested, in two separate systematic reviews, to improve CVD risk and its associated surrogate markers (Hammer et al., 2008, Mulholland et al., 2012, Snel et al., 2012b, Parretti et al., 2016). However, with all these studies in patients not concurrently treated with insulin, the generalisability of the result to patients with more advanced diabetes or those treated with insulin is uncertain. Furthermore, there is limited evidence into the use of formula LED within patients with DKD and OSA (Johansson et al., 2009, Johansson et al., 2011, Rolland et al., 2013, Brown and Taheri, 2018) and none within obese T2D patients treated within insulin.

4.2. Hypothesis

In obese type 2 diabetes patients treated with insulin, a 24-week dietary intervention with a low energy diet (LED) will result in greater weight reduction, leading to short-term beneficial effects on surrogate markers of complications of T2D and obesity when compared to gold standard clinical care (GSC).

4.2.1. Aims

This investigation was a randomised control trial aiming to examine the effect of a low energy formula diet on short-term surrogate markers of complications associated with T2D and obesity compared to gold standard clinical care in obese type 2 diabetes patients treated with insulin.

4.2.2. Outcomes

The primary outcome of this part study was the effect of body weight loss between the LED and GSC groups on the following CVD surrogate markers

- Serum lipid profile;
- Serum liver prolif;
- blood pressure and kidney function;
- sleep quality and obstructive sleep apnoea;
- metabolic syndrome, and CVD risk.

4.3. Methods

The methodology used within this investigation is summarised in detail within Chapter 2 regarding recruitment, study design, intervention details and outcomes measured.

4.3.1. Recruitment data

The recruitment data were identical to that reported in Chapter 3. Please refer the results section of Chapter 3 for details on the recruitment, attrition and flow diagram.

4.4. Results

The baseline characteristics of the subjects are summarised in Table 3.1.

4.4.1. Lipid Profile

At baseline, blood lipid profiles were poorly controlled in reference to suggested diabetes lipid levels (ADA, 2018) within both groups as seen in Table 4.1.

4.4.1.1. Total cholesterol (TC)

Figure 4.1 shows total cholesterol TC levels from baseline to 24 weeks between groups. There was no significant difference in TC between groups at 24 weeks between the LED and GSC with adjusted difference of -0.17 (95% CI -0.63 to 0.28, $p=0.452$) (Table 4.1).

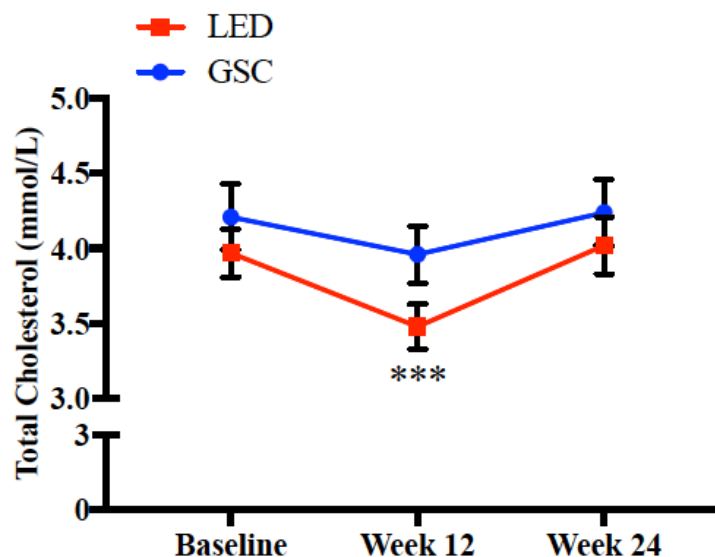


Figure 4. 1 Comparison of total cholesterol between groups at 12 & 24 weeks.

Bar charts represent adjusted mean total cholesterol with standard errors. p values calculated using adjusted mixed linear modelling *** $p<0.001$ Abbreviations; GSC, gold standard clinical care; LED, low energy diet.

4.4.1.2. Low Density Lipoprotein - Cholesterol (LDL-C)

Figure 4.2 shows low-density lipoprotein cholesterol (LDL-C) levels from baseline to 24 weeks between groups. There was a non-significant change in LDL at 24 weeks with an adjusted difference of -0.05 (95% CI -4.41 to 0.30, $p=0.770$).

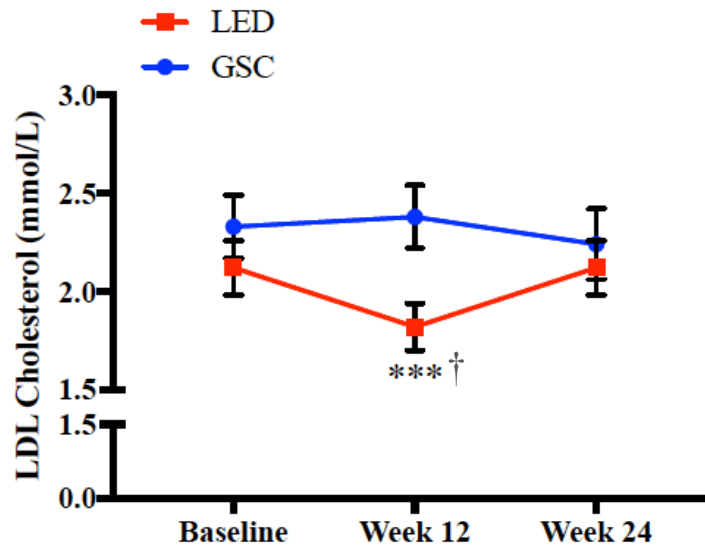


Figure 4. 2 Comparison of low-density lipoprotein cholesterol between groups at 12 & 24 weeks.

Bar charts represent mean LDL cholesterol with standard errors. p values calculated using adjusted mixed linear modelling * $p < 0.05$, *** $p < 0.001$ †: adjusted between group comparison. Abbreviations: LDL, low-density lipoprotein; GSC, gold standard clinical care; LED, low energy diet.

4.4.1.3. High Density Lipoprotein Cholesterol (HDL-C)

There was no significant difference in high-density lipoprotein cholesterol (HDL-C) between groups at 24 weeks. HDL-C increased significantly in both groups at week 24 with an increase of 0.09mmol/L resulting in HDL-C being 1.20mmol/L (SD 0.33) and 1.23mmol/L (SD 0.36) for the LED and GSC group respectively (Figure 4.3).

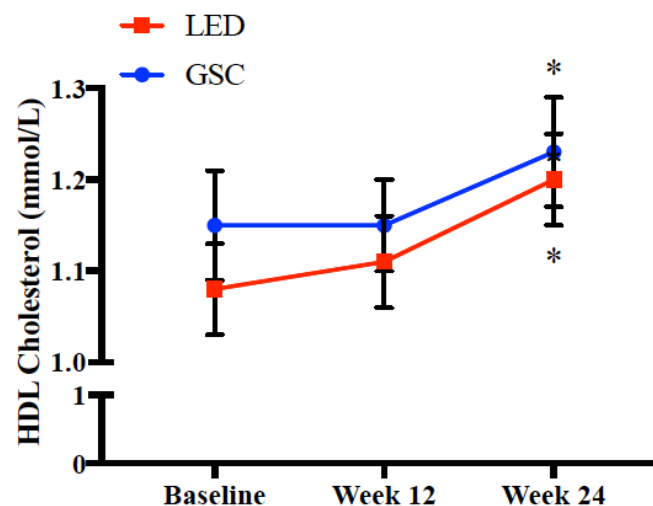


Figure 4. 3 Comparison of high-density lipoprotein cholesterol between groups at 12 & 24 weeks.

Bar charts represent mean HDL cholesterol with standard errors. p values calculated using adjusted mixed linear modelling * $p < 0.05$ Abbreviations: HDL, high-density lipoprotein; GSC, gold standard clinical care; LED, low energy diet

4.4.1.4. Non-high density lipoprotein cholesterol

Non-high density lipoprotein cholesterol (Non-HDL-C) has been linked to increased risk of CVD and is reported to be a better marker than LDL-C alone (Robinson et al., 2009). Non-HDL-C is calculated by subtracting HDL from TC, giving non-HDL-C (Expert Panel on Detection, 2001). Figure 4.4 shows Non-HDL-C levels from baseline to 24 weeks between groups.

There was no significant difference in non-HDL-C between groups at 24 weeks of -0.18 (-0.63 to 0.28, $p=0.445$).

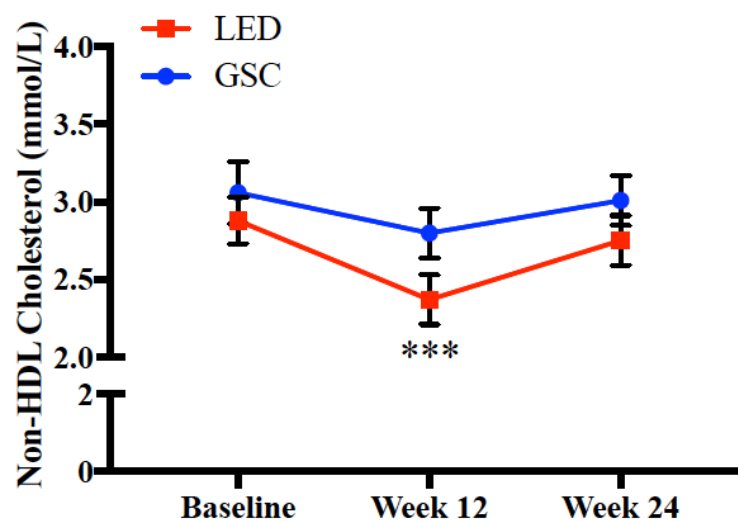


Figure 4. 4 Comparison of non-high density lipoprotein cholesterol between groups at 12 & 24 weeks.

Bar charts represent mean non-HDL cholesterol with standard errors. p values calculated using adjusted mixed linear modelling *** $p<0.001$ Abbreviations: non-HDL non high density lipoprotein; GSC, gold standard clinical care; LED, low energy diet

4.4.1.5. Total Cholesterol to HDL Cholesterol Ratio

Total cholesterol to high-density lipoprotein cholesterol (TC/HDL-C) ratio is used to predict heart disease risk and been shown to related to IR syndrome (Lemieux et al., 2001). Figure 4.5 shows TC/HDL-C ratio levels from baseline to 24 weeks between groups.

There was also no difference in TC/HDL-C ratio between groups at 24 weeks with an adjusted difference of -0.06 (95% CI -0.57 to 0.45, $p=0.832$).

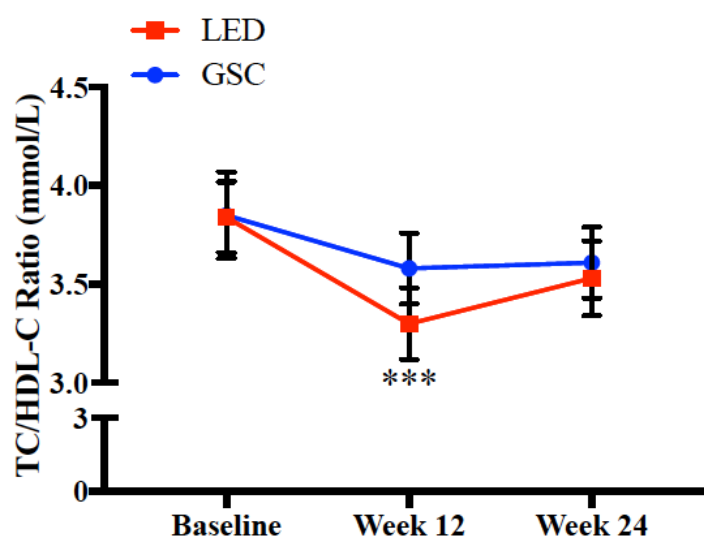


Figure 4. 5 Comparison of total cholesterol to high-density lipoprotein cholesterol ratio between groups at 12 & 24 weeks.

Bar charts represent mean TC/HDL-C ratio with standard errors. p values calculated using adjusted mixed linear modelling ***p<0.001 Abbreviations: TC/HDL, total cholesterol to high density lipoprotein cholesterol; GSC, gold standard clinical care; LED, low energy diet

4.4.1.6. Triglycerides

Figure 4.6 shows triglyceride (TG) levels from baseline to 24 weeks between groups. There was a significant difference in TG between groups at 24 weeks. Mean serum TG decreased by -0.78 mmol/L (SD 0.80, $p < 0.0001$) in the LED group and by -0.42 mmol/L (SD 0.79, $p < 0.0001$) in the GSC group at 24 weeks, resulting in an adjusted difference -0.35 mmol/L (95% CI -0.70 to -0.01 , $p = 0.046$), despite the GSC also having a significant reduction.

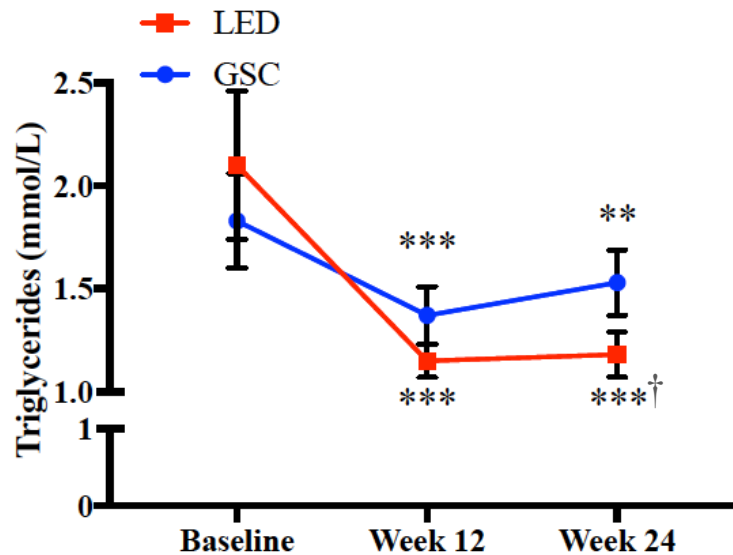


Figure 4. 6 Comparison of triglycerides between groups at 12 & 24 weeks.

Bar charts represent mean triglycerides with standard errors. p values calculated using adjusted mixed linear modelling * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ †: adjusted between group comparison. Abbreviations: GSC, gold standard clinical care; LED, low energy diet

Table 4. 1 Lipid outcomes comparing LED and GSC at 12 and 24 weeks

	Mean (SD)						Intervention effect	
	Baseline	Week 12	Change	Week 24	Change		Estimate (95% CI)	P value
Total Cholesterol (mmol/L)								
LED	3.97 (1.04)	3.48 (0.95)	-0.56 (1.03) ***	4.02 (1.18)	-0.08 (1.04)	0-12	-0.37 (-0.82 to 0.09)	0.112
GSC	4.21 (1.38)	3.96 (1.21)	-0.19 (1.03)	4.24 (1.38)	0.09 (1.03)	12-24	-0.17 (-0.63 to 0.28)	0.452
LDL Cholesterol (mmol/L)								
LED	2.12 (0.87)	1.82 (0.79)	-0.35 (0.80) ***	2.12 (0.90)	-0.11 (0.81)	0-12	-0.44 (-0.79 to -0.09)	0.014
GSC	2.33 (1.01)	2.38 (1.02)	0.87 (0.80)	2.24 (1.14)	-0.05 (0.80)	12-24	-0.05 (-4.41 to 0.30)	0.770
HDL Cholesterol (mmol/L)								
LED	1.08 (0.31)	1.11 (0.34)	0.01 (0.27)	1.20 (0.33)	0.09 (0.27) *	0-12	-0.01 (-0.13 to 0.11)	0.895
GSC	1.15 (0.36)	1.15 (0.32)	0.02 (0.27)	1.23 (0.36)	0.09 (0.27) *	12-24	0.00 (-0.12 to 0.12)	0.987
Triglycerides (mmol/L)								
LED	2.10 (2.25)	1.15 (0.53)	-0.83 (0.79) ***	1.18 (0.70)	-0.78 (0.80) ***	0-12	-0.25 (-0.60 to 0.10)	0.162
GSC	1.83 (1.48)	1.37 (0.90)	-0.58 (0.79) ***	1.53 (0.99)	-0.42 (0.79) **	12-24	-0.35 (-0.70 to -0.01)	0.046
Non-HDL Cholesterol (mmol/L)								
LED	2.88 (0.94)	2.37 (0.90)	-0.57 (1.03) ***	2.75 (1.05)	-0.17 (1.04)	0-12	-0.36 (-0.81 to 0.09)	0.117
GSC	3.06 (1.26)	2.80 (1.15)	-0.21 (1.03)	3.01 (1.29)	0.00 (1.03)	12-24	-0.18 (-0.63 to 0.28)	0.445
TC to HDL Ratio (mmol/L)								
LED	3.84 (1.12)	3.30 (0.95)	-0.55 (1.16) ***	3.53 (1.41)	-0.24 (1.16)	0-12	-0.27 (-0.78 to 0.23)	0.292
GSC	3.85 (1.38)	3.58 (1.26)	-0.27 (1.16)	3.61 (1.17)	-0.30 (1.17)	12-24	-0.06 (-0.57 to 0.45)	0.832

Data represents mean plus standard deviations p values calculated using adjusted mixed linear modelling *p<0.05, **p<0.01, ***p<0.001 within group from baseline.

Intervention effects reported as estimated mean differences (LED minus GSC). LDL, Low-density lipoprotein, HDL, high-density lipoprotein, TC total cholesterol. LED, Low energy diet, GSC Gold standard care

Table 4. 2 Liver function outcomes comparing LED and GSC at 12 and 24 weeks

	Mean (SD)						Intervention effect	
	Baseline	Week 12	Change	Week 24	Change		Estimate (95% CI)	P value
ALT (IU/L)								
LED	29.2 (14.2)	23.6 (9.1)	-5.6 (8.9) ***	23.3 (6.8)	-6.1 (8.9) ***	0-12	-1.2(-5.1 to 2.72)	0.557
GSC	29.4 (18.0)	24.9 (13.6)	-4.5 (8.9) ***	25.3 (13.4)	-4.1 (8.9) ***	12-24	-2.0 (-5.9 to 1.9)	0.324
Alk Phos (IU/L)								
LED	76.1 (22.2)	70.8 (20.1)	-6.3 (14.6)	71.4 (17.4)	-5.4 (14.8) **	0-12	-4.4 (-10.9 to 2.0)	0.180
GSC	84.2 (27.0)	81.2 (26.1)	-1.8 (14.7)	85.9 (28.1)	2.79 (14.7)	12-24	-8.2 (-14.6 to -1.7)	0.014
Albumin (g/L)								
LED	39.5 (3.9)	41.2 (6.5)	1.1 (5.9)	43.2 (8.4)	3.0 (6.0) ***	0-12	1.2 (-1.4 to 3.8)	0.359
GSC	39.7 (4.1)	39.6 (3.4)	-0.1 (5.7)	41.5 (4.1)	1.5 (5.9)	12-24	1.5 (-1.1 to 4.1)	0.247
Bilirubin (μmol/L)								
LED	8.8 (2.9)	12.1 (6.9)	3.1 (5.5) ***	12.3 (5.7)	2.9 (5.7) ***	0-12	3.1 (0.6 to 5.4)	0.012
GSC	9.1 (5.0)	9.3 (5.5)	0.0 (5.4)	9.3 (5.1)	0.1 (5.5)	12-24	2.8 (0.3 to 5.2)	0.025
hsCRP (mg/L)								
LED	5.91 (7.96)	4.28 (6.69)	-1.61 (4.80) *	2.10 (1.50)	-3.79 (4.80) ***	0-12	-1.14 (-3.24 to 0.96)	0.289
GSC	5.83 (5.93)	5.38 (6.76)	-0.47 (4.80)	4.75 (6.13)	-1.10 (4.80)	12-24	-2.69 (-4.79 to -0.59)	0.012

Data represents mean plus standard deviations p values calculated using adjusted mixed linear modelling *p<0.05, **p<0.01, ***p<0.001 within group from baseline.

Intervention effects reported as estimated mean differences (LED minus GSC). ALT, Alanine transaminase; ALP, Alkaline Phosphatase (ALP), hsCRP, High-sensitive C-reactive protein, Led Low energy diet, GSC Gold standard care

4.4.2. Liver Profile

4.4.2.1. Serum Alanine transaminase

Figure 4.7 shows serum alanine transaminase (ALT) levels from baseline to 24 weeks between groups.

There was no significant difference in ALT between the groups at 24 weeks. This may have been due to there being a significant reduction in ALT in both groups at 24 weeks resulting in the adjusted difference not reaching significance. Mean ALT reduced in the LED group by -6.1IU/L (SD 8.9, $p<0.0001$) at 24 weeks. While in the GSC group, ALT fell by -4.1IU/L (SD 8.9, $p=0.003$) at 24 weeks.

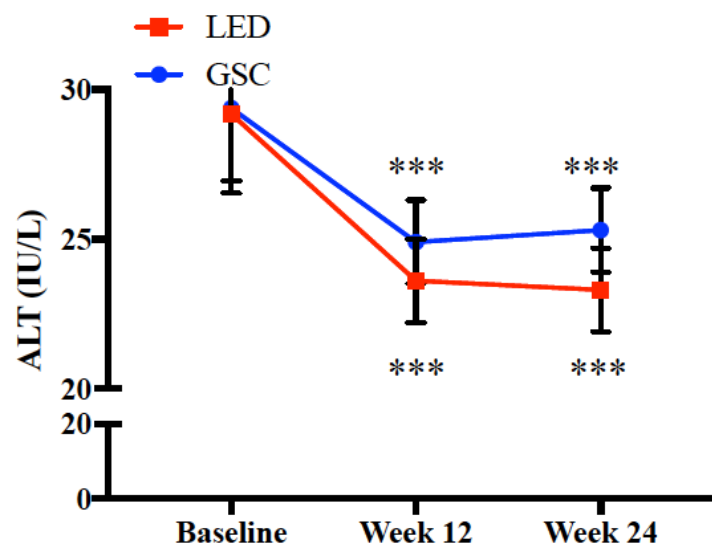


Figure 4. 7 Comparison Alanine transaminase (ALT) of between groups at 12 & 24 weeks.

Bar charts represent mean ALT with standard errors. p values calculated using adjusted mixed linear modelling *** $p<0.001$. Abbreviations: ALT, alanine transaminase; GSC, gold standard clinical care; LED, low energy diet

4.4.2.2. Serum Alkaline Phosphatase (ALP)

There was a significant difference in serum alkaline phosphatase (ALP) between groups at 24 weeks with ALP falling by -5.4IU/L (SD 14.8) in the LED group and increased by 2.79IU/L in the GSC group (adjusted difference -8.2IU/L, -14.6 to -1.7, $p=0.014$).

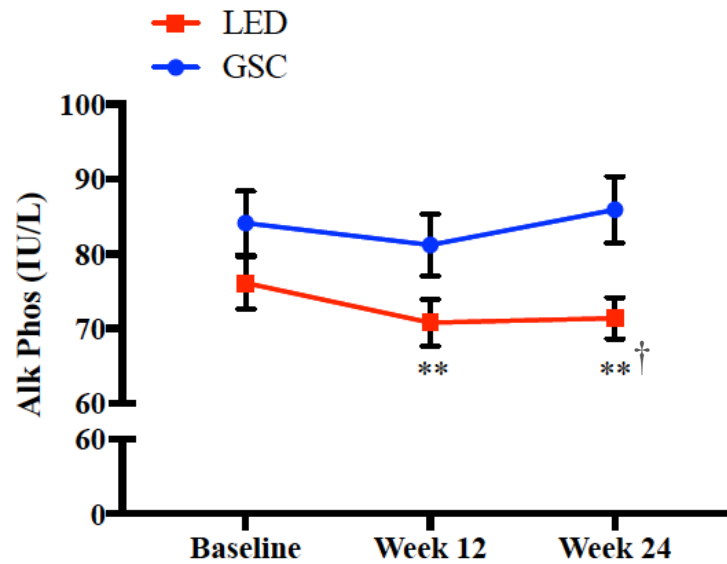


Figure 4. 8 Comparison of alkaline phosphatase between groups at 12 & 24 weeks.

Bar charts represent mean AP with standard errors. p values calculated using adjusted mixed linear modelling
 * $p<0.05$, ** $p<0.01$ †: adjusted between group comparison. Abbreviations: GSC, gold standard clinical care; LED, low energy diet

4.4.2.3. Serum Albumin

Figure 4.9 shows albumin levels from baseline to 24 weeks between groups.

There was no significant difference in albumin between groups at 24 weeks. Mean albumin significantly increased by 3.0g/L (SD 6.0, $p=0.001$) at 24 weeks compared with baseline in the LED group.

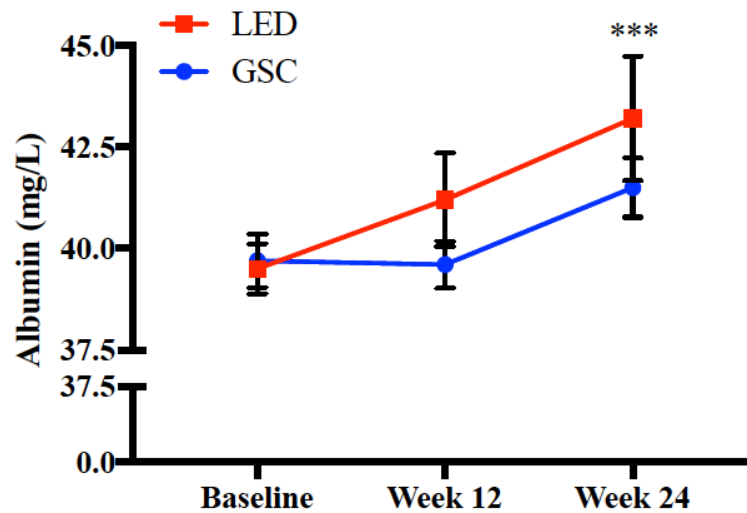


Figure 4. 9 Comparison of albumin between groups at 12 & 24 weeks.

Bar charts represent mean albumin with standard errors. p values calculated using adjusted mixed linear modelling *** $p<0.001$ Abbreviations: GSC, gold standard clinical care; LED, low energy diet

4.4.2.4. Serum Bilirubin

Figure 4.10 shows serum bilirubin levels from baseline to 24 weeks between groups. There was a significant difference in bilirubin between groups at 24 weeks with an adjusted difference of $2.8\mu\text{mol/L}$ (95% CI 0.3 to 5.2, $p=0.025$). At 24 weeks there was a significant increase in bilirubin levels in the LED group of $2.9\mu\text{mol/L}$ (SD 5.7, $p=0.001$).

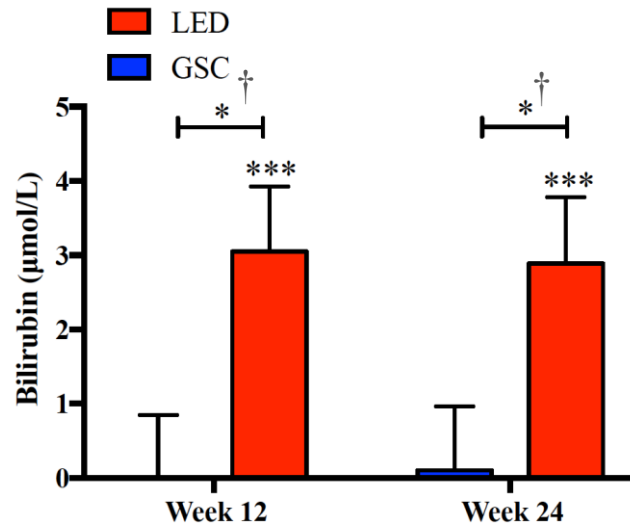


Figure 4. 10 Comparison of bilirubin between groups at 12 & 24 weeks.

Bar charts represent mean bilirubin with standard errors. p values calculated using adjusted mixed linear modelling * $p < 0.05$, *** $p < 0.001$ †: adjusted between group comparison Abbreviations: GSC, gold standard clinical care; LED, low energy diet

4.4.2.5. High-sensitive C-reactive protein (hsCRP)

Figure 4.11 shows high sensitive C-reactive protein levels from baseline to 24 weeks between groups.

At 24 weeks hsCRP decreased by -3.79mg/L (SD 4.80) in the LED group and -1.10mg/L (SD 4.80) in the GSC group with a significant adjusted difference -2.69mg/L (95% CI -4.79 to -0.59 p=0.012). At 24-week there was a significant reduction in hsCRP in the LED group of -3.79 (SD 4.80, p<0.0001). No significant differences were seen in the GSC group.

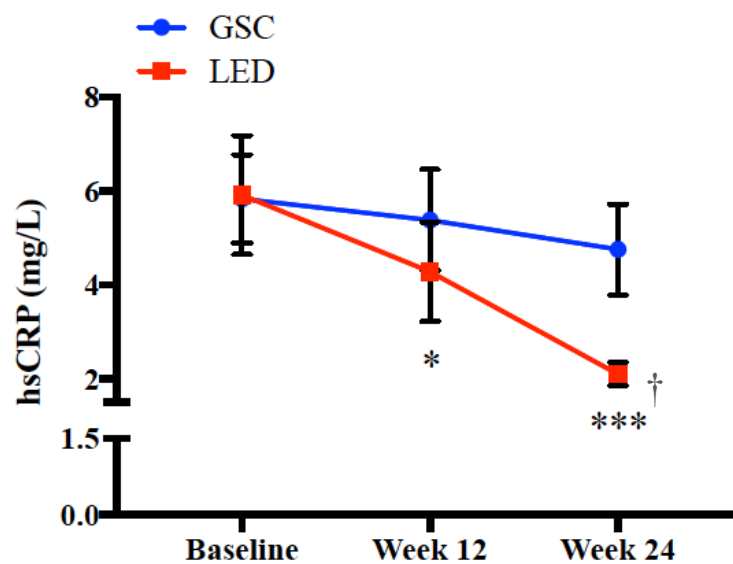


Figure 4. 11 Comparison of High-sensitive C-reactive protein between groups at 12 & 24 weeks.

Bar charts represent mean bilirubin with standard errors. p values calculated using adjusted mixed linear modelling *p<0.05, ***p<0.001 †: adjusted between group comparison. Abbreviations: GSC, gold standard clinical care; LED, low energy diet, hs CRP, high-sensitive C-reactive protein

4.4.3. Blood Pressure

4.4.3.1. Systolic blood pressure

The systolic blood pressure (SBP) at baseline for the GSC group was 133.2mmHg (SD 18.15) while in the LED it was 131.9mmHg (SD 16.2, Table 4.3 and shown in Figure 4.12).

Mean systolic blood pressure fell by -11.2mmHg in the LED group and by -7.4mmHg in the GSC group at 24 weeks, although there was no significant difference between the groups at 24 weeks (-3.8mmHg CI -9.0 to 1.5, $p=0.157$), due to an improvement in SBP within the GSC and a worsening in the LED group.

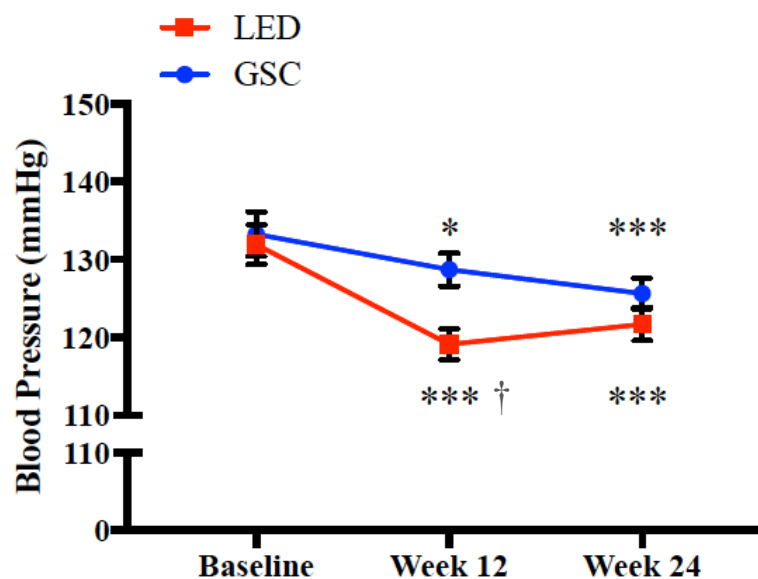


Figure 4.12 Comparison of systolic blood pressure between groups at 12 & 24 weeks.

Bar charts represent mean systolic blood pressure with standard errors. p values calculated using adjusted mixed linear modelling * $p<0.05$, ** $p<0.01$, *** $p<0.001$ †: adjusted between group comparison Abbreviations: SBP, systolic blood pressure; GSC, gold standard clinical care; LED, low energy diet; Δ : change.

4.4.3.2. Diastolic blood pressure

Figure 4.13 shows the change from baseline for diastolic blood pressure (DBP) between LED and GSC at 12 and 24 weeks.

There was a significant difference in DBP between groups at 24 weeks, the adjusted difference was -5.96mmHg (CI -9.91 to -2.01 $p=0.003$). There was a significant reduction in DBP at 24 weeks in subjects on the LED group with DBP falling by -7.66mmHg (SD 9.3, $p<0.001$), respectively. There was no significant change in the GSC group.

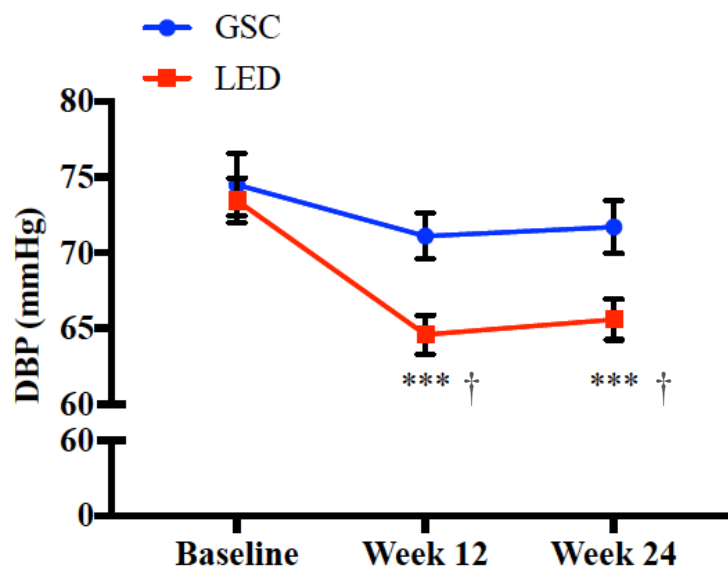


Figure 4. 13 Comparison of diastolic blood pressure between groups at 12 & 24 weeks.

Bar chart represent mean diastolic pressure with standard errors. p values calculated using adjusted mixed linear modelling * $p<0.05$, *** $p<0.001$ †: adjusted between group comparison. Abbreviations: DBP diastolic blood pressure; GSC, gold standard clinical care; LED, low energy diet; Δ : change.

4.4.4. Measures of Kidney Function

Baseline kidney function is summarised in Table 4.3, which lists estimated glomerular filtration rate (eGFR), urinary albumin, urine creatinine, urine albumin to creatinine ratio (ACR) and microalbuminuria (microalbuminuria defined as ACR >2.5mg/mmol in men and >3.5mg/mmol in women). eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) equation (Levey et al., 1999).

4.4.4.1. Glomerular Filtration Rate

There was no difference in estimated glomerular filtration rate (eGFR) at 24 weeks with an adjusted difference of 3.6 mL/min/1.73 m² (95% CI -1.4 to 8.5, p=0.156).

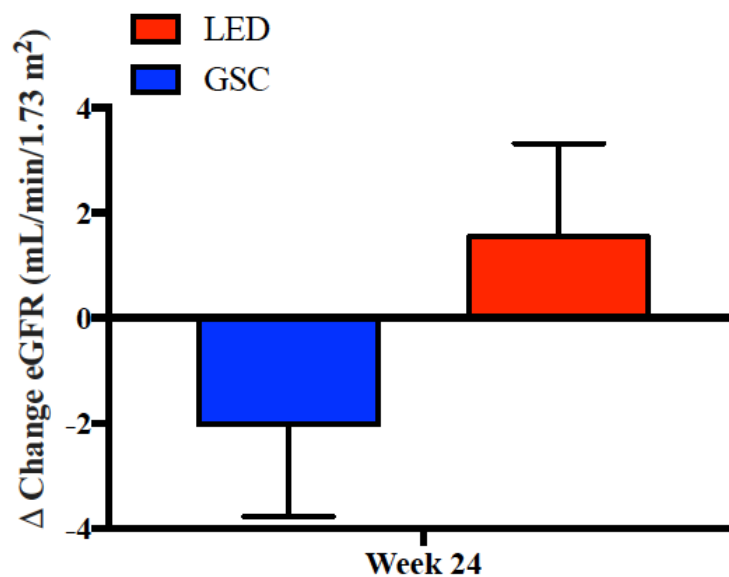


Figure 4. 14 Comparison of estimated glomerular filtration rate between groups at 24 weeks.

Bar charts represent mean eGFR with standard errors. p values calculated using adjusted mixed linear modelling *p<0.05, **p<0.01***p<0.001 †: adjusted between group comparison. Abbreviations: eGFR estimated glomerular filtration rate; GSC, gold standard clinical care; LED, low energy diet; Δ, change

4.4.4.2. Urinary Creatinine

There is no significant difference in urinary creatinine (UCr) between groups at 24 weeks. In addition, there were no differences within groups for both the LED and the GSC. There was a trend for the LED to have a reduction in UCr at 24 weeks, although this did not reach significance (-1.7, SD 6.7, p=0.099).

4.4.4.3. Urinary Albumin

There is no significant difference in urinary albumin (UA) between groups at 24 weeks. In addition, there were not differences within groups for both the LED and the GSC. There was a trend for the LED to have a reduction in UA at 24 weeks (-35.6, SD 132.5, $p=0.089$), although this did not reach significance.

4.4.4.4. Albumin to Creatinine Ratio

There is no significant difference in urinary albumin to creatinine ratio (ACR) between groups at 24 weeks. In addition, there were no differences within groups for both the LED and the GSC. There was a trend for the LED to have a reduction in ACR at 24 weeks (-2.3, SD 2.9, $p=0.420$), although this did not reach significance.

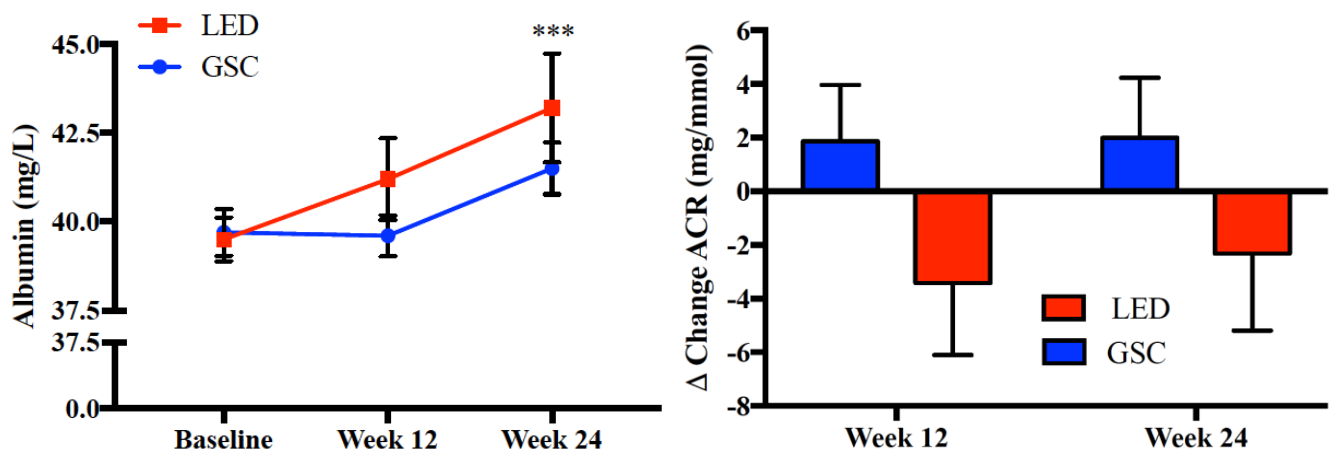


Figure 4. 15 Comparison of change in urinary albumin and albumin to creatinine ratio between groups at 12 & 24 weeks.

Bar charts represent mean UAI and ACR with standard errors. Abbreviations: UAI urinary albumin; ACR, albumin to creatinine ratio; GSC, gold standard clinical care; LED, low energy diet; Δ, change

4.4.4.5. Microalbuminuria

There is no significant difference in the occurrence of microalbuminuria between groups at 24 weeks.

Table 4. 3 Blood Pressure and Renal Function comparing LED and GSC at 12 and 24 weeks

	Mean (SD)						Intervention effect	
	Baseline	Week 12	Change	Week 24	Change		Estimate (95% CI)	P value
Systolic BP (mmHg)								
LED	131.9 (16.2)	119.1 (12.6)	-14.3 (11.7) ***	121.7 (13.5)	-11.2 (12.3) ***	0-12	-10.2 (-15.3 to -5.2)	<0.001
GSC	133.3 (18.2)	128.7 (13.2)	-4.1 (11.3) *	125.6 (12.4)	-7.4 (11.5) ***	12-24	-3.8 (-9.0 to 1.5)	0.157
Diastolic BP (mmHg)								
LED	73.5 (9.4)	64.6 (8.2)	-9.2 (8.9) ***	65.6 (8.5)	-7.7 (9.3) ***	0-12	-7.11 (-11.0 to -3.3)	<0.001
GSC	74.5 (13.0)	71.1 (9.6)	-2.1 (8.6)	71.7 (11.0)	-1.7 (8.6)	12-24	-6.0 (-9.9 to 2.0)	0.003
eGFR (mL/min/1.73 m²)								
LED	75.8 (17.8)	79.7 (17.1)	3.8 (11.2) *	77.4 (13.8)	1.5 (11.3)	0-12	5.4 (0.5 to 10.3)	0.031
GSC	76.6 (22.2)	74.8 (19.5)	-1.6 (11.2)	74.4 (19.6)	-2.0 (11.2)	12-24	3.6 (-1.4 to 8.5)	0.157
Urine Creatinine (mg/L)								
LED	7.5 (4.1)	7.5 (10.1)	0.2 (6.9)	5.9 (3.2)	-1.7 (6.5)	0-12	-0.14 (-3.0 to 2.7)	0.924
GSC	6.9 (3.6)	7.5 (4.6)	0.3 (6.5)	6.8 (4.8)	-0.2 (6.7)	12-24	-1.5 (-4.5 to 1.46)	0.317
Urine Albumin (mg/L)								
LED	47.1 (85)	34.8 (48.2)	-36.4 (121.0)	32.5 (55.3)	-35.6 (132.5)	0-12	-36.4 (-84.7 to 11.9)	0.139
GSC	63.6 (93.6)	58.1 (118.7)	0.03 (98.3)	67.3 (16.0)	5.1 (103.7)	12-24	-40.7 (-92.8 to 11.5)	0.127
ACR (mg/mmol)								
LED	5.8 (8.3)	4.2 (6.25)	-3.4 (17.0)	3.8 (5.9)	-2.3 (18.2)	0-12	-5.3 (-11.9 to 1.4)	0.122
GSC	9.7 (14.3)	9.1 (20.3)	1.9 (13.3)	10.2 (16.0)	2.0 (14.1)	12-24	-4.3 (11.5 to 2.8)	0.237

Data represents mean plus standard deviations p values calculated using adjusted mixed linear modelling *p<0.05, ***p<0.001 within group from baseline. Intervention effects reported as estimated mean differences (LED minus GSC). Abbreviations: BP, blood pressure; eGFR estimated glomerular filtration rate, ACR, albumin to creatinine ratio, LED Low energy diet, GSC Gold Standard care

4.4.5. Sleep

4.4.5.1. Apnoea Hypoxia Index

Apnoea hypoxia index (AHI) was measured as the number of apnoeas or hypopneas recorded over one night, per hour of sleep recording. Figure 4.16 shows AHI from baseline to 24 weeks between groups.

There was no significant difference in AHI between groups at 24 weeks with an adjusted difference of -1.77 (95% CI -4.58 to 1.04, $p=0.218$). There was, however, a significant reduction of AHI in both groups, with the LED group reducing AHI by -3.97 (SD 6.47, $p=0.023$) and -2.13 (SD 6.34, $p=0.034$) in the GSC groups. The reduction in both in likely accounted for the lack of between group differences.

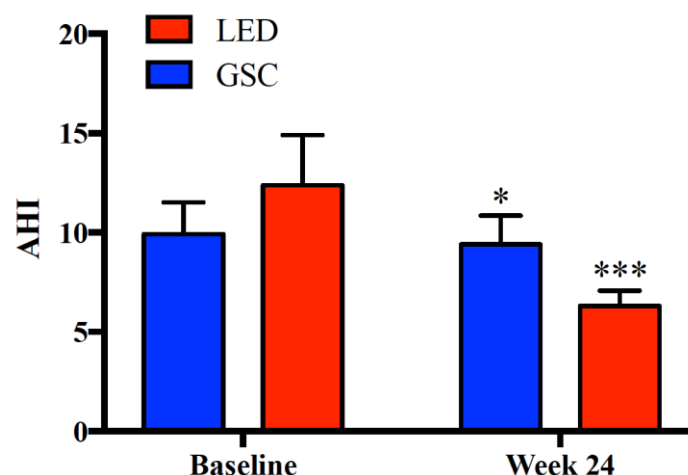


Figure 4. 16 Comparison of apnoea hypoxia index (AHI) between groups at 24 weeks.

Bar charts represent mean AHI with standard errors. p values calculated using adjusted mixed linear modelling * $p<0.05$, *** $p<0.001$. Abbreviations: AHI, apnoea hypoxia index GSC, gold standard clinical care; LED, low energy diet

4.4.5.2. Oxygen Saturation

4.4.5.2.1. Oxygen Desaturation Index

Oxygen desaturation index (ODI) was calculated as the number of times per hour of sleep that the subject's blood oxygen dropped by 4% below baseline. Figure 4.17 shows ODI from baseline to 24 weeks between groups.

There was a significant difference in ODI between groups at 24 weeks with ODI falling by -7.2 (SD 6.9) in the LED group and -3.3 (SD 6.9) in the GSC resulting in an adjusted difference of -3.9 (95% CI -6.9 to -0.8, $p=0.013$). Both groups had a significant reduction in ODI at 24 weeks by -7.2 (SD 6.9, $p<0.001$) and -3.3 (SD 6.9, $p=0.002$).

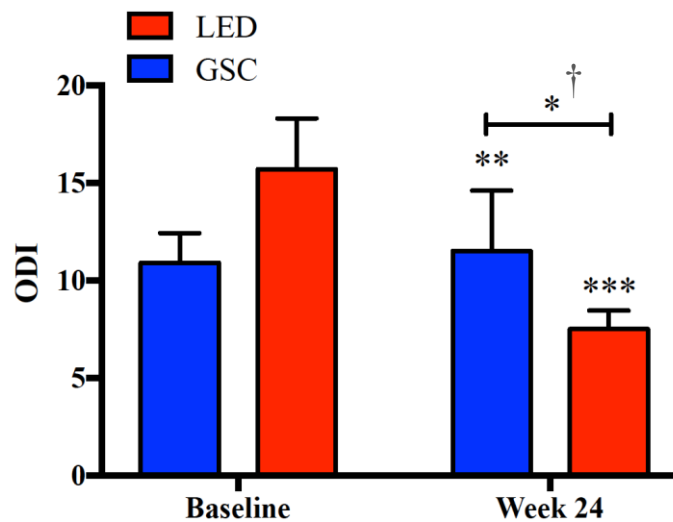


Figure 4. 17 Comparison of oxygen desaturation index between groups at 24 weeks.

Bar charts represent mean ODI with standard errors. p values calculated using adjusted mixed linear modelling * $p<0.05$, ** $p<0.01$, *** $p<0.001$ †: adjusted between group comparison. Abbreviations: ODI, oxygen desaturation index GSC, gold standard clinical care; LED, low energy diet; Δ , change

4.4.5.2.2. Average oxygen saturation

There is no significant difference in average oxygen saturation between groups at 24 weeks. There was a significant increase within treatment groups, with LED rising by 1.2% (SD 2.1, $p<0.0001$) and by 0.8% (SD 2.2, $p=0.025$).

4.4.5.2.3. Lowest oxygen saturation

There is no significant difference in lowest oxygen saturation between groups at 24 weeks with an adjusted difference of -0.7% (95% CI -3.8 to 2.3, $p=0.640$). There were no significant increases seen within groups either.

4.4.5.2.4. Lowest oxygen desaturation

There is no significant difference in lowest oxygen desaturation between groups at 24 weeks. There was a significant increase within treatment groups, with LED rising by 4.3% (SD 5.9, $p<0.0001$) and by 4.6% (SD 5.9, $p<0.0001$) in the GSC group.

4.4.5.3. Epworth Sleepiness Scale

The Epworth sleepiness scale (ESS) is designed to identify self-reported daytime sleepiness. Figure 4.18 shows ESS score change from baseline to 24 weeks between groups.

There is no significant difference in ESS between groups at 24 weeks with adjusted differences of -0.85 (95% CI -2.32 to 0.62, $p=0.256$). At 24 weeks there was significant reduction in the LED with ESS reducing by 0.8 (SD 3.3, $p=0.009$) while the GSC group had a non-significant increase in the ESS of 0.55 (SD 3.26, $p=0.285$).

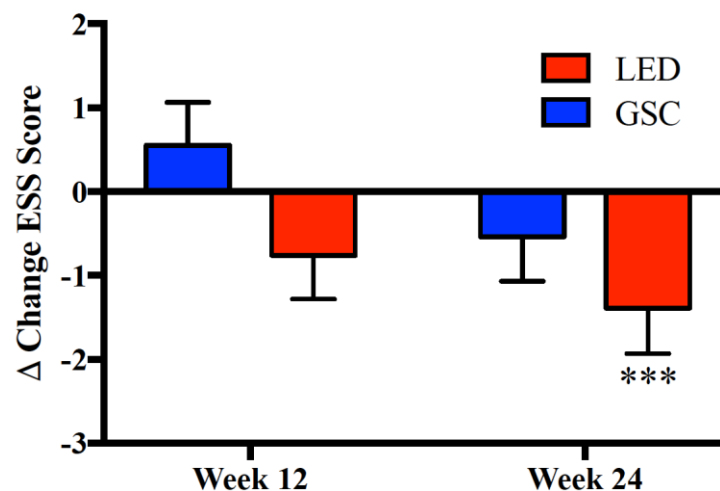


Figure 4. 18 Comparison of Epworth sleep scale between groups at 12 and 24 weeks.

Bar charts represent mean ESS with standard errors. p values calculated using adjusted mixed linear modelling *** $p<0.001$ †: adjusted between group comparison. Abbreviations: ESS, Epworth Sleep Scale; GSC, gold standard clinical care; LED, low energy diet; Δ, change

4.4.5.4. Pittsburgh Sleep Quality Index

The Pittsburgh sleep quality index (PSQI) is designed to examine self-reported sleep quality. Figure 4.19 shows PSQI score change from baseline to 24 weeks between groups.

There is no significant difference in PQSI score between groups at 24 weeks with adjusted differences of -1.3 (95% CI -2.6 to 0.0, $p=0.056$). Although it did not reach significance, there was a trend for an adjusted difference between groups at 24 weeks.

At 24-week there was significant adjusted reduction in the LED with PSQI reducing by -1.0 (SD 3.0, $p=0.034$) while the GSC group had a non-significant increase in the PSQI of 0.3 (SD 2.9, $p=0.571$).

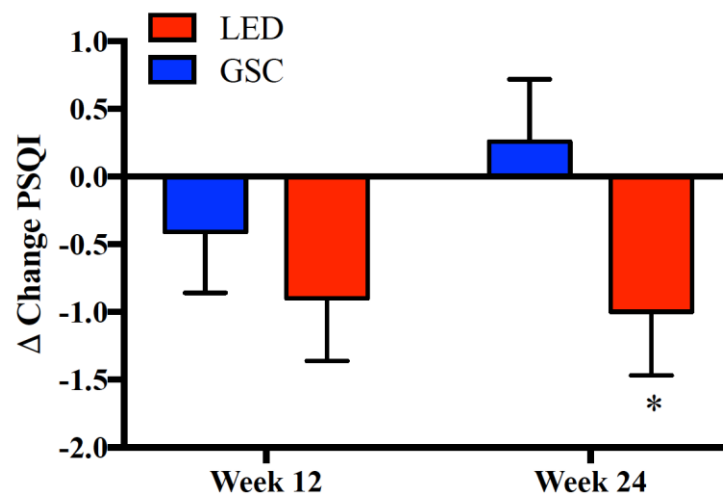


Figure 4. 19 Comparison of Pittsburgh Sleep Quality Index between groups at 12 and 24 weeks.

Bar charts represent mean PSQI with standard errors. . p values calculated using adjusted mixed linear modelling * $p<0.05$ Abbreviations: PSQI, Pittsburgh sleep quality index, GSC, gold standard clinical care; LED, low energy diet; Δ , change

Table 4. 4 Sleep Outcomes comparing LED and GSC at 12 and 24 weeks

	Mean (SD)						Intervention effect	
	Baseline	Week 12	Change	Week 24	Change		Estimate (95% CI)	P value
AHI								
LED	12.4 (15.9)	-	-	6.3 (4.9)	-3.9 (6.5) ***	0-12	-	
GSC	9.9 (10.1)	-	-	9.4 (9.2)	-2.1 (6.3) *	12-24	-1.8 (-4.6 to 1.0)	0.218
ODI								
LED	15.7 (16.4)	-	-	7.5 (6.1)	-7.2 (6.9) ***	0-12	-	
GSC	10.9 (9.7)	-	-	11.5 (19.6)	-3.3 (6.9) **	12-24	-3.9 (-6.9 to -0.8)	0.013
Av Saturation								
LED	92.6 (4.4)	-	-	94.2 (1.8)	1.2 (2.1)	0-12	-	
GSC	93.8 (2.1)	-	-	93.9 (1.4)	0.8 (2.2)	12-24	0.4 (-0.5 to 1.4)	0.384
Low Saturation								
LED	77.7 (11.5)	-		80.0 (6.8)	2.1 (6.9)	0-12	-	
GSC	80.3 (8.9)	-		80.8 (3.9)	1.3 (6.9)	12-24	-0.7 (-3.6 to 2.3)	0.640
Low Desaturation								
LED	80.6 (11.5)	-	-	84.5 (5.6)	4.3 (5.9)	0-12	-	
GSC	81.3 (14.4)	-	-	84.6 (3.7)	4.6 (5.9)	12-24	-0.3 (-2.9 to 2.3)	0.816
ESS								
LED	7.3 (4.7)	6.1 (3.8)	-0.8 (3.3)	5.4 (4.6)	-1.4 (3.4) ***	0-12	-1.3 (-2.7 to 0.1)	0.075
GSC	6.9 (4.5)	7.4 (4.4)	0.6 (3.3)	6.3 (4.4)	-0.5 (3.3)	12-24	-0.85 (-2.3 to 0.6)	0.256
PSQI								
LED	6.7 (3.4)	5.8 (3.2)	-0.9 (2.9)	5.7 (3.6)	-1.0 (3.0) *	0-12	-0.5 (-1.8 to 0.8)	0.453
GSC	6.7 (3.1)	6.3 (3.5)	-0.4 (2.9)	7.0 (4.3)	0.3 (2.9)	12-24	-1.3 (-2.6 to 0.0)	0.056

Data represents mean plus standard deviations p values calculated using adjusted mixed linear modelling *p<0.05, **p<0.01, ***p<0.001 within group from baseline.

Intervention effects reported as estimated mean differences (LED minus GSC). Abbreviations AHI, Apnoea Hypoxia Index, ODI, Oxygen Desaturation Index, ESS, Epworth Sleepiness Scale, PSQI Pittsburgh Sleep Quality Index, LED Low Energy diet, GSC Gold Standard Care

Table 4. 5 Dietary fatty acid intake comparing LED and GSC at 12 and 24 weeks

Mean (SD)						Intervention effect		
	Baseline	Week 12	Change	Week 24	Change	Estimate (95% CI)		P value
SFA (g)								
LED	25.3 (16.3)	3.9 (7.8)	-22.6 (8.9) ***	7.3 (5.1)	-18.5 (9.3) ***	0-12	-12.9 (-16.8 to -9.0)	<0.0001
GSC	27.2 (13.3)	16.8 (7.9)	-9.8 (8.9) ***	15.7 (8.5)	-11.9 (8.9) ***	12-24	-6.6 (-10.6 to -2.6)	0.001
PUFA (g)								
LED	10.6 (5.0)	6.8 (2.0)	-3.8 (4.2) ***	6.9 (3.2)	-3.7 (4.4) ***	0-12	-2.0 (-3.9 to -0.20)	0.029
GSC	10.2 (5.2)	8.3 (5.1)	-1.7 (4.2) *	8.2 (4.6)	-2.3 (4.2) ***	12-24	-1.3 (-3.2 to 0.56)	0.168
MUFA (g)								
LED	22.0 (12.0)	3.7 (5.7)	-19.5 (8.4) ***	8.5 (6.7)	-14.2 (8.9) ***	0-12	-13.8 (-17.5 to -10.0)	<0.0001
GSC	23.7 (12.9)	17.3 (9.0)	-5.7 (8.5) ***	15.7 (8.9)	-8.4 (8.5) ***	12-24	-5.8 (-9.6 to -1.9)	0.003
Salt (g)								
LED	5.0 (2.7)	3.5 (1.1)	-1.8 (1.8) ***	3.2 (1.7)	-2.0 (1.9) ***	0-12	-0.57 (-1.4 to 0.23)	0.161
GSC	5.0 (2.5)	3.9 (1.7)	-1.2 (1.8) ***	3.6 (1.7)	-1.7 (1.8) ***	12-24	-0.34 (-1.2 to 0.48)	0.411

Data represents mean plus standard deviations p values calculated using adjusted mixed linear modelling *p<0.05, **p<0.01, ***p<0.001 within group from baseline. Intervention effects reported as estimated mean differences (LED minus GSC). Abbreviations. SFA Saturated fatty acid, PUFA, Polyunsaturated fatty acid, MUFA, monounsaturated fatty acid, LED Low energy diet, GSC Gold standard care, g grams

4.4.7. Cardiovascular Disease 10-year risk and Coronary Heart Disease 10-year mortality

The reduction of CVD and CHD mortality is key to treatments for an obese T2D population treated with insulin, therefore the QRISK2 and Framingham hard coronary heart disease risk score were calculated to estimate any risk benefit.

At baseline according to the QRISK2 score both groups were classified as high risk (Hippisley-Cox et al., 2008), with a 10-year risk of CVD of 20% in both the LED and GSC groups.

There was a non-significant difference between groups at 24 weeks with an adjusted difference of -1.8% (95% CI -3.5 to 0.0, $p=0.055$, Table 4.7). On assessment of the risk factors included this lack of difference was possibly due to the change in systolic blood pressure affecting the risk score.

Table 4. 6 Predicted 10-year cardiovascular risk using QRISK2 comparing LED and GSC at 12 and 24 week

	GSC	p Value	LED	p value	IE 95% CI	p value
Baseline (%)	20 (11)		20 (8)			
12 weeks (%)	18 (10)	<0.0001	15 (6)	<0.0001	-2.9 (-4.7 to 1.2)	0.001
24 weeks (%)	17 (10)	<0.0001	16 (7)	<0.0001	-1.7 (-3.5 to 0.0)	0.055

Data represents mean 10-year CVD risk with standard deviations. Intervention effects reported as estimated mean differences (LED minus GSC). Abbreviations LED, low energy diet; GSC, gold standard clinical care; % percentage 10-year risk using QRISK2; IE, intervention effect

Figure 4.21 shows change in predicted 10-year CVD risk at 24 weeks between groups

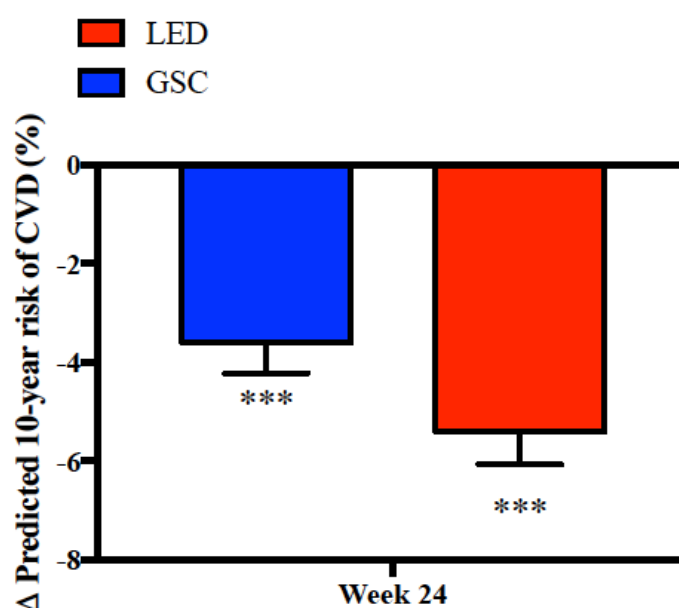


Figure 4. 20 Comparison of the change in predicted 10-year cardiovascular risk using QRISK2 between groups at 24 weeks.

Bar charts represent percentage predicted 10-year risk of CVD. p values calculated using adjusted mixed linear modelling. **p<0.01, ***p<0.001 †: adjusted between group comparison. Abbreviations: GSC, gold standard clinical care; LED, low energy diet; Δ, change

From a point of view of predicted 10-year CHD mortality there was no difference between groups at 24 weeks with an adjusted difference of -0.9% (95% CI -1.9 to 0.1, p=0.074). There was a significant reduction in CHD mortality risk in both groups 24 weeks (Table 4.8)

Table 4. 7 Predicted 10-year coronary heart disease morality risk using Framingham risk score comparing LED and GSC at 12 and 24 week

	GSC	p Value	LED	p value	IE 95% CI	p value
Baseline (%)	13 (4)		13 (5)			
12 weeks (%)	12 (4)	0.008	12 (5)	<0.0001	-0.8 (-1.8 to 0.1)	0.085
24 weeks (%)	12 (4)	<0.0001	11 (5)	<0.0001	-0.9 (-1.9 to 0.1)	0.074

Data represents mean 10-year CVD risk with standard deviations. Intervention effects reported as estimated mean differences (LED minus GSC). Abbreviations LED, low energy diet; GSC, gold standard clinical care; % percentage 10-year mortality risk ; IE, intervention effect

Figure 4.22 shows change of predicted 10-year CVD risk at 24 weeks between groups.

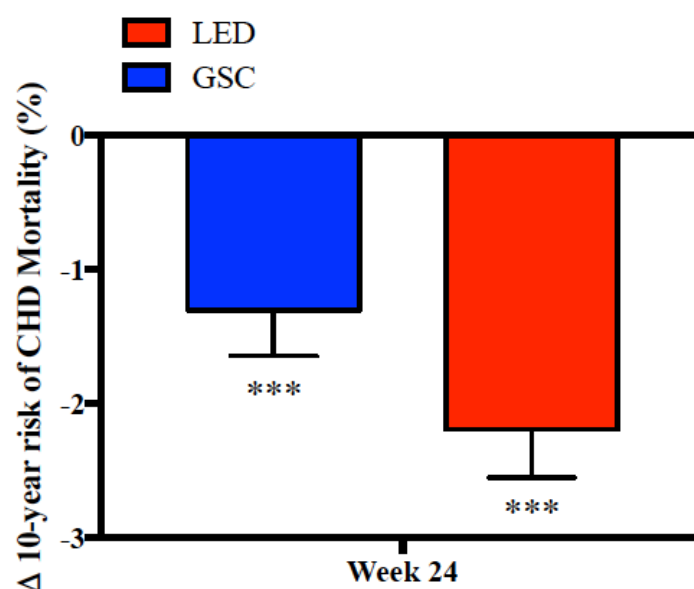


Figure 4. 21 Comparison of change in predicted 10-year coronary heart disease mortality risk between groups at 24 weeks.

Bar charts represent percentage predicted 10-year risk of CHD mortality. p values calculated using adjusted mixed linear modelling **p<0.01, ***p<0.001 †: adjusted between group comparison. Abbreviations: GSC, gold standard clinical care; LED, low energy diet; Δ, change; CHD, coronary heart disease

4.4.8. Energy intake under free-living conditions

Energy and macronutrient intake were measured using a 3-day food diary to assess if there were dietary changes that may be associated with the changes seen with weight and cardiovascular disease risk. Dietary fat is of particular interest to an individual's lipid profile. Therefore, data regarding saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA) and mono-unsaturated fatty acids (MUFA) were analysed. At baseline there were no difference between groups in any dietary parameter. Energy, fat, carbohydrate and fibre outcomes are summarised in Chapter 3 (Table 3.9)

There was a significant adjusted difference during the total diet replacement phase in fat (-34.8g, 95% CI -45.2 to -24.5, p<0.0001), SFA (-12.9g, 95% CI -16.8 to -9.0, p<0.0001), PUFA (-2.0g, 95% CI -3.9 to -0.2, p=0.029) and MUFA (-13.8g, 95% CI -17.5 to 10.0g, p<0.0001).

At 24 weeks there remained a significant difference in fat (-16.0g, 95% CI -26.7 to -5.4, $p=0.003$), SFA (-6.6g, 95% CI -10.6 to -2.6, $p=0.001$) and MUFA (-5.8g, 95% CI -9.6 to -1.9g, $p<0.0001$), although the degree of this difference reduced as food was reintroduced. Although the between group difference of PUFA was not significant, the LED continued to consume less each day (adjusted difference -1.3g, 95% CI -3.2 to 0.56, $p=0.168$).

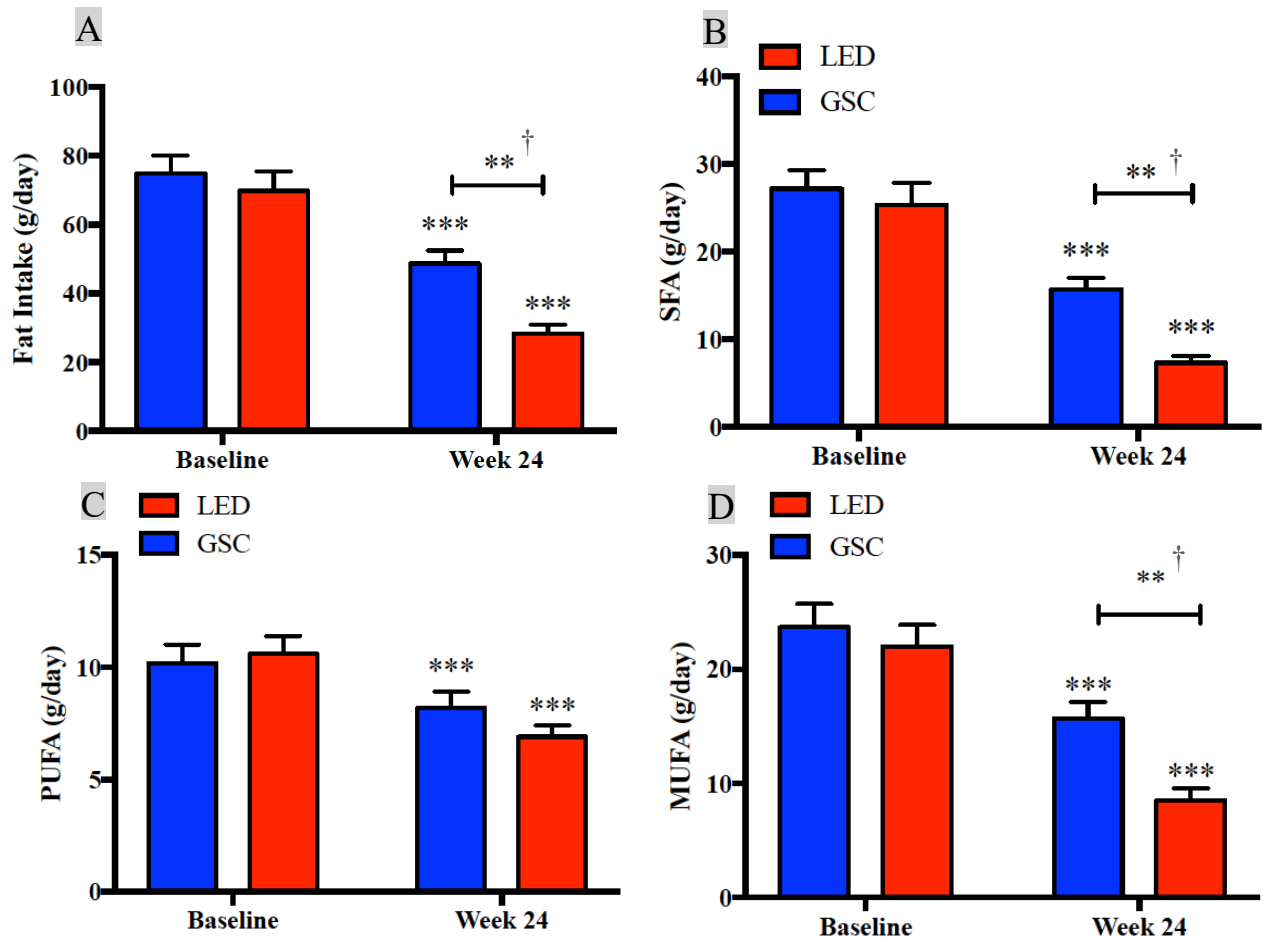


Figure 4. 22 Summary of saturated fat, polyunsaturated fat and monounsaturated fat and total fat intake following LED and GSC at 24 weeks.

Data represents mean plus standard error. (A) Fat (B) SFA (C) PUFA (D) MUFA. p values calculated using adjusted mixed linear modelling * $p<0.05$, ** $p<0.01$, *** $p<0.001$ †: adjusted between group comparison. Abbreviations: LED, low energy diet; GSC, gold standard clinical care; g, grams; SFA, saturated fatty acids; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids

4.5. Discussion

4.5.1. Summary of findings

- Triglycerides (TG) were the only lipids to show a difference between the intervention groups at 24 weeks.
- Bilirubin was significantly greater following the LED intervention at 24 weeks.
- Chronic systemic inflammation (high-sensitive C-reactive protein) was significantly lower at 24 weeks following the LED intervention.
- Systolic and diastolic blood pressure were significantly lower in both groups at 24 weeks although only diastolic blood pressure was significantly different between groups.
- Overall, no significant improvement was seen in renal function over the study duration.
- Oxygen desaturation index was significantly reduced at 24 weeks in both groups, with greater improvements in the LED group.
- 10-year cardiovascular risk score was significantly reduced in both groups at 24 weeks.

4.5.2. Detailed Discussion

The aim of this investigation was to examine the effect of a formula LED on weight loss and surrogate markers of cardiovascular disease (CVD) risk compared with GSC in obese T2D patients concurrently treated with insulin. Previous data from systematic reviews (Mulholland et al., 2012, Parretti et al., 2016) have suggested that there might be benefit to CVD risk with the use of formula VLED. Much of the data, however, were only from obese patients or those treated with oral hypoglycaemic agents (OHA) excluding obese T2D patients treated with insulin.

The results from this study are the first to assess not just one aspect of cardiovascular risk but also a more global view of health including lipid, liver, obstructive sleep apnoea and kidney function. At the end, overall CVD risk is calculated, which presents a unique insight for obese T2D patients concurrently on insulin.

This chapter will attempt to discuss some of these changes in isolation; however, as diseases rarely happen independently, particularly with T2D and obesity, this means that there is significant overlap and interconnectability between discussion points. For example less than 20% of hypertension occurs in isolation and is usually accompanied by one or two additional risk factors (Kannel, 2000). Moreover, in the Multiple Risk Factor Intervention Trial (MRFIT) study (Stamler et al., 1993) a progressive increase of additional risk factors (cholesterol, BP and smoking) on top of T2D resulted in an incremental increase in age-adjusted CVD risk.

The global effect of weight loss will be discussed in each section as this is, for many, the primary driver for the improvements seen in a number but not all of the CVD risk factors.

Benefit of glycaemic control

The benefit of improved glycaemic control on CVD risk was first demonstrated by data from the UKPDS, which showed intensive glycaemic control improved both microvascular and macrovascular complications (UKPDS Study Group, 1998, Stratton et al., 2000, Gerstein et al., 2008, Holman et al., 2008). Over the years, the impact on CVD has been studied extensively however recently there has been suggestion that there is no benefit of intensive glycaemic control over standard care regarding the CVD mortality (Hemmingsen et al., 2011, Hemmingsen et al., 2013), although from the UKPDS this benefit appears to only be after extended follow-up (Holman et al., 2008). There is therefore now an increasing recommendation that glycaemic targets should be individually managed according to risk. With evidence that an HbA1c at both ends of the spectrum can be detrimental to all-cause mortality, it appears that managing within a more conservative range is ultimately more beneficial (Currie et al., 2010, Colayco et al., 2011).

As previously highlighted, HbA1c was significantly improved in both groups over the 24 week period (Table 3.4), there was a greater more sustained improvement following the LED and this was closer to national targets, potentially representing the greater improvement in a number of CVD risk factors. This is likely to translate to a greater reduction in microvascular disease (UKPDS Study Group, 1998). Also, given that HbA1c moved towards the recommended target range this could potentially have an impact on macrovascular disease, if sustained over the long-term (Stratton et al., 2000, Holman et al., 2008). Previous studies

have also seen similar reductions in HbA1c in T2D patients, but specifically within the context of looking at cardiac function. When compared with ILI using meal replacement in subjects with T2D as part of the Look AHEAD study, similar reductions were seen in comparison with the GSC group in the current study (-0.64%, SD 0.02) (Pi-Sunyer et al., 2007). This, however, was significantly less than the 1.4% reduction found within the LED group in this study. Within the Look AHEAD, there was a reduction in CVD risk with improvements seen in blood pressure, LDL-C, HDL-C and fasting glucose that, like HbA1c, were very similar to the reduction seen in the GSC group. This shows clearly that in comparison to best practice, the use of LED is superior in the treatment of T2D patients treated with insulin.

Hypoglycaemic risk and CVD

It has been repeatedly observed that with intensification comes an increased occurrence of severe hypoglycaemia (SH) (UKPDS Study Group, 1998, Stratton et al., 2000, Gerstein et al., 2008). The presence of SH, defined as a hypoglycaemic episode that require assistance, has been shown to be related to an increased risk of both adjusted all-cause and CVD mortality (Cha et al., 2016). Within a cohort study of 906 T2D patients followed over 10.4 years, the presence of SH was higher in those who were older, had longer duration of T2D, and those who were using insulin at baseline, with insulin treatment having a hazard ratio of 3.21 and 3.07 of SH predicting CVD and all-cause mortality (Cha et al., 2016). Therefore, SH would a major concern in a study using LED in T2D patients with longstanding diabetes who are treated with insulin.

Within this current study, there were no reported cases of severe hypoglycaemia. To prevent hypoglycaemia, an insulin titration algorithm was developed, which accommodated the dramatic reduction insulin requirements (Figure 2.3). The reduction of insulin dose algorithm was effective in the majority of patients; however, there were some individuals in both the LED and GSC group who on review had elevated blood sugars. Whether this was related to increased IR or non-adherence is difficult to determine, but it must be noted that frequent review is essential following the introduction of energy restriction in T2D patients treated with insulin.

Interestingly, there was no difference between the number of hypoglycaemic episodes experienced between the two groups at either 12 or 24 weeks; in fact, the GSC group had more episodes of hypoglycaemia per person over both 12-week periods. One possible explanation for this could be heightened sense with the LED group that they might have hypoglycaemia and therefore greater vigilance in testing compared to those in the GSC group. However, further analysis revealed that there were two subjects in the GSC that had an excessive number of hypoglycaemic episodes, which may have biased the data. When these were removed from analysis, the number of episodes reduced in the GSC group to 2.8 (SD 4.2) at 12-weeks and 4.3 (SD 5.6) in the LED group, although the differences between groups remained statistically non-significant. Finally, the standardised carbohydrate intake from the TDR may have made insulin titration and adjustment easier to manage resulting in less glucose variability and hypoglycaemic episodes.

The role of hypoglycaemia during VLED has not been previously studied; therefore, it is difficult to compare the results found here with previous work. However, in a recent study looking at the role of intermittent fasting with T2D subjects on oral OHA which used 2 non-consecutive days of VLED, found an increased risk of hypoglycaemia (RR 2.05) (Corley et al., 2018).

Lipids

It must be highlighted that the majority of the patients were treated with HMG-CoA reductase inhibitors. The benefit of statin use in lowering blood lipids and improving the dyslipidaemia linked with T2D is unequivocal. It should therefore be noted that the potential benefits seen may be conservative, and not represent the true effect on blood lipid profile if they had been stopped.

The role of blood lipids in CVD is well established and particularly in those that have overt diabetes (Chahil and Ginsberg, 2006). Key features of diabetes dyslipidaemia are low plasma HDL-C levels, high triglyceride (TG) levels, and also a higher proportion of small dense low-density lipoprotein that are suggested to be more atherogenic. In addition, in those with significant relative or absolute insulin deficiency this is linked with a decreased clearance of LDL due to a downregulation of the LDL receptor (Kissebah, 1987). LDL-cholesterol is now the primary lipid measurement to measure atherogenic lipoproteins as circulating LDL

particles are taken up by the endothelium of the artery wall, and once oxidised, promote inflammation and finally atherosclerosis (Veniant et al., 2000). LDL-C levels are an independent risk factor for CVD risk: CHD risk increases by nearly 40% for every 39mg/dL increase in LDL-C (Sharrett et al., 2001). More recently, however, it has been suggested that non-HDL-C is a better predictor of CVD risk than LDL-C (Sniderman et al., 2011, Boekholdt et al., 2012).

The only significant between group differences seen in blood lipids was a significant greater reduction of LDL-C at 12 weeks and TG at 24 weeks. All other lipid parameters did not reach statistical significance. Despite this, there did appear to be a transient improvement at 12 weeks in the LED group, with significant within group differences in total cholesterol, LDL-C, triglycerides, non HDL-C and TC to HDL-C ratio.

Although the reduction above did not reach statistical significance over the period of the study, they are superior to those observed with previously reported ILI including the Look AHEAD study (Pi-Sunyer et al., 2007, Schauer et al., 2012). In a systematic review comparing VLED and VLED and behaviour change, there was an inconsistent message regarding CVD risk. While HDL appeared to increase by a mean 0.06mmol/L, there was an apparent increase in LDL of 0.11mmol/L (Parretti et al., 2016). This pattern has not been seen in all studies. In the recent randomised control trial (RCT) using an LED in recently diagnosed T2D subjects, lipid parameters were reported not to change (HDL-C, TC), although like our present study there was a significant reduction in TG (-0.31mmol/L, SD 1.33) with a significant between group difference ($p<0.0001$). In more advanced T2D improvement has been reported in TG (-0 to -1.3mmol/L), TC (-0.6 to -1.1mmol/L), HDL (0 to 0.2mmol/L), LDL (-0.7 to -1.2mmol/L) (Hammer et al., 2008, Snel et al., 2011, Snel et al., 2012a).

The observed alterations in the current study are supported by previous work, where triglycerides have been seen to reduce by around 25%, while TC and LDL-C also generally reduce in those with T2D (Henry and Gumbiner, 1991). In those T2D patients within more advanced diabetes, using a 16-week VLED, there was generally a reduction in TC, TG and LDL-C (Jazet et al., 2007, Hammer et al., 2008, Snel et al., 2012a). The response of HDL-C levels varies amongst previous studies - usually demonstrating either a slight reduction or

remaining stable in the TDR phase, followed by a gradual increase (Christensen et al., 2011, Rolland and Broom, 2011).

HDL-C is an independent inverse predictor of CVD event risk and thought to be cardioprotective. Its role in this process are believed to include inhibition of LDL oxidation, reduction in vascular inflammation, and promotion of endothelial repair (Rye and Barter, 2014). Within the current study, there was no difference between HDL-C at either 12 or 24 weeks. There was, however, an increase between 12 to 24 weeks of 1.2mmol/L in the LED and 1.23mmol/L in the GSC group. The exact reason for this increase is unclear, but may related to the role of the metabolic change of HDL sub-fractions and also physical activity increasing HDL-C (Rolland and Broom, 2011).

IR plays a significant role with the abnormal lipid and lipoprotein metabolism seen in T2D (Chahil and Ginsberg, 2006). In the hyperinsulinemic IR state, there is an overproduction of very low-density lipoproteins, which in turn results in more TG particles, less HDL particles, and more small, dense LDL (Howard, 1999). Following weight loss, it is clear from the data that whole body IR improves with a reduction in insulin levels; therefore, it can be hypothesised that this was a key driver in the reductions seen in lipoprotein levels with the LED and GSC interventions. Finally, it must be highlighted that the role of dietary fats is key to the availability of cholesterol in the formation of lipoproteins in the body. It has therefore been recommended that obese individuals with T2D on insulin not only control their weight, but also look at altering their diet to facilitate reduction in plasma lipids and reduce their blood pressure (Diabetes UK, 2018). In a recent review of intake in the dietary management of diabetes, some key aspects were highlighted around dietary fatty acid intake. It has been recommended that focus should be on the reduction of saturated fat intake and replacement with polyunsaturated fat and not refined carbohydrate (Authors/Task Force Members et al., 2013, Evert et al., 2014, NICE, 2014a, Catapano et al., 2016, Diabetes UK, 2018), limiting intakes of trans fatty acids (Diabetes UK, 2018) and increasing the intake of omega-3 unsaturated fats in the form of oily fish (Burr et al., 1989, Delgado-Lista et al., 2012, Diabetes UK, 2018).

As noted with the previous chapter, dietary intake was analysed using a 3-day food diary- it would appear that there was significant underreporting in relation to all macronutrients and energy intakes, and although dietary fat was assessed this should be interpreted with caution.

There was a significant adjusted difference during the TDR phase in fat, saturated fat, polyunsaturated fat and monounsaturated fatty acids, with the LED group having significantly lower intakes. It can be seen from the product composition (see appendix 4) that each total dietary fat intake was between 16-17%, far less than the recommended (30%) by national guidelines, with a good balance of unsaturated to saturated fats and the inclusion of both alpha-linoleic and alpha-linolenic acids. This could in part explain the reduction in lipids seen during the TDR phase. The slight discrepancy in the values reported from DietPlan software, the nutritional information was due to some subjects eating additional food during the TDR phase. At 24 weeks, the distribution between SFA, PUFA and MUFA changed slightly from being in favour to PUFA to more evenly distributed as food was reintroduced.

The above was in contrast to the GSC group. Although there was a significant reduction in all key elements of fat intake at both 12 and 24 weeks, the relative distribution between the fatty acids, may not have reduced CVD risk with an increased intake of saturated and monounsaturated fats with a lower polyunsaturated. Closer analysis of the dietary intake would be beneficial to see what food components caused this distribution and this may be able to be linked to biomarker data. Clearly, risk is not just limited to the consumption of dietary fat and should not be done independently.

Finally, dietary fibre has been shown in the past to improve blood lipid profiles and reduce CHD and CVD risk (Buil-Cosiales et al., 2014). Dietary fibre is traditionally low in LED, accounting for only 10g per day; this was reflected in the diet diaries that showed a significant reduction in the TDR phase. Of interest was the significant reduction in both the LED and GSC at 24 weeks with intake being very low at 7.8g and 10.4g, respectively. This may have reflected the intake of dietary carbohydrate which was significantly reduced in both groups. Despite these small levels, a recent Cochrane systematic reviews concluded that there was insufficient evidence for dietary fibre in the primary prevention of CVD and whole grain cereal in both primary and secondary prevention of CVD (Hartley et al., 2016, Kelly et al., 2017, Clar et al., 2017). Therefore, at present the advice regarding fibre remains inconclusive regarding CVD risk benefit.

Liver Function

At baseline, the liver function tests including liver enzymes (alanine aminotransferase [ALT] and alkaline phosphatase [ALP]), albumin and bilirubin were controlled and within the normal range, which was not expected for this patient population (Harris, 2005). This might explain the relatively conservative reductions seen within the study population with such a significant weight loss. In previous studies that have shown weight loss improving liver function tests (Dixon et al., 2004), liver parameters were deemed abnormal and associated with NAFLD, which may reflect the significant reductions in LFT seen after weight loss.

The evidence regarding the use of role of VLED and LED in improving liver function is currently limited (Rolland et al., 2013) and no high quality evidence specifically examining those concurrently treated with insulin therapy. The few studies that have reported liver function outcomes often fail to have a comparator group resulting in just the impact of VLED or LED being presented. The result from the current study reflects in part, those seen in previous studies, where there was a reduction in liver function test (ALT and ALP) within the LED group that this did not translate into a between group difference (Arai et al., 1992, Melin et al., 2003, Rolland et al., 2009, Christensen et al., 2011). In the study by Christensen and colleagues, there was a reduction in both ALT of -2IU/L and ALP by -2.31IU/L and an increase in bilirubin of 0.73mmol/L, which was also observed in the current study as discussed below (Christensen et al., 2011). This lack of difference in ALT appears in part due to the significant body weight loss in both groups resulting in equal reductions in ALT.

The only two tests to show difference was that of ALP at 24 weeks and Bilirubin at both 12 and 24, which was associated with a significant increase. Of interest was the effect on bilirubin as this has not been previously reported or highlighted as significant following LED.

Bilirubin

Bilirubin is formed as a result of haemoglobin breakdown and although it has been linked to jaundice in elevated levels, it is now also recognised as an important antioxidant and anti-inflammatory (Stocker et al., 1987). Biliary sludge and formation of gallstones may occur after significant weight loss. However, these are associated with greater increases in bilirubin than observed in the current study. This suggests that the increase in bilirubin with weight

loss could reflect other changes. Interestingly, serum bilirubin concentrations are inversely related to CVD risk (Djousse et al., 2001, Perlstein et al., 2008), with experimental bilirubin inhibiting atherosclerosis and carotid plaque burden (Ishizaka et al., 2001) which is suggestive that raised bilirubin levels in fact may reduce the risk of CVD events. In an analysis of the National Health and Nutrition Examination Survey 1999-2004 data, adjusted multivariate analysis demonstrated that a $1.71\mu\text{mol/L}$ increase in bilirubin was associated with a 9% reduction in the odds of stroke (Perlstein et al., 2008). While in a meta-analysis examining bilirubin levels in men, it was shown that serum concentrations less than $10\mu\text{mol/L}$ was associated with increased CVD risk, while an increase of bilirubin by $1.0\mu\text{mol/L}$ reduced risk of CVD by 6.5% (Novotny and Vitek, 2003).

Instead of the increase in the LED being detrimental, this may in fact be suggestive that there is a cardioprotective element to this increase. This increase following weight loss has been seen elsewhere. In a sub-analysis of the Sibutramine Cardiovascular OUTcome (SCOUT) study (Andersson et al., 2009), an increase in bilirubin was found to be associated with weight loss, where each 1% increase of weight loss was associated with an increase in bilirubin of $0.19\mu\text{mol/L}$ in men and $0.09\mu\text{mol/L}$ in women. This increase in bilirubin has also been seen following using an LED with bilirubin being significantly increased at the end of the TDR phase (Gasteyger et al., 2008, Christensen et al., 2011). This relationship between bilirubin and LED improvement in risk has not been explored previously and presents an interesting avenue of future research.

Weight loss reducing liver fat – link with CVD risk and glycaemic control

Weight loss has been shown to be the most effective way of promoting the reduction in liver fat. Unfortunately, in this study there was no official assessment for the presence of NAFLD; therefore, it is difficult to say if there was a reduction in liver fat over the course of the study and may have been in part responsible for these improvements although this cannot be confirmed. There is, however, a suggestion from previous studies that LED reduces liver fat. Within the bariatric surgery cohort, VLED have been used preoperatively to aid the reduction of liver triglycerides to aid the ease of the operation (Fris, 2004, Colles et al., 2006, Lewis et al., 2006, Bottin, 2014, Nielsen et al., 2016). Using magnetic resonance imaging (MRI), it showed that weight loss was associated with a significant reduction in liver size, liver volume

and liver fat content, with the reduction in liver size being accurately predicted by the degree of weight loss (Fris, 2004). With the additional measures of reduction in waist circumference and lower trunk fat, we could hypothesise that fat loss from the liver has helped to drive these changes. Outside of bariatric surgery, other groups have also demonstrated an association between VLED and reduction in liver fat. In work by the Newcastle group, it was shown that following an 8 week VLED in obese patient with T2D there was a reduction in liver triacylglycerol from 12.8% to 2.9%, furthermore pancreatic fat also fell from 8.0 to 6.2% (Lim et al., 2011).

Blood pressure

Hypertension is a strong and consistent predictor for CVD, CHD, stroke and transient ischemic attacks (Emdin et al., 2015) and is twice as frequent in T2D compared to those with normal glucose tolerance (NGT) (Stokes et al., 1989, Sowers et al., 2001). In isolation, there is an indication from the literature that systolic blood pressure is a better predictor of CVD events than diastolic alone; however, the combination of both blood pressure parameters appears to be a better predictor of overall risk (Kannel, 2000). Reductions in blood pressure with weight loss are associated with dizziness observed when using LED and achieving rapid weight loss.

In a systematic review in obese subjects, it was observed that there was a reduction in both systolic (-1.9 to -8mmHg) and diastolic blood pressure (-1.7 to -6mmHg) over the period of VLED use (Parretti et al., 2016). In other studies of high-risk obese patients, the use of a VLED was associated with a reduction in SBP (-3 to -26mmHg) and DBP (-2.4 to 10mmHg) (Hammer et al., 2008, Jonker et al., 2014, Pedersen et al., 2015), which is similar to the trends to our study. The apparent variability in outcomes may be due to the heterogeneity of the study populations, the VLED or LED used, and the presence of prior hypertension. In the largest study of its kind, the use of a LED in recently diagnosed T2D showed that using an LED allowed discontinuation of blood pressure medication without a significant rise from baseline blood pressure (Lean et al., 2017).

Systolic blood pressure (SBP) was significantly reduced following both the LED and GSC at both time points (Figure 4.12). However, there was only a between group difference at 12-weeks (-10.2mmHg, 95% CI -15.3 to -5.2, $p<0.0001$), where weight loss was maximal. The

lack of difference at 24 weeks is explained by increasing blood pressure in the LED from 12 to 24-weeks. Despite this, there was still a highly significant reduction from baseline and clinically relevant in terms of potential CVD risk. A recent systematic review and meta-analysis reported that for each 10mmHg lowering of systolic blood pressure there was a significant reduction in all-cause mortality, CVD events, CHD events and stroke in patients with diabetes (Emdin et al., 2015).

With the re-introduction of food, despite maintenance of body weight loss, there appears to be association with a potential loss of the beneficial effect for both systolic and diastolic blood pressure in the LED. This may be related to diet quality as a whole and the reintroduction of increased amounts of salt, however this was not reflected from the food diaries, which are limited and demonstrates the unreliability of food diaries.

Diastolic blood pressure (DBP) was significantly lower in the LED and both 12 and 24-weeks compared with GSC. These reductions are likely to be explained by the weight loss achieved within both group over the period of the study.

It should be mentioned that the blood pressure overall was well controlled at baseline in both groups, as a patient population they present a high risk of CVD events, and therefore were on more aggressive management. Hypertensive management was accessed at baseline, revealing that 41.3% were on 2 medication, while 12.5% were on triple therapy. With national guidance recommending a blood pressure of less than 140/90mmHg and 130/80mmHg if in the presence of kidney, eye or cerebrovascular disease (NICE, 2015), this means both SBP and DBP met this criteria. Despite this, there is still call for more aggressive management with several meta-analyses concluding that systolic blood pressure should be managed below 130mmHg, particularly in higher risk patients (Emdin et al., 2015, Ettehad et al., 2016). This might suggest that instead of escalating medication that an LED could be used to reduce an individual's weight and potentially improve their blood pressure more holistically. Blood pressure reduction also has a beneficial effect on diabetes microvascular complications, in particular DKD and retinopathy.

Renal Function

Declining renal function is closely linked with the presence of both obesity and T2D (Maric-Bilkan, 2013). As mentioned previously, DKD is currently the leading cause of end stage renal disease (ESRD) (Ritz et al., 1999, Gross et al., 2005, Tuttle et al., 2014). In addition, obesity is also an independent risk factor for the development and progressing of chronic kidney disease (CKD) (Gelber et al., 2005, Hallan et al., 2006, Navaneethan et al., 2009), meaning that those obese T2D patients are at increased risk of developing ESRD. Once patients are on renal replacement therapy, the risk of mortality is markedly increased. Therefore, preventing DKD, by addressing DKD risk factors early, is essential for preventing more T2D patients on insulin progressing to ESRD.

Weight loss has been shown to attenuate CKD progression, normalising eGFR and reducing proteinuria, while also reducing the risk of CVD by 18% (Mykkanen et al, 1998; de Zeeuw et al., 2004). Bariatric surgery provides the most effective treatment (Alexander et al., 2007; Palomer et al., 2005; Navaro-Diaz et al., 2006; Chagnac et al, 2003; Agrawal et al., 2008). Despite this, the current evidence of the effect of VLED within the CKD population remains unclear (Mulholland et al., 2012, Rolland et al., 2013, Brown and Taheri, 2018) with much of the data being of too poor quality to help give recommendations for clinical practice.

Similar to previous studies that have used formula LED and VLED within patients with background CKD (Rolland et al., 2009), no changes were found in eGFR, urinary creatinine, urinary albumin and albumin to creatinine ratio or percentage of patients with microalbuminuria. The only significant between group difference was in eGFR at 12-weeks with an increase in eGFR of 3.8 mL/min/1.73 m². The lack of significant change seen within this population may be due to the participants having only slight renal impairment at baseline. At present there are only six studies (Vasquez et al., 1984, Saiki et al., 2005, Rolland et al., 2009, Malandrucco et al., 2012, Giordani et al., 2014) that have examined CKD and VLED. The lack of well-controlled studies, methodological heterogeneity, and studies involving differing stages of CKD, means that unpicking the effect of VLED is difficult. With the average eGFR of our population being 75.8 mL/min and 76.6 mL/min for the LED and GSC respectively, the study by Rolland and Colleague reflects our population the most (Rolland et al., 2009). Within the Rolland study, which compared a VLED to a low carbohydrate/high protein diet, there was a significant improvement in eGFR of 5.3 mL/min in the LED. This is

a slightly higher result than in the current study, but the heterogeneity between the two studies makes direct comparisons difficult.

Despite the reduction in ACR not reaching statistical significance, it could be argued that it was clinically relevant. The presence of microalbuminuria has independently been linked to the risk of CVD mortality in T2D patients (Bastard et al., 2000). Evidence from the Heart Outcomes Prevention Evaluation (HOPE) study showed that for every 0.4mg/mmol increase in albumin to creatinine ratio there is an increase in adjusted hazard ratio for major CVD events of 5.9% (Mattock et al., 1992). With an adjusted mean ACR reduction of -2.3mg/mmol, this could be of CVD benefit to these subjects. Indeed, with an increase being seen within the GSC group of 2.0mg/mmol at 24-weeks, the use of an LED in comparison to GSC could be seen as reducing risk.

It would appear that much of the data involving renal function might not have reached significance due to lack of power rather than the LED not improving function. When looking closer at the data, all assessments show a positive trend towards the LED group, but fail to reach statistical significance. There is a need for future research into the role of LED in T2D patients, targeting those with more advanced DKD.

Sleep

Obstructive sleep apnoea (OSA) is one of the most common sleep disturbances, although is regularly undiagnosed (Punjabi, 2008). T2D and OSA share common risk factors with the most important factor being obesity (Young et al., 2004). OSA is associated with increased risk of CVD, inflammation, metabolic disorders, and mortality (Marshall et al., 2008, Somers et al., 2008, Tasali and Ip, 2008, Arnardottir et al., 2009). Even mild OSA is associated with an increased risk of CVD mortality, and there is a dose-response relationship between OSA severity and CVD (Marin et al., 2005).

Currently, the actual prevalence of OSA among insulin treated T2D is unknown, as they are often not distinguished in studies of patients with T2D. Within this current study nearly one-third (29%) of subjects were diagnosed with moderate to severe OSA ($AHI \geq 15/h$ with an $ESS > 10$) at baseline. This is significantly less than reported previously in the secondary care

population, with reports of up to 86% being reported (Foster et al., 2009b), but are similar to the prevalence observed in the primary care population, where 44% of their diabetes population with OSA were treated with insulin (West et al., 2006).

There are very few treatment options currently available for those with OSA (Johansson et al., 2009) with the mainstay of treatment being continuous positive air pressure (CPAP) therapy. Weight loss has been shown to be highly effective in improving OSA symptoms, with a 10% weight loss being associated with a 26% reduction in AHI (Peppard et al., 2000). However, weight loss alone is insufficient to cure OSA unless highly significant. Even with significant weight loss after bariatric surgery, about a quarter of patients with prior OSA will continue to have some degree of OSA.

Despite evidence for the use of VLED and LED in OSA in those with obesity, there is no clear evidence for their use among the obese T2D population and particularly those treated with insulin.

The apnoea hypoxia index (AHI) is calculated as the number of apnoeas or hypopneas recorded over one night, per hour of sleep recording. Figure 4.14 shows AHI from baseline to 24-weeks between groups. AHI did not differ between the 2 groups at 24 weeks with an adjusted difference of -1.8 events/hour (95% CI -4.6 to 1.0, $p=0.218$). This being said, there was a significant reduction in AHI within the LED over the 24-week period with a reduction of -3.9 events/hour, representing a 31.5% reduction in AHI from baseline, similar to reduction seen elsewhere (Tuomilehto et al., 2009). Within this study a VLED was used alongside lifestyle in 72 obese patients with mild sleep apnoea to see if this could be used to help treat OSA. Similar to the LED group they had a reduction of -4.9 events/hour, however they reached statistical significance possibly due to the increase in their control (Tuomilehto et al., 2009).

Those subjects that lost 10kg from their initial weight was associated with a -4.8 events/hour reduction in AHI, while those that lost less had an increase in events of 0.1 per hour ($p=0.06$). Although this did not reach statistical significance, it appears that the degree of weight loss is important to AHI improvement, in line with previous reports (Johansson et al., 2009, Johansson et al., 2011).

Oxygen desaturation index (ODI) is the number of oxygen desaturation events over an hour exceeding 4% from baseline. These episodes of oxygen desaturation have been linked with diabetes risk and reduced insulin sensitivity (Lindberg et al., 2012). ODI was shown to be significantly reduced in the LED by -7.2 events per hour, with those that lose the greatest weight loss showing the greatest alleviation of ODI event ($p=0.04$). Previous studies have also seen a significant reduction in ODI (-26.3 event/hour) after a VLED and that this reduction can be seen in the long-term (Kajaste et al., 2004). The impact on ODI is particularly significant, given that hypoxaemia is a key driver of the pathophysiology associated with OSA (Dewan et al., 2015).

One key feature that would have benefited this investigation would have been recording neck circumference. This was reported in two of the studies (Johansson et al., 2009, Johansson et al., 2011) and has been identified as an independent predictor of overnight hypoxic dipping in OSA (Stradling and Crosby, 1991). Despite there being some similarities within the data presenting in several of the studies investigating the role of VLED and OSA it has to be noted that there is also a significant heterogeneity in terms of patient population being studied (Kajaste et al., 2004, Johansson et al., 2009, Johansson et al., 2011). Much of the prevailing literature focused on moderate to severe sleep apnoea and uses polysomnography to diagnose OSA meaning that the direct comparison in treatment effect is difficult. Other data from obtained regarding oxygenation parameters were not significant either within or between group (Table 4.5). This is likely to be due to the low number of cases with very severe OSA.

The current study also examined sleepiness using the Epworth Sleep Scale (ESS) (Johns, 1991) and sleep quality using the Pittsburgh Sleep Quality Index (PQSI) (Buysse et al., 1989) were assessed. The analyses from both these questionnaires indicated that there was no between group difference in either daytime sleepiness or sleep quality between the LED and GSC groups.

Despite there being no apparent difference between groups, the questionnaires were able to identify some interesting data regarding the subject population. The data from the ESS showed that there was no excessive daytime sleepiness from baseline scores with group scores both being below 10. At 24 weeks, daytime sleepiness reduced significantly in the LED reducing by -1.4 (SD 1.4, $p<0.001$) suggesting that within the group, the average sleep propensity was reduced. Similar to our study, Tuomilehto found that ESS reduced

significantly within LED with a drop of -2.1, but no change was then found between groups (Tuomilehto et al., 2009). Within the direct comparison with baseline Johansson and colleagues also reported a significant reduction in ESS of -2 (-1 to -3) following the VLED however again this was not maintained, despite weight maintenance (Johansson et al., 2011).

Data from the PSQI identified that obese T2D patients have poor sleep quality with both groups having score of 6.7, where greater than 5 indicates poor sleep (Buysse et al., 1989). At 24 weeks similar, to the ESS, there was a significant reduction in PSQI, with a reduction of -1.0 (SD 3.0, $p < 0.05$). Poor sleep quality has previously been reported in patients with T2D (Lecube et al., 2016), with almost half being reported as “poor sleepers”. Within the current study, similar figures were observed with approximately 50% identifying that they had poor sleep, with no difference being found between the groups at either 12 and 24 weeks ($p = 0.912$, $p = 0.256$, respectively). From the literature, poor sleep has been linked to worsening glucose control, quality of life, diabetes complications and hyperglycaemia (Lamond et al., 2000, Knutson et al., 2006, Tsai et al., 2012, Lecube et al., 2016). There is also likely to be a reciprocal relationship between sleep and glycaemic control.

Based on data from the current study no benefits to sleep appear to occur with the initial 12-week TDR phase of the LED. Instead both sleep quality and daytime sleepiness improvements were delayed until Week 24. This may correspond with weight stabilising, as there has been a side effect of sleepiness have been reported during VLED TDR phases (Christensen et al., 2011, Lean et al., 2017) that there are sleep disturbances, which would correspond with the changes seen. Alternatively, the impact of weight changes on sleep quality may manifest after a longer period than is seen in the current study.

CVD risk assessment

T2D patients with longer than 10 years’ diabetes duration and using insulin therapy have the greatest presence of CHD and CVD (Hu et al., 2001, Wannamethee et al., 2011, Rana et al., 2016). This chapter so far has presented data that demonstrate that there is a reduction in individual surrogate markers of CVD risk, but this fails to demonstrate a reduction in CVD risk. The use of modelling to assess and predict CVD risk within clinical practice to help guide treatment has now been recognised (Hippisley-Cox et al., 2008, Simmons et al., 2009), and as a result, The National Institute for Health and Care Excellence (NICE) guidelines

recommend the use of predictive models (NICE, 2014a). Two models were chosen from the multitude available as they each answered different questions. First the impact on CVD risk was assessed. The QRISK2 model was chosen as it has been recommended by NICE; in addition, it includes multiple other risk factors including T2D, ethnicity, and CKD. (Hippisley-Cox et al., 2008, Hippisley-Cox et al., 2017). The QRISK2 model is updated from the QResearch Database, helping to ensure the modelling is accurate. The second model employed was the Framingham coronary heart disease risk score (Wilson et al., 1998) as a prediction of 10 year mortality. It must be considered that risk algorithms all have various limitations that have been extensively discussed by others (Simmons et al., 2009, Kengne et al., 2010).

From data included within the model, there was a significant between group difference in 10-years CVD risk, with there being a -5.8% reduction in the LED group and a smaller -2.9% in the GSC. This benefit unfortunately was reduced at 24 weeks meaning that although there was a difference, it was no longer statistically significant (adjusted difference -1.8%, 95% CI -3.5 to 0.0, $p=0.055$, Table 4.7). This change in risk was probably due to the alteration in systolic blood pressure seen at 24 weeks in the LED group. Despite this, there was a significant reduction in both groups, showing the overall benefit of weight loss, reduction in blood pressure, and improvement of blood lipids on risk.

CHD mortality risk showed no difference using an LED compared to GSC at either 12 or 24 weeks. However, there was a significant within group difference for both LED and GSC. Ten-year heart disease risk estimators have previously only been used once in other studies using VLED (Jazet et al., 2007). Although there was no difference between those VLED with or without exercise, this previous study did show a positive benefit in 10-year CHD risk estimates from baseline.

One key mechanism could be energy restriction itself, whether using the LED or GSC. In a study looking at the effect of extended energy restriction on atherosclerosis, it was shown that, compared to a typical American diet, there was a significant improvement in inflammation, SBP, DBP, all lipid measures and fasting glucose. Furthermore, carotid artery intima thickness was significantly reduced, demonstrating the potential benefit of chronic energy restriction (Fontana et al., 2004). Although the current study was not specifically

designed to elicit this type of data, the analyses of the risk models do show the overall benefit of improving surrogate markers of CVD and CHD risk.

Chapter 5: Conclusions

5.1. Summary of main findings of study

- This is the largest study to assess the role of a formula low energy diet intervention in those with advanced T2D treated with insulin.
- The use of a formula low energy diets in obese patients with T2D mellitus was observed to be an effective way of managing weight loss compared to gold standard clinical care.
- Total insulin usage was significantly reduced alongside a reduction in HbA1c with the use of the LED approach.
- Thirteen subjects in the LED group discontinued insulin over the 24-week period, with the majority achieving this in the initial 12 weeks.
- Improvements in whole body insulin resistance appear to drive the improvements seen in glycaemic control in insulin treated T2D patients rather than an increase in insulin secretion.
- The effect of LED on liver metabolism and the increase in serum bilirubin levels might have a beneficial role.
- It remains unclear whether the LED approach improves kidney, liver and lipid profiles greater than gold standard clinical care.

5.2 Conclusions and Limitations

This body of work in this thesis was designed to evaluate the potential role of a formula low energy diet approach within obese T2D patients concurrently treated with insulin. These investigations found that subjects on a LED lost significantly more weight, had greater improvements in their glycaemic control and a greater reduction in their insulin usage. These glycaemic benefits appear to be related to an improvement in whole body insulin sensitivity rather than an improvement in insulin secretion, although future data is needed to confirm this.

Like many studies there were strengths and limitations to the study and these will be discussed in more detail below.

The comparator arm of this study is unique in that it was designed to elicit weight loss as well. This approach was also shown to have significant beneficial effects, but less than those

observed with the LED. In addition unlike other studies within this patient group, subjects continued to use their insulin due to the advanced stages of their diabetes. This makes the study more applicable to those patients within clinical practice that would be unable to stop all diabetes medications due to poor glycaemia control and beta-cell failure. It will be important to assess cost-effectiveness in the future to determine the best approach to complex obese T2D patients in daily clinical practice. The use of the LED approach may obviate the use of bariatric surgery in select patients. Many patients prefer a medical approach to their obesity and diabetes and the LED approach appears to provide an important option for these patients. It will be necessary to conduct a health economic analysis prior to introduction of the LED into clinical services.

In relation to the recent data from Lean and colleagues (Lean et al., 2017), this study adds and starts to provide a more complete picture of how weight loss influences outcome in more advanced stage of T2D. The findings reinforce previous work (Snel et al., 2012a) indicating that with advanced T2D patients, insulin resistance and the liver play a key role glycaemic improvement.

From previous data, there have been concerns whether patients on insulin would respond like those with greater residual pancreatic function and less medication burden, as data had suggested they would not (Steven and Taylor, 2015). The findings in this thesis suggest that some individuals respond better than others, coming off insulin treatment. Developing approaches to identify the degree of response to LED may allow greater tailoring of this intervention approach.

In order to understand further the mechanisms through which LED regulates energy intake during and after weight loss experimental work was carried out using both subjective and objective measures. An unexpected finding was in relation to the effect that the LED on appetite. Unlike previous published data there was a significant increase in subjective fullness and a trend towards reduced hunger, desire to eat and appetite for a meal. This requires further study including measures of appetite hormones.

The limitations of the study have been discussed throughout the thesis. The major limitation is the short-term nature of the study. It will be important to examine the longer-term outcomes of the intervention, and this is currently underway. Some of the limitations of the

work are related to the methodologies employed to measure various parameters. For example, the assessment of dietary intake is an inherent problem in nutrition research and has been shown to result in under reporting of actual dietary intake. This means that any conclusion drawn regarding dietary change following the LED is possibly biased and do not report what has really happened. It is therefore the plan to use urinary samples to measure the metabolomic profile that enable a more accurate assessment of dietary intake. This will be the plan for future work as 24-hour urine collections were taken at the 3 assessment visits and will aid to see how diet changed over time using biomarkers.

A variety of confounding variables that could not be controlled for due to the patient group under study may have masked the degree to which the LED benefit some parameters such as the use of HMG-CoA reductase inhibitors influence on changes in lipoprotein and hypertensive medication on blood pressure. Stopping blood pressure medication may have given a clearer picture of the potential benefits of the LED on blood pressure. HMG-CoA reductase inhibitors are, however, cost-effective and can be used in conjunction with the LED approach. In the future algorithms to adjust these medications would be of benefit.

The role of fasting insulin with the T2D patient treated with insulin is difficult to unpick often due to the use of exogenous insulin. From the current data it is difficult to tease out the effect of intervention on fasting insulin, as subjects remained on exogenous insulin throughout the study, apart from those that ceased treatment. Due to the advanced years of diabetes and high insulin doses, stopping insulin for long periods of time like other studies that previously enrolled this patient population was not practical. In retrospect, the measurement of C-peptide levels may well have been a better indicator of β -cell function than insulin due to the continued use of exogenous insulin by many of the subject. In the future, clamp studies may clarify the metabolic pathways involved in T2D treated with insulin that respond to LED intervention.

There remain several unanswered questions regarding the precise mechanism by which the LED brings about the improvement in glycaemic control in those treated with insulin. From the present data this appears to be related to improvement insulin sensitivity and disposal of glucose in a postprandial state. Although an important limitation is that the present study used surrogate indices of insulin sensitivity some of which were not appropriate for use with insulin patients and therefore the results should be viewed with caution. In addition, this does

not answer if there is any change in lipid metabolism, which might be expected to play a role within the improvements seen due to the role of lipotoxicity in T2D aetiology. More detailed study is required including insulin clamps, imaging of key organs such as the liver and the pancreas, and more in-depth profiling of systemic and organ-level metabolic changes.

Due to the advanced stages of disease progression, it seems logical that the β -cell is unable to “reawaken” easily like those found among patients with early onset diabetes. Therefore, the role of pancreatic triglycerides may not be key in this patient population, although this needs confirmation with

Despite this being the largest randomised study within this specific patient population and was powered to reliability answer the primary outcome hypothesis; the sample size may have been underpowered to address all the secondary outcomes and also account for missing data. This can be seen with several outcomes that although have shown trends toward improvement, did not reach statistical significance. To minimise this appropriate statistical analyses were conducted and avoidance of violating assumptions.

Taken together this study represents a significant step forward in the treatment of obese T2D patients treated with insulin. With nearly half the patients achieving 15kg weight loss at 24-weeks, this is far greater than any other dietary study within the same population that have continued IT during the LED intervention. The significant reduction in insulin usage in combination with a reduction of HbA1c, improvements in triglyceride and inflammation is of great clinical relevance to not just diabetes related outcomes but also to patient quality of life. If these observations are shown to be sustained in the longer-term, this will translate to incorporation of the LED intervention into clinical care and future services.

Appendix 1. Patient Information Sheet



Professor Gary Frost

Division of Diabetes, Endocrinology and Metabolism
6th Floor Commonwealth Building
Imperial College Hammersmith Campus
DuCane Road
London, W12 0NN

PATIENT INFORMATION LEAFLET – Invitation

A Randomised Controlled Trial Assessing the Impact of a Low Calorie Diet on Weight Loss in Obese Patients with Type 2 Diabetes Mellitus Treated with Insulin

Dear Patient,

Thank you for taking time to read this. You have been invited to take part in this research study because you have type 2 diabetes and are on insulin therapy. We are looking to see if the use of low calorie diets could be an effective treatment for weight loss and reducing or stopping your insulin therapy. If you would like to take part in the study we will need you to provide consent.

This information sheet will provide you with more detail about the study so that you can make an informed decision whether or not to take part. Please ask if there is anything that is not clear or if you would like more information.

Thank you for your time.

Best wishes,

A handwritten signature in black ink, appearing to read "G Frost".

Professor Gary Frost
Chief Investigator

Introduction

What is the purpose of this study?

The main purpose of the research is to assess if using, a liquid meal replacement low calorie diet produces better weight loss and reduces or stops the need for insulin compared to good clinical care alone.

You will be allocated to either receive treatment with the formula low calorie diet (Cambridge Weight Plan) or good clinical care. The number of patients in each treatment group will be the same and you will have an equal chance of receiving either treatment. The allocation will be decided by chance, which is much like tossing a coin. This allocation process is called 'randomisation.'

Why is the research needed?

Type 2 Diabetes is a major risk factor for cardiovascular disease renal failure and other complications. Insulin therapy is often needed in type 2 diabetes to help control your blood sugars to help reduce these complications. However, insulin therapy can lead to weight gain. Therefore, there is a need to develop interventions to help patients reduce or even come off insulin therapy. Research has shown that using low calorie diets can produce significant weight loss. This research study will test if weight loss through a liquid meal replacement low calorie diet can help you lose weight and reduce or even help you come off your insulin and help reduce the complications associated with your diabetes.

Why have I been invited?

You have been invited because you are between aged 18-70 years, have type 2 diabetes, are on insulin, and have a BMI greater or equal to 30kg/m². This study is looking for 90 patients to take part.

Do I have to take part?

No. It is up to you to decide whether or not to take part. Only when you are satisfied that you have been given enough information about the study and have decided that you would like to take part, will you be asked to sign the consent form. If you decide to take part, you will be asked to sign a consent form and will be given a copy of this

information sheet along with a copy of your signed consent for you to keep for your own records. You will be given up to 2 weeks to decide whether or not you would like to participate. If you decide to take part you are still free to withdraw your consent at any time and without giving a reason. A decision to withdraw or not to take part will not affect the standard of care you receive in the future.

Who cannot take part in this research study?

Before you consent to participate in this research study we will ask you questions about your general health. If you have certain medical problems then we will kindly ask you not to participate because taking the formula low calorie diet could further complicate or worsen your current medical condition. The following conditions will mean that you will be unable to take part in the study.

- Pregnant or planning to become pregnant within the next 12 month.
- Currently participating in a weight loss research study or have participated in weight loss study in the past 3-months.
- If any problems arise during the study which may worsen your condition then we will immediately tell you to stop participating and we will notify your GP for suitable treatment.
- Hypoglycaemia unawareness – this means you are not aware you are having a low blood sugar (less than 4 mmol/l). As there is a risk of having low blood sugars during the study it is important that you are aware of this to ensure that you are safe.
- Fasting c-peptide less than 600 if you have been on insulin for longer than 10 years – To avoid putting you at risk of very high blood sugars, we will measure your c-peptide at screening to ensure that you are still able to produce your own insulin.

What will I have to do?

You will be asked to attend 11 visits over a period of 12 months. A final appointment will be scheduled at 24 months to assess the maintenance of weight loss. Therefore, you are required to have a lot of participation in this study. The first visit is a screening visit for the research team to assess that you are eligible to take part in the study which will take approximately 2 hours. Thereafter, the additional 10 visits will

make up key assessments and check-ups. The check-ups will also last approximately 1 hour and the 3 key assessments will each last up to 6 hours.

Will participating in this research affect my routine care?

This research will happen alongside your routine care. We will try and co-ordinate your visits as much as possible to be carried at your local GP practice to avoid unnecessary visits. If any abnormalities are found during the study that may affect your health and wellbeing, we will inform your clinical team and your GP, if appropriate, so that any issues can be addressed. If you choose to withdraw at any time from the study, you will continue to be provided with the appropriate routine clinical care.

What are the risks of taking part?

The risks associated with taking part within the study are minimal. Although they include:

- Hypoglycaemia – The team will help educate you to manage your low blood sugar and therefore reduce the risk. In addition we will ask you to monitor your blood sugars to see if you are at risk of low blood sugars. This is very important and will help reduce a risk associated with having a low blood sugar.
- Time – this study will require you to attend the Imperial College Hammersmith Campus on a regular basis and therefore this should be considered before you consent to taking part.
- Blood sample – during the study we will take blood samples at screening and 4 larger assessment visits. At screening and 6 months we will take up to 30ml of blood and at the 3 larger assessment visits we will take up to 250ml. These will be 3 months apart. To put this into context a blood donation is 470ml of blood each time and this can be done every 3 months.

What are the possible benefits of taking part?

There are no direct benefits from you taking part in the study. You will be involved in a study that will hopefully help to improve the management of patients within type 2 diabetes on insulin.

Will my taking part in the study be kept confidential?

Your participation in the research study will be kept strictly confidential. We will follow ethical and legal practice in accordance with the Data Protection Act (1998). Your information will be respected and handled in confidence unless you disclose that you, or someone else, are in immediate danger of serious harm. Access to identifiable data (name, address, etc) will be limited only to selected members of the research team and will be anonymised. Your personal details will not be included in data analysis, publications or reports. You will be given a unique number and all information collected from you during the study will be recorded under this code so that you cannot be identified.

All data will be kept on secure password protected computers and in locked filing cabinets within locked offices at Imperial College London. If you consent to take part in the study, your medical records will be looked at by people from regulatory authorities and ethics committees to check that the study is being or has been carried out properly. If you give us permission we may contact your GP to ask them to provide information from your records, which are required for the study. However, your name and identity will not be recorded or disclosed outside the study clinic.

What happens when the research study stops?

At the end of the study, a newsletter with the main findings of the study will be sent to all the patients who were involved in the research by your study doctor. The chief investigator will also be happy to speak to local support groups and GP surgeries and engage the public with the specific findings of this study. Your study doctor and your GP will discuss future treatment options with you.

Taking Part in the Research

What if new information becomes available?

If new information becomes available during the research, we will discuss this with you and it is up to you to decide whether you wish to continue or withdraw from the study. If you decide to continue, we will ask you to sign a new consent form.

What will happen if I do not want to carry on with the research study?

The study is voluntary so if you decide to withdraw at anytime, without giving a reason, this will not affect your usual care. If you should decide to withdraw from the research then you should tell your study doctor or member of the research team. They will make sure that proper procedures are followed and a final visit is made for your safety. If you should withdraw we will ask your permission to use the data collected up to the time you withdraw. If you say no or we do not receive an answer, your samples will be destroyed.

When can I be taken out of the research?

You will only be taken out of the research if the study cancels; you need treatment that is not part of this research; continuing in this research is harmful to your health, you do not follow the research procedures, or if you become pregnant.

What will happen if I do not understand something, or there is a problem?

Imperial College London holds insurance policies which apply to this study. If you experience serious and enduring harm or injury as a result of taking part in this study, 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 Investigator, Gary Frost, 02083838037). The normal National Health Service complaints mechanisms are also available to you. If you are still not satisfied with the response, you may contact the Imperial AHSC Joint Research Compliance Office.

If you wish to complain formally, you can do this through the normal complaint mechanisms available to you through the NHS by contacting Patient Advice and

Liaison Service (PALS) through their website www.pals.nhs.uk or at the following numbers for your area.

Imperial College London: Charing Cross, Hammersmith and Queen Charlotte's & Chelsea hospitals on 020 3313 0088; Email: pals@imperial.nhs.uk

Who is organising and carrying out this research?

This study is being organised by Professor Gary Frost, who is a professor at Imperial College London. The study will be run by Imperial College London. .

Who is funding this research?

This research is funded by the Cambridge Manufacturing Company Limited, UK. Your study doctor or any other members of the research team that are involved in your treatment and care are not paid for entering you into this research and will not receive payment for carrying out this study.

What insurance do people who are carrying out this research have?

The Imperial College London is legally liable as the sponsor and has in force a Public Liability Policy and Clinical Trials policy, which provides cover for claims, negligent, and non-negligent harm arising from the activities are included within that coverage.

What will happen to the results of this study?

Anonymised results of this study will be presented at relevant conferences and symposiums (e.g. Diabetes UK conferences) as a means of early communication. Full reports and papers will then be prepared for publication in high impact medical, nutrition, and diabetes related journals (e.g. British Medical Journal, British Journal of Clinical Nutrition, Diabetes, and American Journal of Clinical Nutrition). The reports and papers that are published about the research will not identify you or any other patient who participated in this research.

Who has reviewed the research?

All research in the NHS is looked at by an independent group of people called a Research Ethics Committee to protect your safety, rights, wellbeing, and dignity. This research has been reviewed and given favourable opinion by the NRES Committee West Midlands-Solihull ..

When do I have to decide by if I want to take part in this study?

You can take up to 2 weeks to decide if you wish to take part in this study.

Contact details

If you have any further questions please contact Adrian Brown on 07989599549 or email on weightloss@imperial.ac.uk

If you have any questions regarding your rights as a study participant or about the way this study is conducted please contact your

Research team project manager: Becky Ward,

Address: Joint Research Compliance Office, Imperial College London, Room 221, Medical School Building, St Marys Campus, Norfolk Place, London, W2 1PG

Tel: 0207 594 9459

We would like to thank you for reading the Patient information sheet and for considering taking part in this study. Please feel free to talk to your family and friends about the study. If you have any further questions please feel free talk to the study doctor before considering entry into this study.

Appendix 2. Consent Form



CONSENT FORM

LOW CALORIE DIET: TYPE 2 DIABETES ON INSULIN

A Randomised Controlled Trial Assessing the Impact of a Low Calorie Diet on Weight Loss in Obese Patients with Type 2 Diabetes Treated with Insulin

Name of Chief Investigator: Professor Gary Frost

Patient ID: _____

Please
initial
here

1. I confirm that I have read and understood the patient information sheet for the Low Calorie Diet: Type 2 Diabetes. I have had the opportunity to ask questions and have had these answered to my satisfaction. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving a reason, and without my medical care or legal rights being affected. ☐
3. I understand that all information collected from me will be held in confidence and that my personal details will not appear in any publication. ☐
4. I understand that sections of my medical notes may be looked at by researchers and the regulatory authorities. I give permission for these individuals to have access to my records. ☐
5. I understand that my GP will be notified of my participation in this study. ☐
6. I understand that blood samples collected may be stored for research purposes. ☐
7. I agree to take part in the above research study. ☐

Name of patient

Signature

Date

Name of investigator/designee

Signature

Date

Appendix 3. Energy summary of test meals

Standardised Test Meal at start of MMTT

Standardised Meal	Approximate Analysis	Per 100ml	Per bottle
Ensure Plus (220ml)	Energy (kcal)	150	330
	Fat (g)	4.92	10.8
	Carbohydrate (g)	20.20	44.4
	Including sugar (g)	6.50	14.3
	Protein (g)	6.25	13.8

Standardised *Ad Libitum* Meal

Standardised Meal	Ingredients	Quantity
Tomato & Herb Pasta <i>Ad Libitum</i> Meal	Sainsbury's pasta shapes, basic (cooked as per manufacturers instructions)	1000g
	Sainsbury's pasta sauce, tomato and herbs (cooked as per manufacturers instructions)	1000g
	Sainsbury's olive oil	50ml

Appendix 4. Nutritional Breakdown of Formula Low Energy Diet Products

Cambridge[®]
Pro800

INGREDIENT AND NUTRITIONAL INFORMATION:

Soups

NUTRITION INFORMATION: Typical analysis when mixed with water

How to make your tasty soup:

It only takes a couple of minutes to create the perfect soup. Simply pour a minimum of 200ml of hot (not boiling) water into a container, then add the contents of the sachet to the water. Using a blender or whisk, mix until completely smooth. Once thoroughly mixed, drink your delicious product within 15 minutes for full nutritional benefit.

PLEASE DO NOT MICROWAVE.

Additional information:

For best results, store your soup sachets in a cool, dry place.

You need to drink at least 2.25 litres (4 pints) of calorie-free liquid throughout the day when following any Pro800 programme.

		Per 100ml when mixed with water	% of RDA per 100ml	Per 54g serving	% of RDA per serving	Per 4 servings
Energy	kJ	350.0		878.0		3512.0
	kcal	83.4		209.0		836.0
Protein	g	5.1		12.9		51.5
Carbohydrate	g	11.7		29.6		118.4
of which sugars	g	3.0		7.6		20.4
starch	g	7.8		19.8		79.2
Fat	g	1.5		3.8		15.2
of which saturates	g	0.3		0.8		3.2
mono-unsaturates	g	0.4		1.0		4.0
polyunsaturates	g	0.7		1.9		7.6
linoleic acid	g	0.7		1.7		6.8
linolenic acid	g	0.08		0.2		0.8
cholesterol	mg	0.3		0.7		2.8
Fibre	g	1.0		2.5		10.0
Sodium	g	0.2		0.6		2.4
Vitamin A	µg	103.9	13	264.0	33	1056.0
Vitamin D	µg	0.6	13	1.7	33	6.6
Vitamin E	mg	1.6	13	4.0	33	15.8
Vitamin C	mg	10.4	13	26.4	33	105.6
Thiamin	mg	0.1	13	0.4	33	1.5
Riboflavin	mg	0.2	13	0.5	34	1.9
Niacin	mg	2.1	13	5.4	34	21.6
Vitamin B6	mg	0.2	13	0.5	33	1.8
Folacin	µg	26.0	13	66.0	33	264.0
Vitamin B12	µg	0.3	13	0.8	33	3.3
Biotin	µg	6.5	13	16.5	33	66.0
Pantothenic acid	mg	0.8	13	2.0	33	7.9
Vitamin K	µg	9.8	13	24.8	33	99.2
Calcium	mg	108.2	14	274.9	34	1100.0
Phosphorus	mg	98.7	14	250.7	36	1003.0
Iron	mg	2.0	14	5.2	37	20.6
Magnesium	mg	49.4	13	125.5	33	502.1
Zinc	mg	1.5	15	3.8	38	15.2
Iodine	µg	20.3	14	51.6	34	206.3
Potassium	mg	307.0	15	781.0	39	3124.0
Chloride	mg	433.0	54	1100.0	138	4400.0
Copper	mg	0.2	19	0.5	47	1.9
Manganese	mg	0.4	18	0.9	45	3.6
Selenium	µg	8.0	15	20.4	37	81.5
Molybdenum	µg	6.8	14	17.3	35	69.4
Chromium	µg	5.4	13	13.6	34	54.4
Fluoride	mg	0.5	13	1.2	33	4.7

Each Pro800 soup contains at least 33% of the RDA (Recommended Daily Allowance) as given in Commission Directive 2008/100/EC and complies fully with Directive 96/8/EC (Foods Intended for Use in Energy Restricted Diets for Weight Reduction).

INGREDIENTS

Mushroom flavour: Maltodextrin, Soya flour, Skimmed milk powder, Soya protein isolate, Flavouring, Potassium chloride, Salt, Hydrolysed vegetable protein, Dried mushrooms, Calcium carbonate, Compound vitamin and mineral mixture*, Stabiliser: xanthan gum, Magnesium oxide, Soya lecithin, Monocalcium phosphate, Malt extract. **Allergens:** Contains gluten, milk and soya. Suitable for vegetarians.

Vegetable flavour: Maltodextrin, Soya flour, Skimmed milk powder, Soya protein isolate, Flavouring, Salt, Dried vegetables (carrot, onion), Potassium chloride, Compound vitamin and mineral mixture*, Calcium carbonate, Inulin, Stabiliser: xanthan gum, Magnesium oxide, Soya lecithin, Monocalcium phosphate, Colour: beta-carotene. **Allergens:** Contains gluten, milk, celery and soya. Suitable for vegetarians.

***Compound vitamin and mineral mixture:** Maltodextrin, Ascorbic acid, Ferrrous fumarate, Nicotinamide, Vitamin E acetate, Sodium fluoride, Zinc oxide, Calcium D-pantothenate, Copper gluconate, Vitamin A acetate, Pyridoxine hydrochloride, Thiamin hydrochloride, Riboflavin, Potassium iodate, Chromic chloride, Folic acid, Sodium molybdate, Sodium selenite, Vitamin K, Manganese sulphate, D-Biotin, Vitamin D3, Vitamin B12.

Important allergen information: All soups have been produced in a factory that handles nuts and peanuts.

Cambridge[®]
Weight Plan

Cambridge Weight Plan
Hatton House, Hunters Road,
Corby, Northants, NN17 5JE

t 01536 403344 f 01536 202396
e medical@cambridgeweightplan.co.uk
w cambridgeweightplan.co.uk

PRO800 CAD013 February 2012

Shakes

NUTRITION INFORMATION: Typical analysis when mixed with water

How to make your tasty shake:

If only takes a couple of minutes to create the perfect shake. Simply pour a minimum of 200ml of cold water into a container, then add the contents of the sachet to the water. Using a blender or whisk, mix until completely smooth. Once thoroughly mixed, drink your delicious product within 15 minutes for full nutritional benefit.

Please note: Some shake flavours also make a delightful warm drink — simply mix with warm water (not boiling). PLEASE DO NOT MICROWAVE.

Cambridge Mix-a-Mousse[®] can also be used to convert any shake flavour into a delicious dessert. Simply follow the instructions on the Mix-a-Mousse container.

Additional information:

For best results, store your shake sachets in a cool, dry place.

You need to drink at least 2.25 litres (4 pints) of calorie-free liquid throughout the day when following any Pro800 programme.

		Per 100ml when mixed with water	% of RDA per 100ml	Per 54g serving	% of RDA per serving	Per 4 servings
Energy	kJ	335.0 (350.0)		848.0 (878.0)		3392.0 (3512.0)
	kcal	79.7 (83.4)		202.0 (209.0)		808.0 (836.0)
Protein	g	5.1		12.9		51.5
Carbohydrate	g	11.7		29.6		118.4
of which sugars	g	7.6 (3.0)		19.4 (7.6)		77.6 (20.4)
starch	g	3.9 (7.8)		9.9 (19.8)		39.6 (79.2)
Fat	g	1.2 (1.5)		3.0 (3.8)		12.0 (15.2)
of which saturates	g	0.3		0.8		3.2
mono-unsaturates	g	0.3 (0.4)		0.7 (1.0)		2.8 (4.0)
polyunsaturates	g	0.5 (0.7)		1.3 (1.9)		5.2 (7.6)
linoleic acid	g	0.5 (0.7)		1.2 (1.7)		4.8 (6.8)
linolenic acid	g	0.04 (0.08)		0.1 (0.2)		0.4 (0.8)
cholesterol	mg	1.7 (0.3)		4.2 (0.7)		16.8 (2.8)
Fibre	g	1.0		2.5		10.0
Sodium	g	0.08 (0.2)		0.2 (0.6)		0.8 (2.4)
Vitamin A	µg	103.9	13	264.0	33	1056.0
Vitamin D	µg	0.6	13	1.7	33	6.6
Vitamin E	mg	1.6	13	4.0	33	15.8
Vitamin C	mg	10.4	13	26.4	33	105.6
Thiamin	mg	0.1	13	0.4	33	1.5
Riboflavin	mg	0.2	13	0.5	34	1.9
Niacin	mg	2.1	13	5.4	34	21.6
Vitamin B6	mg	0.2	13	0.5	33	1.8
Folacin	µg	26.0	13	66.0	33	264.0
Vitamin B12	µg	0.3	13	0.8	33	3.3
Biotin	µg	6.5	13	16.5	33	66.0
Pantothenic acid	mg	0.8	13	2.0	33	7.9
Vitamin K	µg	9.8	13	24.8	33	99.2
Calcium	mg	179.7 (108.2)	22 (14)	456.5 (274.9)	57 (34)	1826 (1100)
Phosphorus	mg	141.8 (98.7)	20 (14)	360.1 (250.7)	51 (36)	1440 (1003)
Iron	mg	2.0	14	5.2	37	20.6
Magnesium	mg	49.4	13	125.5	33	502.1
Zinc	mg	1.5	15	3.8	38	15.2
Iodine	µg	20.3	14	51.6	34	206.3
Potassium	mg	307.0	15	781.0	39	3124.0
Chloride	mg	197.0 (433)	25 (54)	500.0 (1100)	62.5 (138)	2000.0 (4400)
Copper	mg	0.2	19	0.5	47	1.9
Manganese	mg	0.4	18	0.9	45	3.6
Selenium	µg	8.0	15	20.4	37	81.5
Molybdenum	µg	6.8	14	17.3	35	69.4
Chromium	µg	5.4	13	13.6	34	54.4
Fluoride	mg	0.5	13	1.2	33	4.7

Each Pro800 shake contains at least 33% of the RDA (Recommended Daily Allowance) as given in Commission Directive 2008/100/EC and complies fully with Directive 96/8/EC (Foods Intended for Use in Energy Restricted Diets for Weight Reduction). Figures in brackets refer to Summer Fruits.

INGREDIENTS

Banana flavour (with sweetener):

Skimmed milk powder, Maltodextrin, Inulin, Refined soya oil, Flavouring, Potassium chloride, Stabiliser: xanthan gum, Compound vitamin and mineral mixture**, Magnesium oxide, Milk protein, Sweetener: aspartame*, Colour: beta-carotene. **Allergens:** Contains milk and soya. Suitable for vegetarians.

Chocolate flavour (with sweetener):

Skimmed milk powder, Maltodextrin, Reduced fat cocoa powder, Refined soya oil, Inulin, Flavouring, Stabiliser: xanthan gum, Compound vitamin and mineral mixture**, Potassium chloride, Magnesium oxide, Milk protein, Sweetener: aspartame*. **Allergens:** Contains milk and soya. Suitable for vegetarians.

Summer Fruits flavour (with sweetener):

Maltodextrin, Soya protein isolate, Inulin, Refined soya oil, Potassium chloride, Vegetable oil, Tricalcium phosphate, Flavouring, Salt, Compound vitamin and mineral mixture**, Stabiliser: xanthan gum, Soya lecithin, Monocalcium phosphate, Colour: beetroot powder, Magnesium oxide, Milk protein, Sweetener: aspartame*. **Allergens:** Contains milk and soya. Suitable for vegetarians.

Vanilla flavour (with sweetener):

Skimmed milk powder, Maltodextrin, Inulin, Refined soya oil, Potassium chloride, Flavouring, Stabiliser: xanthan gum, Compound vitamin and mineral mixture**, Magnesium oxide, Milk protein, Sweetener: aspartame*. **Allergens:** Contains milk and soya. Suitable for vegetarians.

*Contains source of phenylalanine

**Compound vitamin and mineral mixture:

Maltodextrin, Ascorbic acid, Ferrous fumarate, Nicotinamide, Vitamin E acetate, Sodium fluoride, Zinc oxide, Calcium D-pantothenate, Copper gluconate, Vitamin A acetate, Pyridoxine hydrochloride, Thiamin hydrochloride, Riboflavin, Potassium iodate, Chromic chloride, Folic acid, Sodium molybdate, Sodium selenite, Vitamin K, Manganese sulphate, D-Biotin, Vitamin D3, Vitamin B12.

Important allergen information: All shakes have been produced in a factory that handles nuts and peanuts.

Appendix 5. Questionnaire Booklet



A Randomised Controlled Trial Assessing the Impact of a Low Calorie Diet on Weight Loss in Obese Patients with Type 2 Diabetes Mellitus Treated with Insulin

Patient ID:

--	--	--	--	--	--

Practice:

--	--	--	--	--	--

Date:

--	--	--	--	--	--

QUESTIONNAIRE: Baseline/ Three Months / Six Months / Twelve Months

Dear Participant,

Thank you for taking part in the above mentioned Study. This booklet contains some questions about your health, sleep and your diabetes. Your answers will help us understand your health and well-being regarding your diabetes. The answers you give will remain anonymous and confidential to the study team.

Read the directions carefully and tick a box (☑) or circle the number (⑤) that corresponds to the answer that best describes how you feel. Please do not select more than one answer per question unless you are asked to do so – just answer the question as best you can. Please remember:

THERE ARE NO RIGHT OR WRONG ANSWERS!

If you have any questions or concerns about completing this questionnaire booklet, please speak to the Adrian Brown whose details are at the bottom of this page.

Thank you for taking the time to help us with this study.

Adrian Brown 6th Floor
Commonwealth Building
Imperial College Hammersmith Campus
DuCane Road
London, W12 0NN
Email: weightloss@imperial.ac.uk

We won't ask for your name in this booklet, but we would like to start by asking about you.

How would you describe your marital status?

☐	Single, never married	☐	Divorced
☐	Married	☐	Widowed
☐	Living with partner	☐	Civil partnership
☐	Separated		

How would you describe your ethnic background?

☐	White: British	☐	Any other Chinese background
☐	White: Irish	☐	Asian or Asian British: Indian
☐	Any other White background	☐	Asian or Asian British: Pakistani
☐	Mixed: White and Black Caribbean	☐	Asian or Asian British: Bangladeshi
☐	Mixed: White and Black African	☐	Any other Asian background
☐	Mixed: White and Asian	☐	Black or Black British: Caribbean
☐	Any other Mixed Race background	☐	Black or Black British: African
☐	Chinese	☐	Any other Black background

What is your current smoking status?

☐	Non-smoker
☐	Ex-smoker
☐	Current smoker

If you are an ex-smoker, when did you stop smoking? years ago

If you are an ex-smoker, how many cigarettes did you smoke per day?

If you are a current smoker, how many cigarettes do you smoke per day?

Epworth Sleepiness Scale

Use the following scale to choose the most appropriate number for each situation:

- 0 = no chance of dozing
- 1 = slight chance of dozing
- 2 = moderate chance of dozing
- 3 = high chance of dozing

- | | |
|--|--------------------------|
| 1. Sitting and reading | ☐ |
| 2. Watching TV | ☐ |
| 3. Sitting inactive in a public place (ie. theatre or meeting) | ☐ |
| 4. As a passenger in a car for an hour without a break | ☐ |
| 5. Lying down to rest in the afternoon when circumstances permit | ☐ |
| 6. Sitting and talking to someone | ☐ |
| 7. Sitting quietly after a lunch without alcohol | ☐ |
| 8. In a car while stopped for a few minutes in traffic | ☐ |

TOTAL
.....

The Pittsburgh Sleep Quality Index (PSQI)

Instructions: The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

During the past month,

1. When have you usually gone to bed? _____
2. How long (in minutes) has it taken you to fall asleep each night? _____
3. When have you usually gotten up in the morning? _____
4. How many hours of actual sleep do you get at night? (this may be different from the number of hours you spend in bed) _____

5. During the past month, how often have you had trouble sleeping because you...	Not during the past month (0)	Less than once a week (1)	Once or twice a week (2)	Three or more times a week (3)
a. Cannot get to sleep within 30 minutes				
b. Wake up in the middle of the night or early morning				
c. Have to get up to use the bathroom				
d. Cannot breathe comfortably				
e. Cough or snore loudly				
f. Feel too cold				
g. Feel too hot				
h. Have bad dreams				
i. Have pain				
j. Other reason(s), please describe, including how often you have had trouble sleeping because of this reason(s):				
6. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?				
7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?				

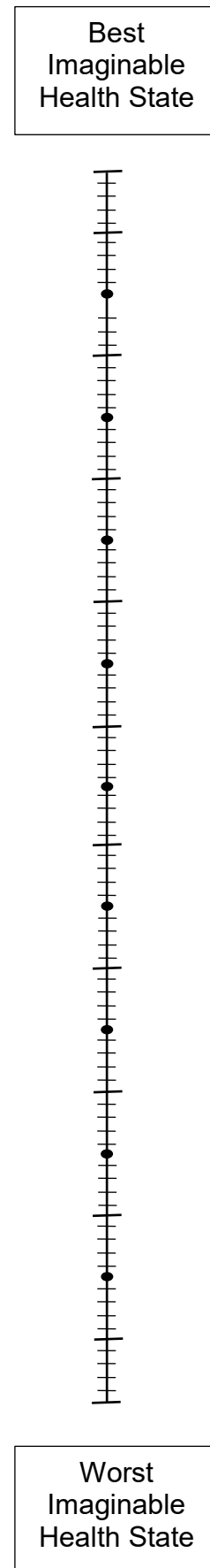
8. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?				
--	--	--	--	--

	Very good (0)	Fairly good (1)	Fairly bad (2)	Very bad (3)
9. During the past month, how would you rate your sleep quality overall?				

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.

**Your own
health state
today**



Blood Glucose Monitoring

We would like to know about your blood glucose levels.

Please use the space below to write down your blood glucose readings (BG) taken before and 2 hours after each main meal on three consecutive days. This can be recorded on your food diary below

Three Day Food Diary

Please fill out the diary as accurately as possible. Use the example food diary on the next page as a guide of how to fill in your Diary.

- 1) Write down all the food and drinks you consume and what activity you do for at least 3 days (including a weekend). Include all snacks and/or supplements if you have any. Include all alcoholic and all non-alcoholic drinks you consume.
- 2) Record the time that you eat the meal or snack.
- 3) Describe the food in as much detail as possible e.g. cooking methods and portion size.
- 4) Rate your hunger before you eat using the hunger scale below.
- 5) Write down where you are and who you are with when eating and drinking.
- 6) Record your mood/feelings and/or any symptoms and the time they occur.
- 7) Review your diary after you have completed it to see where you could do things differently. Identify which times/ places/ people/ feelings/ moods put you at high risk of moving away from your healthy lifestyle plan.
- 8) Remember this food diary is for you so that you can become more aware of your food and drink intake.

Hunger Scale:

1	2	3	4	5	6	7	8	9	10
Physically Faint	Ravenous	Fairly Hungry	Slightly Hungry	Neutral	Pleasantly satisfied	Full	Stuffed	Bloated	Nauseous

An example of how to fill in your diary

Date: _____

Food/Drink/Activity	Quantity	Where/Who with	Feelings/Symptoms
Breakfast (8.00am) Cornflakes Milk, blue top White sugar Tea Milk, green top White sugar Walk slow pace	6 tbsps ¼ pint 2 tsp ½ cup 1 tbsp 1 tsp 10 minutes	At home, sitting at the table Alone Alone around the block	Hunger=3
Mid morning (11.00am) Walkers salt and vinegar crisps Ribena	1 packet (28g) 2 tbsps made up to 1 glass	At work With 1 colleague-Sue	Hunger=5 Bored
Lunch (12.30pm) Large medium sliced white bread Scrape of butter Boiled ham Tomato Diet Ski yoghurt Coca-cola	2 slices 1 tsp 1 thin slice (25g) 1 medium 1 pot (150g) 1 can (330ml)	At work With colleagues-Martin, Edward and Tina	Hunger=4 Feeling bloated
Mid afternoon (3.30pm) McVitie's milk chocolate digestive biscuits Coffee made with ½ green top milk White sugar	3 ½ cup of milk 1 tsp	At work With colleagues-Savan	Hunger=5 Friends all eating as well
(5.00 pm) raw carrots	2 medium	At home Alone	Hunger=5 Cooking tea and handy to nibble
Evening meal (7.30pm) Ross beef burgers (grilled) Baked beans Mashed potatoes with added butter and milk Walls vanilla ice cream	2 2 tbsps 2 tbsps of potato with 1 tbsp butter and 2 tbsps milk 3 scoops	At home With partner	Hunger=3 Felt full after I had eaten
Bedtime (9.00pm) Hot chocolate made with silver top milk Rich tea biscuits	1 cup of milk 3	At home watching television Alone	Hunger=5 Habit, routine, biscuits

This as an example of a diary- not a suggestion of what to eat.

Date: _____

Food/Drink/Activity	Quantity	Where/Who with	Feelings/Symptoms
Breakfast ()			Hunger= BG Before = BG 2 hrs After =
Mid morning ()			Hunger=
Lunch ()			Hunger= BG Before = BG 2 hrs After =
Mid afternoon ()			Hunger=
Evening meal ()			Hunger= BG Before = BG 2 hrs After =
Bedtime ()			Hunger=

Appendix 6. Summary of weight loss strategies to aid weight loss with insulin

This table summaries the effects of the different weight loss strategies on weight loss, surrogate markers of cardiovascular disease and insulin reduction within the literature at present

Weight Loss Strategies	FU (months)	WL (kg)	HbA1c (%)	FPG	BP (mmHg)		Lipids (mmol/mol)		Insulin Reduction (unit)	Insulin (U kg ⁻¹ day ⁻¹)
					SBP	DBP	T.Chol	LDL		
Intensive Lifestyle	6-60	-7.7 to +3.5	-1- to +0.3	-0.8	-1.9 to -4	-2 to -4.2	-0.2	-0.1	-	-
Orlistat	6-12	-3.9 to -7.1	-0.62	-	-	-	-	-	-8.1 to -30	0.64-0.85
GLP-1	6	-1.4 to -5.7	-0.1 to -1.81	-	7	2	-0.12	-0.07	-18.1 to -29	0.25-0.89
Pramlintide	4-12	-1.1 to -2.5	-0.53 to -0.73	-1.6 to -1.7	-	-	-	-	-2.1 to 11.7	0.48-0.73
SGLT-2 Inhibitor	12-24	-0.9 to -3.5	-0.58 to -1.01	-1.05 to -1.89	-0.5 to -7.5	-1.2 to -2.9	-4.1 to +0.7	-9.3 to +1.7	-0.9 to +4.1	0.61-0.9
Low CHO diets	6-24	-0.7 to -11.1	-0.02 to -1.5	-0.1 to -1.1	-16.6 to +5	-8.1 to +0.2	-0.1 to -0.7	-0.5 to +0.1	-7 to -49.3	0.45-0.69
VLED - Initial	1-4	-12.4 to -27.2	-	-	-	-	-	-	-	-
VLED – Long term	4-18	-8.6 to -15.4	-0.2 to -3.8	-0.3 to -4.3	-16 to +1	-9 to +1	-0.02 to -1.1		-	0-0.34
Bariatric Surgery	12-60	53.8 to 68.8 (%EWL)	-1 to -2.1	-2.3 to -4	-3.3 to -8.3	-5.8 to -8.1		-0.5 to +0.1	-62.3 to -72	0.47-0.77

NOTE: FU = follow up, WL = weight loss, EBWL = excess body weight loss, HbA1c = glycated haemoglobin, FBG = fasting blood glucose, BP = blood pressure, SBP = systolic blood pressure, DBP diastolic blood pressure, TChol = total cholesterol, LDL= low density lipoproteins, DM=diabetes, U kg⁻¹ day⁻¹=units per kilogram per day. Taken from (Brown et al., 2017).

References

- AAS, A. M., OHRVIK, J., MALMBERG, K., RYDEN, L. & BIRKELAND, K. I. 2009. Insulin-induced weight gain and cardiovascular events in patients with type 2 diabetes. A report from the DIGAMI 2 study. *Diabetes Obes Metab*, 11, 323-9.
- ABEL, E. D., PERONI, O., KIM, J. K., KIM, Y. B., BOSS, O., HADRO, E., MINNEMANN, T., SHULMAN, G. I. & KAHN, B. B. 2001. Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver. *Nature*, 409, 729-33.
- ABRAIRA, C., DUCKWORTH, W. C. & MORITZ, T. 2009. Glycaemic separation and risk factor control in the Veterans Affairs Diabetes Trial: an interim report. *Diabetes Obes Metab*, 11, 150-6.
- ADA, A. D. A. 2014a. Diagnosis and classification of diabetes mellitus. *Diabetes Care*, 37 Suppl 1, S81-90.
- ADA, A. D. A. 2014b. Standards of medical care in diabetes-2014. *Diabetes Care*, 37 Suppl 1, S14-80.
- ADA, A. D. A. 2016. Standards of Medical Care in Diabetes - Approaches to Glycemic Treatment. *Diabetes Care*, 39 Suppl 1, S52-9.
- ADA, A. D. A. 2018. Cardiovascular Disease and Risk Management: Standards of Medical Care in Diabetes 2018. *Diabetes Care*, 41, S86-S104.
- ADAMS, T. D., ARTERBURN, D. E., NATHAN, D. M. & ECKEL, R. H. 2016. Clinical Outcomes of Metabolic Surgery: Microvascular and Macrovascular Complications. *Diabetes Care*, 39, 912-23.
- ADLER, A. I. 2008. UKPDS-modelling of cardiovascular risk assessment and lifetime simulation of outcomes. *Diabet Med*, 25 Suppl 2, 41-6.
- AFSHIN, A., FOROUZANFAR, M. H., REITSMA, M. B., SUR, P., ESTEP, K., LEE, A., MARCZAK, L., MOKDAD, A. H., MORADI-LAKEH, M., NAGHAVI, M., SALAMA, J. S., VOS, T., ABATE, K. H., ABBAFATI, C., AHMED, M. B., AL-ALY, Z., ALKERWI, A., AL-RADDADI, R., AMARE, A. T., AMBERBIR, A., AMEGAH, A. K., AMINI, E., AMROCK, S. M., ANJANA, R. M., ARNLOV, J., ASAYESH, H., BANERJEE, A., BARAC, A., BAYE, E., BENNETT, D. A., BEYENE, A. S., BIADGILIGN, S., BIRYUKOV, S., BJERTNESS, E., BONEYA, D. J., CAMPOS-NONATO, I., CARRERO, J. J., CECILIO, P., CERCY, K., CIOBANU, L. G., CORNABY, L., DAMTEW, S. A., DANDONA, L., DANDONA, R., DHARMARATNE, S. D., DUNCAN, B. B., ESHRATI, B., ESTEGHAMATI, A., FEIGIN, V. L., FERNANDES, J. C., FURST, T., GEBREHIWOT, T. T., GOLD, A., GONA, P. N., GOTO, A., HABTEWOLD, T. D., HADUSH, K. T., HAFEZINEJAD, N., HAY, S. I., HORINO, M., ISLAMI, F., KAMAL, R., KASAEIAN, A., KATIKIREDDI, S. V., KENGNE, A. P., KESAVACHANDRAN, C. N., KHADER, Y. S., KHANG, Y. H., KHUBCHANDANI, J., KIM, D., KIM, Y. J., KINFU, Y., KOSEN, S., KU, T., DEFO, B. K., KUMAR, G. A., LARSON, H. J., LEINSALU, M., LIANG, X., LIM, S. S., LIU, P., LOPEZ, A. D., LOZANO, R., MAJEED, A., MALEKZADEH, R., MALTA, D. C., MAZIDI, M., MCALINDEN, C., MCGARVEY, S. T., MENGISTU, D. T., MENSAH, G. A., MENSINK, G. B. M., MEZGEBE, H. B., MIRRAKHIMOV, E. M., MUELLER, U. O., NOUBIAP, J. J., OBERMEYER, C. M., OGBO, F. A., OWOLABI, M. O., PATTON, G. C., et al. 2017. Health Effects of Overweight and Obesity in 195 Countries over 25 Years. *N Engl J Med*, 377, 13-27.

- ALLER, E. E., LARSEN, T. M., CLAUS, H., LINDROOS, A. K., KAFATOS, A., PFEIFFER, A., MARTINEZ, J. A., HANDJIEVA-DARLENSKA, T., KUNESOVA, M., STENDER, S., SARIS, W. H., ASTRUP, A. & VAN BAAK, M. A. 2014. Weight loss maintenance in overweight subjects on ad libitum diets with high or low protein content and glycemic index: the DIOGENES trial 12-month results. *Int J Obes (Lond)*, 38, 1511-7.
- ANDERSON, J. W., KONZ, E. C., FREDERICH, R. C. & WOOD, C. L. 2001. Long-term weight-loss maintenance: a meta-analysis of US studies. *Am J Clin Nutr*, 74, 579-84.
- ANDERSSON, C., WEEKE, P., FOSBOL, E. L., BRENDORP, B., KOBER, L., COUTINHO, W., SHARMA, A. M., VAN GAAL, L., FINER, N., JAMES, W. P., CATERSON, I. D., RODE, R. A. & TORP-PEDERSEN, C. 2009. Acute effect of weight loss on levels of total bilirubin in obese, cardiovascular high-risk patients: an analysis from the lead-in period of the Sibutramine Cardiovascular Outcome trial. *Metabolism*, 58, 1109-15.
- ARAI, K., MIURA, J., OHNO, M., YOKOYAMA, J. & IKEDA, Y. 1992. Comparison of clinical usefulness of very-low-calorie diet and supplemental low-calorie diet. *Am J Clin Nutr*, 56, 275s-276s.
- ARDESTANI, A., RHOADS, D. & TAVAKKOLI, A. 2015. Insulin cessation and diabetes remission after bariatric surgery in adults with insulin-treated type 2 diabetes. *Diabetes Care*, 38, 659-64.
- ARNARDOTTIR, E. S., MACKIEWICZ, M., GISLASON, T., TEFF, K. L. & PACK, A. I. 2009. Molecular Signatures of Obstructive Sleep Apnea in Adults: A Review and Perspective. *Sleep*, 32, 447-70.
- ARNER, P. 1995. Differences in lipolysis between human subcutaneous and omental adipose tissues. *Ann Med*, 27, 435-8.
- ARTERBURN, D. E., BOGART, A., SHERWOOD, N. E., SIDNEY, S., COLEMAN, K. J., HANEUSE, S., O'CONNOR, P. J., THEIS, M. K., CAMPOS, G. M., MCCULLOCH, D. & SELBY, J. 2013. A multisite study of long-term remission and relapse of type 2 diabetes mellitus following gastric bypass. *Obes Surg*, 23, 93-102.
- ASCASO, J. F., PARDO, S., REAL, J. T., LORENTE, R. I., PRIEGO, A. & CARMENA, R. 2003. Diagnosing insulin resistance by simple quantitative methods in subjects with normal glucose metabolism. *Diabetes Care*, 26, 3320-5.
- ASTRUP, A., MEINERT LARSEN, T. & HARPER, A. 2004. Atkins and other low-carbohydrate diets: hoax or an effective tool for weight loss? *Lancet*, 364, 897-9.
- ASTRUP, A. & ROSSNER, S. 2000. Lessons from obesity management programmes: greater initial weight loss improves long-term maintenance. *Obes Rev*, 1, 17-9.
- AUTHORS/TASK FORCE MEMBERS, AU - RYDÉN, L., AU - GRANT, P. J., AU - ANKER, S. D., AU - BERNE, C., AU - COSENTINO, F., AU - DANCHIN, N., AU - DEATON, C., AU - ESCANED, J., AU - HAMMES, H.-P., AU - HUIKURI, H., AU - MARRE, M., AU - MARX, N., AU - MELLBIN, L., AU - OSTERGREN, J., AU - PATRONO, C., AU - SEFEROVIC, P., AU - UVA, M. S., AU - TASKINEN, M.-R., AU - TENDERA, M., AU - TUOMILEHTO, J., AU - VALENSI, P., AU - ZAMORANO, J. L., (CPG), A.-E. C. F. P. G., AU - ZAMORANO, J. L., AU - ACHENBACH, S., AU - BAUMGARTNER, H., AU - BAX, J. J., AU - BUENO, H., AU - DEAN, V., AU - DEATON, C., AU - EROL, Ç., AU - FAGARD, R., AU - FERRARI, R., AU - HASDAI, D., AU - HOES, A. W., AU - KIRCHHOF, P., AU - KNUUTI, J., AU - KOLH, P., AU - LANCELLOTTI, P., AU - LINHART, A., AU - NIHOYANNOPOULOS, P., AU - PIEPOLI, M. F., AU - PONIKOWSKI, P., AU - SIRNES, P. A., AU - TAMARGO, J. L., AU - TENDERA, M., AU - TORBICKI, A., AU - WIJNS, W., AU -

- WINDECKER, S., REVIEWERS, A.-D., AU - DE BACKER, G., AU - SIRNES, P. A., AU - EZQUERRA, E. A., AU - AVOGARO, A., AU - BADIMON, L., AU - BARANOVA, E., AU - BAUMGARTNER, H., AU - BETTERIDGE, J., AU - CERIELLO, A., AU - FAGARD, R., AU - FUNCK-BRENTANO, C., AU - GULBA, D. C., AU - HASDAI, D., AU - HOES, A. W., AU - KJEKSHUS, J. K., AU - KNUUTI, J., AU - KOLH, P., AU - LEV, E., AU - MUELLER, C., AU - NEYSES, L., AU - NILSSON, P. M., AU - PERK, J., AU - PONIKOWSKI, P., AU - REINER, Ž., AU - SATTAR, N., AU - SCHÄCHINGER, V., AU - SCHEEN, A., AU - SCHIRMER, H., AU - STRÖMBERG, A., AU - SUDZHAIEVA, S., AU - TAMARGO, J. L., AU - VIIGIMAA, M., AU - VLACHOPOULOS, C. & AU - XUEREB, R. G. 2013. ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD The Task Force on diabetes, pre-diabetes, and cardiovascular diseases of the European Society of Cardiology (ESC) and developed in collaboration with the European Association for the Study of Diabetes (EASD). *European Heart Journal*, 34, 3035-3087.
- AVENELL, A., BROOM, J., BROWN, T. J., POOBALAN, A., AUCOTT, L., STEARNS, S. C., SMITH, W. C., JUNG, R. T., CAMPBELL, M. K. & GRANT, A. M. 2004. Systematic review of the long-term effects and economic consequences of treatments for obesity and implications for health improvement. *Health Technol Assess*, 8, iii-iv, 1-182.
- BAILYES, E. M., NAVE, B. T., SOOS, M. A., ORR, S. R., HAYWARD, A. C. & SIDDLE, K. 1997. Insulin receptor/IGF-I receptor hybrids are widely distributed in mammalian tissues: quantification of individual receptor species by selective immunoprecipitation and immunoblotting. *Biochem J*, 327 (Pt 1), 209-15.
- BALKAU, B., HOME, P. D., VINCENT, M., MARRE, M. & FREEMANTLE, N. 2014. Factors associated with weight gain in people with type 2 diabetes starting on insulin. *Diabetes Care*, 37, 2108-13.
- BARRATT, R., FROST, G., MILLWARD, D. J. & TRUBY, H. 2008. A randomised controlled trial investigating the effect of an intensive lifestyle intervention v. standard care in adults with type 2 diabetes immediately after initiating insulin therapy. *Br J Nutr*, 99, 1025-31.
- BARRY, E., ROBERTS, S., FINER, S., VIJAYARAGHAVAN, S. & GREENHALGH, T. 2015. Time to question the NHS diabetes prevention programme. *BMJ : British Medical Journal*, 351.
- BASTARD, J. P., JARDEL, C., BRUCKERT, E., BLONDY, P., CAPEAU, J., LAVILLE, M., VIDAL, H. & HAINQUE, B. 2000. Elevated levels of interleukin 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. *J Clin Endocrinol Metab*, 85, 3338-42.
- BELFORT, R., MANDARINO, L., KASHYAP, S., WIRFEL, K., PRATIPANAWATR, T., BERRIA, R., DEFRONZO, R. A. & CUSI, K. 2005. Dose-Response Effect of Elevated Plasma Free Fatty Acid on Insulin Signaling. *Diabetes*, 54, 1640-1648.
- BERGMAN, R. N. 2000. Non-esterified fatty acids and the liver: why is insulin secreted into the portal vein? *Diabetologia*, 43, 946-52.
- BERRINGTON DE GONZALEZ, A., HARTGE, P., CERHAN, J. R., FLINT, A. J., HANNAN, L., MACINNIS, R. J., MOORE, S. C., TOBIAS, G. S., ANTON-CULVER, H., FREEMAN, L. B., BEESON, W. L., CLIPP, S. L., ENGLISH, D. R., FOLSOM, A. R., FREEDMAN, D. M., GILES, G., HAKANSSON, N., HENDERSON, K. D., HOFFMAN-BOLTON, J., HOPPIN, J. A., KOENIG, K. L., LEE, I. M., LINET, M. S., PARK, Y., POCOBELLI, G., SCHATZKIN, A., SESSO, H. D., WEIDERPASS, E., WILLCOX, B. J., WOLK, A., ZELENIUCH-

- JACQUOTTE, A., WILLETT, W. C. & THUN, M. J. 2010. Body-mass index and mortality among 1.46 million white adults. *N Engl J Med*, 363, 2211-9.
- BLIDDAL, H., LEEDS, A. R., STIGSGAARD, L., ASTRUP, A. & CHRISTENSEN, R. 2011. Weight loss as treatment for knee osteoarthritis symptoms in obese patients: 1-year results from a randomised controlled trial. *Ann Rheum Dis*, 70, 1798-803.
- BLUNDELL, J., DE GRAAF, C., HULSHOF, T., JEBB, S., LIVINGSTONE, B., LLUCH, A., MELA, D., SALAH, S., SCHURING, E., VAN DER KNAAP, H. & WESTERTERP, M. 2010. Appetite control: methodological aspects of the evaluation of foods. *Obes Rev*, 11, 251-70.
- BOEKHOLDT, S. M., ARSENAULT, B. J., MORA, S., PEDERSEN, T. R., LAROSA, J. C., NESTEL, P. J., SIMES, R. J., DURRINGTON, P., HITMAN, G. A., WELCH, K. M., DEMICCO, D. A., ZWINDERMAN, A. H., CLEARFIELD, M. B., DOWNS, J. R., TONKIN, A. M., COLHOUN, H. M., GOTTO, A. M., JR., RIDKER, P. M. & KASTELEIN, J. J. 2012. Association of LDL cholesterol, non-HDL cholesterol, and apolipoprotein B levels with risk of cardiovascular events among patients treated with statins: a meta-analysis. *Jama*, 307, 1302-9.
- BOLLI, G. B. & DEVRIES, J. H. 2015. New long-acting insulin analogs: from clamp studies to clinical practice. *Diabetes Care*, 38, 541-3.
- BORG, P., KUKKONEN-HARJULA, K., FOGELHOLM, M. & PASANEN, M. 2002. Effects of walking or resistance training on weight loss maintenance in obese, middle-aged men: a randomized trial. *Int J Obes Relat Metab Disord*, 26, 676-83.
- BOTTIN, J., BALOGUN, B., THOMAS, E.L., FITZPATRICK, J.A., MOORTHY, K., LEEDS, A.R., BELL, J., FROST, G. 2014. Changes in body composition induced by pre-operative liquid low-calorie diet in morbid obese patients undergoing Roux-en-Y gastric bypass. *Obesity Reviews*, 15, 129-176.
- BOULE, N. G., HADDAD, E., KENNY, G. P., WELLS, G. A. & SIGAL, R. J. 2001. Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. *Jama*, 286, 1218-27.
- BRETZEL, R. G., NUBER, U., LANDGRAF, W., OWENS, D. R., BRADLEY, C. & LINN, T. 2008. Once-daily basal insulin glargine versus thrice-daily prandial insulin lispro in people with type 2 diabetes on oral hypoglycaemic agents (APOLLO): an open randomised controlled trial. *Lancet*, 371, 1073-84.
- BROD, M., KONGSO, J. H., LESSARD, S. & CHRISTENSEN, T. L. 2009. Psychological insulin resistance: patient beliefs and implications for diabetes management. *Qual Life Res*, 18, 23-32.
- BROWN, A., FROST G AND TAHERI, S 2015. Is there a place for low-energy formula diets in weight management. *British Journal of Obesity*, 3, 84-119.
- BROWN, A., GOULDSTONE, A., FOX, E., FIELD, A., TODD, W., SHAKHER, J., BELLARY, S., TEH, M. M., AZAM, M., JOHN, R., JAGIELSKI, A., ARORA, T., THOMAS, G. N. & TAHERI, S. 2015. Description and preliminary results from a structured specialist behavioural weight management group intervention: Specialist Lifestyle Management (SLiM) programme. *BMJ Open*, 5, 1-10.
- BROWN, A., GUESS, N., DORNHORST, A., TAHERI, S. & FROST, G. 2017. Insulin-associated weight gain in obese type 2 diabetes mellitus patients: What can be done? *Diabetes Obes Metab*, 19, 1655-1668.
- BROWN, A. & TAHERI, S. 2018. Very-low-energy diets for weight loss in patients with kidney disease. *Journal of Kidney Care*, 3, 14-22.
- BROWN, J. B., CONNER, C. & NICHOLS, G. A. 2010. Secondary failure of metformin monotherapy in clinical practice. *Diabetes Care*, 33, 501-6.

- BROWN, J. B., NICHOLS, G. A. & PERRY, A. 2004. The burden of treatment failure in type 2 diabetes. *Diabetes Care*, 27, 1535-40.
- BRUNZELL, J. D. & AYYOBI, A. F. 2003. Dyslipidemia in the metabolic syndrome and type 2 diabetes mellitus. *Am J Med*, 115 Suppl 8A, 24s-28s.
- BUCHWALD, H., AVIDOR, Y., BRAUNWALD, E., JENSEN, M. D., PORIES, W., FAHRBACH, K. & SCHOELLES, K. 2004. Bariatric surgery: a systematic review and meta-analysis. *Jama*, 292, 1724-37.
- BUCHWALD, H., ESTOK, R., FAHRBACH, K., BANEL, D., JENSEN, M. D., PORIES, W. J., BANTLE, J. P. & SLEDGE, I. 2009. Weight and type 2 diabetes after bariatric surgery: systematic review and meta-analysis. *Am J Med*, 122, 248-256.e5.
- BUIL-COSIALES, P., ZAZPE, I., TOLEDO, E., CORELLA, D., SALAS-SALVADO, J., DIEZ-ESPINO, J., ROS, E., FERNANDEZ-CREUET NAVAJAS, J., SANTOS-LOZANO, J. M., AROS, F., FIOL, M., CASTANER, O., SERRA-MAJEM, L., PINTO, X., LAMUELA-RAVENTOS, R. M., MARTI, A., BASTERRA-GORTARI, F. J., SORLI, J. V., VERDU-ROTELLAR, J. M., BASORA, J., RUIZ-GUTIERREZ, V., ESTRUCH, R. & MARTINEZ-GONZALEZ, M. A. 2014. Fiber intake and all-cause mortality in the Prevencion con Dieta Mediterranea (PREDIMED) study. *Am J Clin Nutr*, 100, 1498-507.
- BUMBU, A., MOUTAIROU, A., MATAR, O., FUMERON, F., VELHO, G., RIVELINE, J. P., GAUTIER, J. F., MARRE, M., ROUSSEL, R. & POTIER, L. 2018. Non-severe hypoglycaemia is associated with weight gain in patients with type 1 diabetes: Results from the Diabetes Control and Complication Trial. *Diabetes Obes Metab*, 20, 1289-1292.
- BURR, M. L., FEHILY, A. M., GILBERT, J. F., ROGERS, S., HOLLIDAY, R. M., SWEETNAM, P. M., ELWOOD, P. C. & DEADMAN, N. M. 1989. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). *Lancet*, 2, 757-61.
- BUSE, J. B., CAPRIO, S., CEFALU, W. T., CERIELLO, A., DEL PRATO, S., INZUCCHI, S. E., MCLAUGHLIN, S., PHILLIPS, G. L., 2ND, ROBERTSON, R. P., RUBINO, F., KAHN, R. & KIRKMAN, M. S. 2009. How do we define cure of diabetes? *Diabetes Care*, 32, 2133-5.
- BUSE, J. B., GINSBERG, H. N., BAKRIS, G. L., CLARK, N. G., COSTA, F., ECKEL, R., FONSECA, V., GERSTEIN, H. C., GRUNDY, S., NESTO, R. W., PIGNONE, M. P., PLUTZKY, J., PORTE, D., REDBERG, R., STITZEL, K. F. & STONE, N. J. 2007. Primary prevention of cardiovascular diseases in people with diabetes mellitus: a scientific statement from the American Heart Association and the American Diabetes Association. *Diabetes Care*, 30, 162-72.
- BUTLAND, B., JEBB, S., KOPELMAN, P., MCPHERSON, K., THOMAS, S., MARDELL, J. & PARRY, V. 2007. Tackling Obesities: Future Choices - Project Report, 2nd Edition.
- BUTRYN, M. L., WEBB, V. & WADDEN, T. A. 2011. Behavioral treatment of obesity. *Psychiatr Clin North Am*, 34, 841-59.
- BUYSSE, D. J., REYNOLDS, C. F., 3RD, MONK, T. H., BERMAN, S. R. & KUPFER, D. J. 1989. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res*, 28, 193-213.
- CALTON, J. B. 2010. Prevalence of micronutrient deficiency in popular diet plans. *J Int Soc Sports Nutr*, 7, 24.
- CALVERT, M. J., MCMANUS, R. J. & FREEMANTLE, N. 2007. Management of type 2 diabetes with multiple oral hypoglycaemic agents or insulin in primary care: retrospective cohort study. *Br J Gen Pract*, 57, 455-60.

- CARVALHO, E., KOTANI, K., PERONI, O. D. & KAHN, B. B. 2005. Adipose-specific overexpression of GLUT4 reverses insulin resistance and diabetes in mice lacking GLUT4 selectively in muscle. *Am J Physiol Endocrinol Metab*, 289, E551-61.
- CASELLA, G., ABBATINI, F., CALI, B., CAPOCCIA, D., LEONETTI, F. & BASSO, N. 2011. Ten-year duration of type 2 diabetes as prognostic factor for remission after sleeve gastrectomy. *Surg Obes Relat Dis*, 7, 697-702.
- CATAPANO, A. L., GRAHAM, I., DE BACKER, G., WIKLUND, O., CHAPMAN, M. J., DREXEL, H., HOES, A. W., JENNINGS, C. S., LANDMESSER, U., PEDERSEN, T. R., REINER, Z., RICCARDI, G., TASKINEN, M. R., TOKGOZOGLU, L., VERSCHUREN, W. M. M., VLACHOPOULOS, C., WOOD, D. A., ZAMORANO, J. L. & COONEY, M. T. 2016. 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias. *Eur Heart J*, 37, 2999-3058.
- CERSOSIMO, E., SOLIS-HERRERA, C., TRAUTMANN, M. E., MALLOY, J. & TRIPLITT, C. L. 2014. Assessment of Pancreatic β -Cell Function: Review of Methods and Clinical. *Curr Diabetes Rev*, 10, 2-42.
- CHA, S. A., YUN, J. S., LIM, T. S., HWANG, S., YIM, E. J., SONG, K. H., YOO, K. D., PARK, Y. M., AHN, Y. B. & KO, S. H. 2016. Severe Hypoglycemia and Cardiovascular or All-Cause Mortality in Patients with Type 2 Diabetes. *Diabetes Metab J*, 40, 202-10.
- CHAHIL, T. J. & GINSBERG, H. N. 2006. Diabetic dyslipidemia. *Endocrinol Metab Clin North Am*, 35, 491-510, vii-viii.
- CHASTON, T. B., DIXON, J. B. & O'BRIEN, P. E. 2007. Changes in fat-free mass during significant weight loss: a systematic review. *Int J Obes (Lond)*, 31, 743-50.
- CHAUDHURY, A., DUVOOR, C., REDDY DENDI, V. S., KRALETI, S., CHADA, A., RAVILLA, R., MARCO, A., SHEKHAWAT, N. S., MONTALES, M. T., KURIAKOSE, K., SASAPU, A., BEEBE, A., PATIL, N., MUSHAM, C. K., LOHANI, G. P. & MIRZA, W. 2017. Clinical Review of Antidiabetic Drugs: Implications for Type 2 Diabetes Mellitus Management. *Front Endocrinol (Lausanne)*, 8.
- CHEARSKUL, S., DELBRIDGE, E., SHULKES, A., PROIETTO, J. & KRIKETOS, A. 2008. Effect of weight loss and ketosis on postprandial cholecystokinin and free fatty acid concentrations. *Am J Clin Nutr*, 87, 1238-46.
- CHRISTENSEN, B. J., IEPSSEN, E. W., LUNDGREN, J., HOLM, L., MADSBAD, S., HOLST, J. J. & TOREKOV, S. S. 2017a. Instrumentalization of Eating Improves Weight Loss Maintenance in Obesity. *Obes Facts*, 10, 633-647.
- CHRISTENSEN, P., BARTELS, E. M., RIECKE, B. F., BLIDDAL, H., LEEDS, A. R., ASTRUP, A., WINTHER, K. & CHRISTENSEN, R. 2012. Improved nutritional status and bone health after diet-induced weight loss in sedentary osteoarthritis patients: a prospective cohort study. *Eur J Clin Nutr*, 66, 504-9.
- CHRISTENSEN P, B. H., BARTELS E, LEEDS, A., ASTRUP, A. AND CHRISTENSEN, R. 2014. Long-term intervention with weight loss in patients with concomitant obesity and knee osteoarthritis: the LIGHT study – a randomised clinical trial. T5: S25.54. *Obesity Reviews*, 15, 152.
- CHRISTENSEN, P., BLIDDAL, H., RIECKE, B. F., LEEDS, A. R., ASTRUP, A. & CHRISTENSEN, R. 2011. Comparison of a low-energy diet and a very low-energy diet in sedentary obese individuals: a pragmatic randomized controlled trial. *Clinical Obesity*, 1, 31-40.
- CHRISTENSEN, P., FREDERIKSEN, R., BLIDDAL, H., RIECKE, B. F., BARTELS, E. M., HENRIKSEN, M., JUUL, S. R. T., GUDBERGSEN, H., WINTHER, K., ASTRUP, A. & CHRISTENSEN, R. 2013. Comparison of three weight maintenance

- programs on cardiovascular risk, bone and vitamins in sedentary older adults. *Obesity (Silver Spring)*, 21, 1982-90.
- CHRISTENSEN, P., HENRIKSEN, M., BARTELS, E. M., LEEDS, A. R., LARSEN, T. M., GUDBERGSEN, H., RIECKE, B. F., ASTRUP, A., HEITMANN, B. L., BOESEN, M., CHRISTENSEN, R. & BLIDDAL, H. 2017b. Long-term weight-loss maintenance in obese patients with knee osteoarthritis: a randomized trial. *The American Journal of Clinical Nutrition*, 106, 755-763.
- CHRISTIANSEN, M. P., LINFOOT, P. A., NEESE, R. A. & HELLERSTEIN, M. K. 2000. Effect of dietary energy restriction on glucose production and substrate utilization in type 2 diabetes. *Diabetes*, 49, 1691-9.
- CLAR, C., AL-KHUDAIRY, L., LOVEMAN, E., KELLY, S. A., HARTLEY, L., FLOWERS, N., GERMANO, R., FROST, G. & REES, K. 2017. Low glycaemic index diets for the prevention of cardiovascular disease. *Cochrane Database Syst Rev*, 7, Cd004467.
- CLIFTON, P. M., CONDO, D. & KEOGH, J. B. 2014. Long term weight maintenance after advice to consume low carbohydrate, higher protein diets--a systematic review and meta analysis. *Nutr Metab Cardiovasc Dis*, 24, 224-35.
- COBELLI, C., TOFFOLO, G. M., DALLA MAN, C., CAMPIONI, M., DENTI, P., CAUMO, A., BUTLER, P. & RIZZA, R. 2007. Assessment of beta-cell function in humans, simultaneously with insulin sensitivity and hepatic extraction, from intravenous and oral glucose tests. *Am J Physiol Endocrinol Metab*, 293, E1-e15.
- COLAYCO, D. C., NIU, F., MCCOMBS, J. S. & CHEETHAM, T. C. 2011. A1C and cardiovascular outcomes in type 2 diabetes: a nested case-control study. *Diabetes Care*, 34, 77-83.
- COLLES, S. L., DIXON, J. B., MARKS, P., STRAUSS, B. J. & O'BRIEN, P. E. 2006. Preoperative weight loss with a very-low-energy diet: quantitation of changes in liver and abdominal fat by serial imaging. *Am J Clin Nutr*, 84, 304-11.
- CORLEY, B. T., CARROLL, R. W., HALL, R. M., WEATHERALL, M., PARRY-STRONG, A. & KREBS, J. D. 2018. Intermittent fasting in Type 2 diabetes mellitus and the risk of hypoglycaemia: a randomized controlled trial. *Diabet Med*, 35, 588-594.
- CRESPIN, S. R., GREENOUGH, W. B., 3RD & STEINBERG, D. 1973. Stimulation of insulin secretion by long-chain free fatty acids. A direct pancreatic effect. *J Clin Invest*, 52, 1979-84.
- CUDDIHY, R. M., PHILIS-TSIMIKAS, A. & NAZERI, A. 2011. Type 2 diabetes care and insulin intensification: is a more multidisciplinary approach needed? Results from the MODIFY survey. *Diabetes Educ*, 37, 111-23.
- CUMMINGS, D. E., WEIGLE, D. S., FRAYO, R. S., BREEN, P. A., MA, M. K., DELLINGER, E. P. & PURNELL, J. Q. 2002. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *N Engl J Med*, 346, 1623-30.
- CURRIE, C. J. & HOLDEN, S. E. 2014. Optimizing clinical outcomes resulting from glucose-lowering therapies in type 2 diabetes: increased confidence about the DPP-4 inhibitors and continued concerns regarding sulphonylureas and exogenous insulin. *Diabetes, Obesity and Metabolism*, 16, 881-884.
- CURRIE, C. J., PETERS, J. R., TYNAN, A., EVANS, M., HEINE, R. J., BRACCO, O. L., ZAGAR, T. & POOLE, C. D. 2010. Survival as a function of HbA(1c) in people with type 2 diabetes: a retrospective cohort study. *Lancet*, 375, 481-9.
- CUSI, K., MAEZONO, K., OSMAN, A., PENDERGRASS, M., PATTI, M. E., PRATIPANAWATR, T., DEFRONZO, R. A., KAHN, C. R. & MANDARINO, L. J.

2000. Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. *J Clin Invest*, 105, 311-20.
- DAMMS-MACHADO, A., WESER, G. & BISCHOFF, S. C. 2012. Micronutrient deficiency in obese subjects undergoing low calorie diet. *Nutr J*, 11, 34.
- DE VOS, A., HEIMBERG, H., QUARTIER, E., HUYPENS, P., BOUWENS, L., PIPELEERS, D. & SCHUIT, F. 1995. Human and rat beta cells differ in glucose transporter but not in glucokinase gene expression. *J Clin Invest*, 96, 2489-95.
- DEFRONZO, R. A. 2009. Banting Lecture. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes*, 58, 773-95.
- DEFRONZO, R. A. 2015. Pathogenesis of type 2 diabetes mellitus. In: DEFRONZO, R. A., FERRANNINI, E., ZIMMIT, P., ALBERTI, K.G.M.M. (ed.) *International Textbook of Diabetes Mellitus* Fourth ed. United Kingdom: John Wiley & Sons Ltd.
- DEFRONZO, R. A., TOBIN, J. D. & ANDRES, R. 1979. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol*, 237, E214-23.
- DEFRONZO, R. A. & TRIPATHY, D. 2009. Skeletal Muscle Insulin Resistance Is the Primary Defect in Type 2 Diabetes. *Diabetes Care*, 32, S157-63.
- DEL PRATO, S. 2003. Loss of early insulin secretion leads to postprandial hyperglycaemia. *Diabetologia*, 46 Suppl 1, M2-8.
- DEL PRATO, S. 2009. Role of glucotoxicity and lipotoxicity in the pathophysiology of Type 2 diabetes mellitus and emerging treatment strategies. *Diabet Med*, 26, 1185-92.
- DELGADO-LISTA, J., PEREZ-MARTINEZ, P., LOPEZ-MIRANDA, J. & PEREZ-JIMENEZ, F. 2012. Long chain omega-3 fatty acids and cardiovascular disease: a systematic review. *Br J Nutr*, 107 Suppl 2, S201-13.
- DEPARTMENT OF HEALTH 2010. Department of Health—Spending Review 2010.
- DEWAN, N. A., NIETO, F. J. & SOMERS, V. K. 2015. Intermittent Hypoxemia and OSA: Implications for Comorbidities. *Chest*, 147, 266-74.
- DHINDSA, P., SCOTT, A. R. & DONNELLY, R. 2003. Metabolic and cardiovascular effects of very-low-calorie diet therapy in obese patients with Type 2 diabetes in secondary failure: outcomes after 1 year. *Diabet Med*, 20, 319-24.
- DIABETES, U. 2014. State of the Nation Challenges for 2015 and beyond. London.
- DIABETES UK 2005. Type 2 Diabetes & Obesity: A Heavy Burden. .
- DIABETES UK 2012. Diabetes UK State of the Nation 2012.
- DIABETES UK. 2017. *Diabetes Prevalence 2017 (November 2017)* [Online]. Available: <https://www.diabetes.org.uk/professionals/position-statements-reports/statistics/diabetes-prevalence-2017> [Accessed 24/8/2018 2018].
- DIABETES UK, N. W. G. 2018. Evidence-based nutrition guidelines for the prevention and management of diabetes. March 2018. In: DIABETES, U. (ed.). London.
- DICKINSON PJ, D. A. F. G. 2002. A retrospective case-control study of initiating insulin therapy in type 2 diabetes. *Pract Diabetes Int*, 19, 67-70.
- DIXON, J. B., BHATHAL, P. S., HUGHES, N. R. & O'BRIEN, P. E. 2004. Nonalcoholic fatty liver disease: Improvement in liver histological analysis with weight loss. *Hepatology*, 39, 1647-54.
- DIXON, J. B., O'BRIEN, P. E., PLAYFAIR, J., CHAPMAN, L., SCHACHTER, L. M., SKINNER, S., PROIETTO, J., BAILEY, M. & ANDERSON, M. 2008. Adjustable gastric banding and conventional therapy for type 2 diabetes: a randomized controlled trial. *Jama*, 299, 316-23.
- DJOUSSE, L., LEVY, D., CUPPLES, L. A., EVANS, J. C., D'AGOSTINO, R. B. & ELLISON, R. C. 2001. Total serum bilirubin and risk of cardiovascular disease in the Framingham offspring study. *Am J Cardiol*, 87, 1196-200; a4, 7.

- DOGGRELL, S. A. & CHAN, V. 2014. Adherence to insulin treatment in diabetes: can it be improved? *J Diabetes*, 7, 315-21.
- DONNELLY, J. E., BLAIR, S. N., JAKICIC, J. M., MANORE, M. M., RANKIN, J. W. & SMITH, B. K. 2009a. American College of Sports Medicine Position Stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. *Med Sci Sports Exerc*, 41, 459-71.
- DONNELLY, J. E., BLAIR, S. N., JAKICIC, J. M., MANORE, M. M., RANKIN, J. W., SMITH, B. K. & AMERICAN COLLEGE OF SPORTS, M. 2009b. American College of Sports Medicine Position Stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. *Med Sci Sports Exerc*, 41, 459-71.
- DUCKWORTH, W., ABRAIRA, C., MORITZ, T., REDA, D., EMANUELE, N., REAVEN, P. D., ZIEVE, F. J., MARKS, J., DAVIS, S. N., HAYWARD, R., WARREN, S. R., GOLDMAN, S., MCCARREN, M., VITEK, M. E., HENDERSON, W. G. & HUANG, G. D. 2009. Glucose control and vascular complications in veterans with type 2 diabetes. *N Engl J Med*, 360, 129-39.
- DYSON, P. A., KELLY, T., DEAKIN, T., DUNCAN, A., FROST, G., HARRISON, Z., KHATRI, D., KUNKA, D., MCARDLE, P., MELLOR, D., OLIVER, L. & WORTH, J. 2011. Diabetes UK evidence-based nutrition guidelines for the prevention and management of diabetes. *Diabet Med*, 28, 1282-8.
- EEG-OLOFSSON, K., CEDERHOLM, J., NILSSON, P. M., ZETHELIUS, B., NUNEZ, L., GUDBJORNSDOTTIR, S. & ELIASSON, B. 2009. Risk of cardiovascular disease and mortality in overweight and obese patients with type 2 diabetes: an observational study in 13,087 patients. *Diabetologia*, 52, 65-73.
- EEG-OLOFSSON, K., CEDERHOLM, J., NILSSON, P. M., ZETHELIUS, B., SVENSSON, A. M., GUDBJORNSDOTTIR, S. & ELIASSON, B. 2010. New aspects of HbA1c as a risk factor for cardiovascular diseases in type 2 diabetes: an observational study from the Swedish National Diabetes Register (NDR). *J Intern Med*, 268, 471-82.
- EGUCHI, K. & NAGAI, R. 2017. Islet inflammation in type 2 diabetes and physiology. *J Clin Invest*, 127, 14-23.
- ELFHAG, K. & ROSSNER, S. 2005. Who succeeds in maintaining weight loss? A conceptual review of factors associated with weight loss maintenance and weight regain. *Obes Rev*, 6, 67-85.
- ELRICK, H., STIMMLER, L., HLAD, C. J., JR. & ARAI, Y. 1964. PLASMA INSULIN RESPONSE TO ORAL AND INTRAVENOUS GLUCOSE ADMINISTRATION. *J Clin Endocrinol Metab*, 24, 1076-82.
- EMDIN, C. A., RAHIMI, K., NEAL, B., CALLENDER, T., PERKOVIC, V. & PATEL, A. 2015. Blood pressure lowering in type 2 diabetes: a systematic review and meta-analysis. *Jama*, 313, 603-15.
- ERNST, B., THURNHEER, M., SCHMID, S. M. & SCHULTES, B. 2009. Evidence for the necessity to systematically assess micronutrient status prior to bariatric surgery. *Obes Surg*, 19, 66-73.
- ETTEHAD, D., EMDIN, C. A., KIRAN, A., ANDERSON, S. G., CALLENDER, T., EMBERSON, J., CHALMERS, J., RODGERS, A. & RAHIMI, K. 2016. Blood pressure lowering for prevention of cardiovascular disease and death: a systematic review and meta-analysis. *Lancet*, 387, 957-967.
- EUROPEAN FOOD SAFETY AUTHORITY PANEL ON DIETETIC PRODUCTS, N. A. A. N. 2015. Scientific Opinion on the essential composition of total diet replacements for weight control. *EFSA Journal*, 13, 3957.

- EVERT, A. B., BOUCHER, J. L., CYPRESS, M., DUNBAR, S. A., FRANZ, M. J., MAYER-DAVIS, E. J., NEUMILLER, J. J., NWANKWO, R., VERDI, C. L., URBANSKI, P. & YANCY, W. S., JR. 2014. Nutrition therapy recommendations for the management of adults with diabetes. *Diabetes Care*, 37 Suppl 1, S120-43.
- EXPERT PANEL ON DETECTION, E., AND TREATMENT OF HIGH BLOOD CHOLESTEROL IN ADULTS (ADULT TREATMENT PANEL III) 2001. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) *Jama*, 285, 2486-97.
- FERRANNINI, E., BUZZIGOLI, G., BONADONNA, R., GIORICO, M. A., OLEGGINI, M., GRAZIADEI, L., PEDRINELLI, R., BRANDI, L. & BEVILACQUA, S. 1987. Insulin resistance in essential hypertension. *N Engl J Med*, 317, 350-7.
- FLOYD, J. C., JR., FAJANS, S. S., CONN, J. W., KNOPF, R. F. & RULL, J. 1966. Insulin secretion in response to protein ingestion. *J Clin Invest*, 45, 1479-86.
- FOGELHOLM, M., KUKKONEN-HARJULA, K., NENONEN, A. & PASANEN, M. 2000. Effects of walking training on weight maintenance after a very-low-energy diet in premenopausal obese women: a randomized controlled trial. *Arch Intern Med*, 160, 2177-84.
- FONTANA, L., MEYER, T. E., KLEIN, S. & HOLLOSZY, J. O. 2004. Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans. *Proc Natl Acad Sci U S A*, 101, 6659-63.
- FORETZ, M., GUIGAS, B., BERTRAND, L., POLLAK, M. & VIOLET, B. 2014. Metformin: from mechanisms of action to therapies. *Cell Metab*, 20, 953-66.
- FOSTER, G. D., BORRADAILE, K. E., SANDERS, M. H., MILLMAN, R., ZAMMIT, G., NEWMAN, A. B., WADDEN, T. A., KELLEY, D., WING, R. R., PI-SUNYER, F. X., REBOUSSIN, D. & KUNA, S. T. 2009a. A randomized study on the effect of weight loss on obstructive sleep apnea among obese patients with type 2 diabetes: the Sleep AHEAD study. *Arch Intern Med*, 169, 1619-26.
- FOSTER, G. D., SANDERS, M. H., MILLMAN, R., ZAMMIT, G., BORRADAILE, K. E., NEWMAN, A. B., WADDEN, T. A., KELLEY, D., WING, R. R., SUNYER, F. X., DARCEY, V. & KUNA, S. T. 2009b. Obstructive sleep apnea among obese patients with type 2 diabetes. *Diabetes Care*, 32, 1017-9.
- FOSTER, G. D., WADDEN, T. A., PETERSON, F. J., LETIZIA, K. A., BARTLETT, S. J. & CONILL, A. M. 1992. A controlled comparison of three very-low-calorie diets: effects on weight, body composition, and symptoms. *Am J Clin Nutr*, 55, 811-7.
- FRANZ, M. J., BOUCHER, J. L., RUTTEN-RAMOS, S. & VANWORMER, J. J. 2015. Lifestyle weight-loss intervention outcomes in overweight and obese adults with type 2 diabetes: a systematic review and meta-analysis of randomized clinical trials. *J Acad Nutr Diet*, 115, 1447-63.
- FRIS, R. J. 2004. Preoperative low energy diet diminishes liver size. *Obes Surg*, 14, 1165-70.
- FROST, G., K., M., C., K., M., K., U., H., P., H., R., W. & J., S. 1991. A new method of energy prescription to improve weight loss. *Journal of Human Nutrition and Dietetics*, 4, 369-373.
- GABORIT, B., ABDESSELAM, I., KOBER, F., JACQUIER, A., RONSIN, O., EMUNGANIA, O., LESAVRE, N., ALESSI, M. C., MARTIN, J. C., BERNARD, M. & DUTOIR, A. 2015. Ectopic fat storage in the pancreas using 1H-MRS: importance of diabetic status and modulation with bariatric surgery-induced weight loss. *Int J Obes (Lond)*, 39, 480-7.
- GAMBLE, J. M., SIMPSON, S. H., EURICH, D. T., MAJUMDAR, S. R. & JOHNSON, J. A. 2010. Insulin use and increased risk of mortality in type 2 diabetes: a cohort study. *Diabetes Obes Metab*, 12, 47-53.

- GANDASI, N. R., YIN, P., OMAR-HMEADI, M., OTTOSSON LAAKSO, E., VIKMAN, P. & BARG, S. 2018. Glucose-Dependent Granule Docking Limits Insulin Secretion and Is Decreased in Human Type 2 Diabetes. *Cell Metab*, 27, 470-478.e4.
- GARBER, A. J., ABRAHAMSON, M. J., BARZILAY, J. I., BLONDE, L., BLOOMGARDEN, Z. T., BUSH, M. A., DAGOGO-JACK, S., DAVIDSON, M. B., EINHORN, D., GARVEY, W. T., GRUNBERGER, G., HANDELSMAN, Y., HIRSCH, I. B., JELLINGER, P. S., MCGILL, J. B., MECHANICK, J. I., ROSENBLIT, P. D., UMPIERREZ, G. E. & DAVIDSON, M. H. 2013. American Association of Clinical Endocrinologists' comprehensive diabetes management algorithm 2013 consensus statement--executive summary. *Endocr Pract*, 19, 536-57.
- GARDNER, C. D., KIM, S., BERSAMIN, A., DOPLER-NELSON, M., OTTEN, J., OELRICH, B. & CHERIN, R. 2010. Micronutrient quality of weight-loss diets that focus on macronutrients: results from the A TO Z study. *Am J Clin Nutr*, 92, 304-12.
- GARVEY, W. T. & HERMAYER, K. L. 1998. Clinical implications of the insulin resistance syndrome. *Clin Cornerstone*, 1, 13-28.
- GARVEY, W. T., MAIANU, L., HANCOCK, J. A., GOLICHOWSKI, A. M. & BARON, A. 1992. Gene expression of GLUT4 in skeletal muscle from insulin-resistant patients with obesity, IGT, GDM, and NIDDM. *Diabetes*, 41, 465-75.
- GARVEY, W. T., MAIANU, L., HUECKSTEADT, T. P., BIRNBAUM, M. J., MOLINA, J. M. & CIARALDI, T. P. 1991. Pretranslational suppression of a glucose transporter protein causes insulin resistance in adipocytes from patients with non-insulin-dependent diabetes mellitus and obesity. *J Clin Invest*, 87, 1072-81.
- GASTALDELLI, A., FERRANNINI, E., MIYAZAKI, Y., MATSUDA, M. & DEFRONZO, R. A. 2004. Beta-cell dysfunction and glucose intolerance: results from the San Antonio metabolism (SAM) study. *Diabetologia*, 47, 31-9.
- GASTEYGER, C., LARSEN, T. M., VERCRUYSE, F. & ASTRUP, A. 2008. Effect of a dietary-induced weight loss on liver enzymes in obese subjects. *Am J Clin Nutr*, 87, 1141-7.
- GELBER, R. P., KURTH, T., KAUSZ, A. T., MANSON, J. E., BURING, J. E., LEVEY, A. S. & GAZIANO, J. M. 2005. Association between body mass index and CKD in apparently healthy men. *Am J Kidney Dis*, 46, 871-80.
- GELDSZUS, R., MAYR, B., HORN, R., GEISTHOVEL, F., VON ZUR MUHLEN, A. & BRABANT, G. 1996. Serum leptin and weight reduction in female obesity. *Eur J Endocrinol*, 135, 659-62.
- GERSTEIN, H. C., MILLER, M. E., BYINGTON, R. P., GOFF, D. C., JR., BIGGER, J. T., BUSE, J. B., CUSHMAN, W. C., GENUTH, S., ISMAIL-BEIGI, F., GRIMM, R. H., JR., PROBSTFIELD, J. L., SIMONS-MORTON, D. G. & FRIEDEWALD, W. T. 2008. Effects of intensive glucose lowering in type 2 diabetes. *N Engl J Med*, 358, 2545-59.
- GHIO, B. J., A.; CORCELLES, R.; FLORES, L.; LACY, A.; VIDAL, J. 2017. Midterm effects bariatric surgery in patients with insulin-treated type 2 diabetes. *Surgery for Obesity and Related Diseases*, in Press. Available from: [http://www.soard.org/article/S1550-7289\(16\)30894-2/fulltext](http://www.soard.org/article/S1550-7289(16)30894-2/fulltext).
- GIBSON, A. A., SEIMON, R. V., LEE, C. M. Y., AYRE, J., FRANKLIN, J., MARKOVIC, T. P., CATERSON, I. D. & SAINSBURY, A. 2015. Do ketogenic diets really suppress appetite? A systematic review and meta-analysis. *Obesity Reviews*, 16, 64-76.
- GILBERT, J. A., DRAPEAU, V., ASTRUP, A. & TREMBLAY, A. 2009. Relationship between diet-induced changes in body fat and appetite sensations in women. *Appetite*, 52, 809-12.

- GIORDANI, I., MALANDRUCCO, I., DONNO, S., PICCONI, F., DI GIACINTO, P., DI FLAVIANI, A., CHIOMA, L. & FRONTONI, S. 2014. Acute caloric restriction improves glomerular filtration rate in patients with morbid obesity and type 2 diabetes. *Diabetes Metab*, 40, 158-60.
- GOLDSTEIN, D. J. 1992. Beneficial health effects of modest weight loss. *Int J Obes Relat Metab Disord*, 16, 397-415.
- GOODPASTER, B. H., HE, J., WATKINS, S. & KELLEY, D. E. 2001. Skeletal muscle lipid content and insulin resistance: evidence for a paradox in endurance-trained athletes. *J Clin Endocrinol Metab*, 86, 5755-61.
- GOUGH, S. C., HARRIS, S., WOO, V. & DAVIES, M. 2013. Insulin degludec: overview of a novel ultra long-acting basal insulin. *Diabetes Obes Metab*, 15, 301-9.
- GRACE, C. 2011. A review of one-to-one dietetic obesity management in adults. *J Hum Nutr Diet*, 24, 13-22.
- GREGG, E. W., CHEN, H., WAGENKNECHT, L. E., CLARK, J. M., DELAHANTY, L. M., BANTLE, J., POWNALL, H. J., JOHNSON, K. C., SAFFORD, M. M., KITABCHI, A. E., PI-SUNYER, F. X., WING, R. R. & BERTONI, A. G. 2012. Association of an intensive lifestyle intervention with remission of type 2 diabetes. *Jama*, 308, 2489-96.
- GRIPETEG, L., TORGERSON, J., KARLSSON, J. & LINDROOS, A. K. 2010. Prolonged refeeding improves weight maintenance after weight loss with very-low-energy diets. *Br J Nutr*, 103, 141-8.
- GROSS, J. L., DE AZEVEDO, M. J., SILVEIRO, S. P., CANANI, L. H., CARAMORI, M. L. & ZELMANOVITZ, T. 2005. Diabetic nephropathy: diagnosis, prevention, and treatment. *Diabetes Care*, 28, 164-76.
- GUARE, J. C., WING, R. R. & GRANT, A. 1995. Comparison of obese NIDDM and nondiabetic women: short- and long-term weight loss. *Obes Res*, 3, 329-35.
- GUIDONE, C., MANCO, M., VALERA-MORA, E., IACONELLI, A., GNIULI, D., MARI, A., NANNI, G., CASTAGNETO, M., CALVANI, M. & MINGRONE, G. 2006. Mechanisms of recovery from type 2 diabetes after malabsorptive bariatric surgery. *Diabetes*, 55, 2025-31.
- GULLI, G., FERRANNINI, E., STERN, M., HAFFNER, S. & DEFRONZO, R. A. 1992. The metabolic profile of NIDDM is fully established in glucose-tolerant offspring of two Mexican-American NIDDM parents. *Diabetes*, 41, 1575-86.
- HAFFNER, S. J. & CASSELLS, H. 2003. Hyperglycemia as a cardiovascular risk factor. *Am J Med*, 115 Suppl 8A, 6s-11s.
- HALL, K. D. & JORDAN, P. N. 2008. Modeling weight-loss maintenance to help prevent body weight regain. *Am J Clin Nutr*, 88, 1495-503.
- HALLAN, S., DE MUTSERT, R., CARLSEN, S., DEKKER, F. W., AASAROD, K. & HOLMEN, J. 2006. Obesity, smoking, and physical inactivity as risk factors for CKD: are men more vulnerable? *Am J Kidney Dis*, 47, 396-405.
- HAMMER, S., SNEL, M., LAMB, H. J., JAZET, I. M., VAN DER MEER, R. W., PIJL, H., MEINDERS, E. A., ROMIJN, J. A., DE ROOS, A. & SMIT, J. W. 2008. Prolonged caloric restriction in obese patients with type 2 diabetes mellitus decreases myocardial triglyceride content and improves myocardial function. *J Am Coll Cardiol*, 52, 1006-12.
- HARPER, W., CLEMENT, M., GOLDENBERG, R., HANNA, A., MAIN, A., RETNAKARAN, R., SHERIFALI, D., WOO, V. & YALE, J. F. 2013. Pharmacologic management of type 2 diabetes. *Can J Diabetes*, 37 Suppl 1, S61-8.
- HARRIS, E. H. 2005. Elevated Liver Function Tests in Type 2 Diabetes. *Clinical Diabetes*, 23, 115-119.

- HARRIS, M. I., KLEIN, R., WELBORN, T. A. & KNUIMAN, M. W. 1992. Onset of NIDDM occurs at least 4-7 yr before clinical diagnosis. *Diabetes Care*, 15, 815-9.
- HARTLEY, L., MAY, M. D., LOVEMAN, E., COLQUITT, J. L. & REES, K. 2016. Dietary fibre for the primary prevention of cardiovascular disease. *Cochrane Database Syst Rev*, Cd011472.
- HASKELL, W., LEE, I., PATE, R., POWELL, K., BLAIR, S. & FRANKLIN, B. 2007. Physical activity and public health: updated recommendation for adults from the American college of sports medicine and the American heart association. *Med Sci Sports Exerc*, 39, 1423 - 1434.
- HASLAM, D. W., WAINE, C. & LEEDS, A.R. . 2010. Medical Management During Effective Weight-Loss. [Accessed 27th July 2015].
- HAYES, R. P., FITZGERALD, J. T. & JACOBBER, S. J. 2008. Primary care physician beliefs about insulin initiation in patients with type 2 diabetes. *Int J Clin Pract*, 62, 860-8.
- HEALTH AND SOCIAL CARE INFORMATION CENTRE 2015. National Diabetes Audit 2012-2013 and 2014-2015 Report 1: Care Processes and Treatment Targets.
- HEINEMANN, L. 2002. Variability of insulin absorption and insulin action. *Diabetes Technol Ther*, 4, 673-82.
- HEISE, T. & PIEBER, T. R. 2007. Towards peakless, reproducible and long-acting insulins. An assessment of the basal analogues based on isoglycaemic clamp studies. *Diabetes Obes Metab*, 9, 648-59.
- HEMMINGSSEN, B., LUND, S. S., GLUUD, C., VAAG, A., ALMDAL, T., HEMMINGSSEN, C. & WETTERSLEV, J. 2011. Intensive glycaemic control for patients with type 2 diabetes: systematic review with meta-analysis and trial sequential analysis of randomised clinical trials. *Bmj*, 343, 1-20.
- HEMMINGSSEN, B., LUND, S. S., GLUUD, C., VAAG, A., ALMDAL, T. P., HEMMINGSSEN, C. & WETTERSLEV, J. 2013. Targeting intensive glycaemic control versus targeting conventional glycaemic control for type 2 diabetes mellitus. *Cochrane Database Syst Rev*, 11, 1-380.
- HENQUIN, J. C. 2009. Regulation of insulin secretion: a matter of phase control and amplitude modulation. *Diabetologia*, 52, 739-51.
- HENQUIN, J. C., RAVIER, M. A., NENQUIN, M., JONAS, J. C. & GILON, P. 2003. Hierarchy of the beta-cell signals controlling insulin secretion. *Eur J Clin Invest*, 33, 742-50.
- HENRY, R. R. & GUMBINER, B. 1991. Benefits and limitations of very-low-calorie diet therapy in obese NIDDM. *Diabetes Care*, 14, 802-23.
- HENRY, R. R., GUMBINER, B., DITZLER, T., WALLACE, P., LYON, R. & GLAUBER, H. S. 1993. Intensive conventional insulin therapy for type II diabetes. Metabolic effects during a 6-mo outpatient trial. *Diabetes Care*, 16, 21-31.
- HERDMAN, M., GUDEX, C., LLOYD, A., JANSSEN, M., KIND, P., PARKIN, D., BONSEL, G. & BADIA, X. 2011. Development and preliminary testing of the new five-level version of EQ-5D (EQ-5D-5L). *Qual Life Res*, 20, 1727-36.
- HEX, N., BARTLETT, C., WRIGHT, D., TAYLOR, M. & VARLEY, D. 2012. Estimating the current and future costs of Type 1 and Type 2 diabetes in the UK, including direct health costs and indirect societal and productivity costs. *Diabet Med*, 29, 855-62.
- HEYMSFIELD, S. B., HARP, J. B., REITMAN, M. L., BEETSCH, J. W., SCHOELLER, D. A., ERONDU, N. & PIETROBELLI, A. 2007. Why do obese patients not lose more weight when treated with low-calorie diets? A mechanistic perspective. *Am J Clin Nutr*, 85, 346-54.
- HILL, J. O. 2006. Understanding and addressing the epidemic of obesity: an energy balance perspective. *Endocr Rev*, 27, 750-61.

- HILL, J. O., WYATT, H., PHELAN, S. & WING, R. 2005. The National Weight Control Registry: is it useful in helping deal with our obesity epidemic? *J Nutr Educ Behav*, 37, 206-10.
- HILL, J. O., WYATT, H. R., REED, G. W. & PETERS, J. C. 2003. Obesity and the environment: where do we go from here? *Science*, 299, 853-5.
- HILL, R. L. A. K., S.E. 2015. Pancreatic morphology in normal and diabetic states. *In*: DEFRONZO, R. A., FERRANNINI, E., ZIMMIT, P., ALBERTI, K.G.M.M. (ed.) *International Textbook of Diabetes Mellitus*. Fourth ed. United Kingdom: John Willey & Sons Ltd.
- HIPPISLEY-COX, J., COUPLAND, C. & BRINDLE, P. 2017. Development and validation of QRISK3 risk prediction algorithms to estimate future risk of cardiovascular disease: prospective cohort study. *Bmj*, 357, 1-21.
- HIPPISLEY-COX, J., COUPLAND, C., VINOGRADOVA, Y., ROBSON, J., MINHAS, R., SHEIKH, A. & BRINDLE, P. 2008. Predicting cardiovascular risk in England and Wales: prospective derivation and validation of QRISK2. *Bmj*, 336, 1475-82.
- HOENIG, M. & SHARP, G. W. 1986. Glucose induces insulin release and a rise in cytosolic calcium concentration in a transplantable rat insulinoma. *Endocrinology*, 119, 2502-7.
- HOERGER, T. J., SEGEL, J. E., GREGG, E. W. & SAADDINE, J. B. 2008. Is glycemic control improving in U.S. adults? *Diabetes Care*, 31, 81-6.
- HOLDEN, S. E., GALE, E. A., JENKINS-JONES, S. & CURRIE, C. J. 2014. How many people inject insulin? UK estimates from 1991 to 2010. *Diabetes Obes Metab*, 16, 553-9.
- HOLDEN, S. E., JENKINS-JONES, S., MORGAN, C. L., SCHERNTHANER, G. & CURRIE, C. J. 2015. Glucose-lowering with exogenous insulin monotherapy in type 2 diabetes: dose association with all-cause mortality, cardiovascular events and cancer. *Diabetes, Obesity and Metabolism*, 17, 350-362.
- HOLDEN SE, S. G., JENKINS-JONES S, CURRIE CJ. 2013. Exogenous insulin and risk of all-cause mortality in type 2 diabetes: a dose-response association - Abstracts of the 49th Annual Meeting of the EASD *Diabetologia*, 56, S96.
- HOLMAN, R. R., PAUL, S. K., BETHEL, M. A., MATTHEWS, D. R. & NEIL, H. A. 2008. 10-year follow-up of intensive glucose control in type 2 diabetes. *N Engl J Med*, 359, 1577-89.
- HOTAMISLIGIL, G. S. 2006. Inflammation and metabolic disorders. *Nature*, 444, 860-7.
- HOTAMISLIGIL, G. S., PERALDI, P., BUDAVARI, A., ELLIS, R., WHITE, M. F. & SPIEGELMAN, B. M. 1996. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. *Science*, 271, 665-8.
- HOWARD, A. N. 1989. The historical development of very low calorie diets. *Int J Obes*, 13 Suppl 2, 1-9.
- HOWARD, B. V. 1999. Insulin resistance and lipid metabolism. *Am J Cardiol*, 84, 28j-32j.
- HU, F. B., STAMPFER, M. J., SOLOMON, C. G., LIU, S., WILLETT, W. C., SPEIZER, F. E., NATHAN, D. M. & MANSON, J. E. 2001. The impact of diabetes mellitus on mortality from all causes and coronary heart disease in women: 20 years of follow-up. *Arch Intern Med*, 161, 1717-23.
- HUBERT, H. B., FEINLEIB, M., MCNAMARA, P. M. & CASTELLI, W. P. 1983. Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. *Circulation*, 67, 968-77.
- HUNTER, S. J. & GARVEY, W. T. 1998. Insulin action and insulin resistance: diseases involving defects in insulin receptors, signal transduction, and the glucose transport effector system. *Am J Med*, 105, 331-45.

- IDF, I. D. F. 2002. The DAWN (Diabetes Attitudes, Wishes and Needs) study. *Practical Diabetes International*, 19, 22-24a.
- INTERNATIONAL DIABETES FEDERATION 2013. IDF Diabetes Atlas.
- INZUCCHI, S. E., BERGENSTAL, R. M., BUSE, J. B., DIAMANT, M., FERRANNINI, E., NAUCK, M., PETERS, A. L., TSAPAS, A., WENDER, R. & MATTHEWS, D. R. 2012. Management of hyperglycemia in type 2 diabetes: a patient-centered approach position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes care*, 35, 1364-1379.
- IOZZO, P., PRATIPANAWATR, T., PIJL, H., VOGT, C., KUMAR, V., PIPEK, R., MATSUDA, M., MANDARINO, L. J., CUSI, K. J. & DEFRONZO, R. A. 2001. Physiological hyperinsulinemia impairs insulin-stimulated glycogen synthase activity and glycogen synthesis. *Am J Physiol Endocrinol Metab*, 280, E712-9.
- ISHIZAKA, N., ISHIZAKA, Y., TAKAHASHI, E., YAMAKADO, M. & HASHIMOTO, H. 2001. High Serum Bilirubin Level Is Inversely Associated With the Presence of Carotid Plaque. *Stroke*, 32, 580-583.
- ITANI, S. I., RUDERMAN, N. B., SCHMIEDER, F. & BODEN, G. 2002. Lipid-induced insulin resistance in human muscle is associated with changes in diacylglycerol, protein kinase C, and IkappaB-alpha. *Diabetes*, 51, 2005-11.
- JAIS, A. & BRUNING, J. C. 2017. Hypothalamic inflammation in obesity and metabolic disease. *J Clin Invest*, 127, 24-32.
- JANSEN, H. J., VERVOORT, G., VAN DER GRAAF, M. & TACK, C. J. 2010. Pronounced weight gain in insulin-treated patients with type 2 diabetes mellitus is associated with an unfavourable cardiometabolic risk profile. *Neth J Med*, 68, 359-66.
- JANSSEN, I., FORTIER, A., HUDSON, R. & ROSS, R. 2002. Effects of an energy-restrictive diet with or without exercise on abdominal fat, intermuscular fat, and metabolic risk factors in obese women. *Diabetes Care*, 25, 431-8.
- JANSSEN, I. & ROSS, R. 1999. Effects of sex on the change in visceral, subcutaneous adipose tissue and skeletal muscle in response to weight loss. *Int J Obes Relat Metab Disord*, 23, 1035-46.
- JAZET, I. M., DE CRAEN, A. J., VAN SCHIE, E. M. & MEINDERS, A. E. 2007. Sustained beneficial metabolic effects 18 months after a 30-day very low calorie diet in severely obese, insulin-treated patients with type 2 diabetes. *Diabetes Res Clin Pract*, 77, 70-6.
- JAZET, I. M., OUWENS, D. M., SCHAART, G., PIJL, H., KEIZER, H., MAASSEN, J. A. & MEINDERS, A. E. 2005a. Effect of a 2-day very low-energy diet on skeletal muscle insulin sensitivity in obese type 2 diabetic patients on insulin therapy. *Metabolism*, 54, 1669-78.
- JAZET, I. M., PIJL, H., FROLICH, M., ROMIJN, J. A. & MEINDERS, A. E. 2005b. Two days of a very low calorie diet reduces endogenous glucose production in obese type 2 diabetic patients despite the withdrawal of blood glucose-lowering therapies including insulin. *Metabolism*, 54, 705-12.
- JAZET, I. M., PIJL, H., FROLICH, M., SCHOEMAKER, R. C. & MEINDERS, A. E. 2005c. Factors predicting the blood glucose lowering effect of a 30-day very low calorie diet in obese Type 2 diabetic patients. *Diabet Med*, 22, 52-5.
- JAZET, I. M., SCHAART, G., GASTALDELLI, A., FERRANNINI, E., HESSELINK, M. K., SCHRAUWEN, P., ROMIJN, J. A., MAASSEN, J. A., PIJL, H., OUWENS, D. M. & MEINDERS, A. E. 2008. Loss of 50% of excess weight using a very low energy diet improves insulin-stimulated glucose disposal and skeletal muscle insulin signalling in obese insulin-treated type 2 diabetic patients. *Diabetologia*, 51, 309-19.
- JENSEN, P., ZACHARIAE, C., CHRISTENSEN, R., GEIKER, N. R., SCHAADT, B. K., STENDER, S., ASTRUP, A., HANSEN, P. R. & SKOV, L. 2014. Effect of weight

- loss on the cardiovascular risk profile of obese patients with psoriasis. *Acta Derm Venereol*, 94, 691-4.
- JENSEN, P., ZACHARIAE, C., CHRISTENSEN, R., GEIKER, N. R., SCHAADT, B. K., STENDER, S., HANSEN, P. R., ASTRUP, A. & SKOV, L. 2013. Effect of weight loss on the severity of psoriasis: a randomized clinical study. *JAMA Dermatol*, 149, 795-801.
- JIMENEZ, A., CASAMITJANA, R., FLORES, L., VIAPLANA, J., CORCELLES, R., LACY, A. & VIDAL, J. 2012. Long-term effects of sleeve gastrectomy and Roux-en-Y gastric bypass surgery on type 2 diabetes mellitus in morbidly obese subjects. *Ann Surg*, 256, 1023-9.
- JOHANSSON, K., HEMMINGSSON, E., HARLID, R., TROLLE LAGERROS, Y., GRANATH, F., ROSSNER, S. & NEOVIUS, M. 2011. Longer term effects of very low energy diet on obstructive sleep apnoea in cohort derived from randomised controlled trial: prospective observational follow-up study. *Bmj*, 342, 1-9.
- JOHANSSON, K., NEOVIUS, M. & HEMMINGSSON, E. 2014. Effects of anti-obesity drugs, diet, and exercise on weight-loss maintenance after a very-low-calorie diet or low-calorie diet: a systematic review and meta-analysis of randomized controlled trials. *Am J Clin Nutr*, 99, 14-23.
- JOHANSSON, K., NEOVIUS, M., LAGERROS, Y. T., HARLID, R., ROSSNER, S., GRANATH, F. & HEMMINGSSON, E. 2009. Effect of a very low energy diet on moderate and severe obstructive sleep apnoea in obese men: a randomised controlled trial. *Bmj*, 339, 1-9.
- JOHNS, M. & HOCKING, B. 1997. Daytime sleepiness and sleep habits of Australian workers. *Sleep*, 20, 844-9.
- JOHNS, M. W. 1991. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep*, 14, 540-5.
- JOHNSON, R. K. 2002. Dietary Intake—How Do We Measure What People Are Really Eating? *Obesity Research*, 10, 63S-68S.
- JONKER, J. T., SNEL, M., HAMMER, S., JAZET, I. M., VAN DER MEER, R. W., PIJL, H., MEINDERS, A. E., DE ROOS, A., SMIT, J. W., ROMIJN, J. A. & LAMB, H. J. 2014. Sustained cardiac remodeling after a short-term very low calorie diet in type 2 diabetes mellitus patients. *Int J Cardiovasc Imaging*, 30, 121-7.
- KAJASTE, S., BRANDER, P. E., TELAKIVI, T., PARTINEN, M. & MUSTAJOKI, P. 2004. A cognitive-behavioral weight reduction program in the treatment of obstructive sleep apnea syndrome with or without initial nasal CPAP: a randomized study. *Sleep Med*, 5, 125-31.
- KALANTAR-ZADEH, K., ABBOTT, K. C., KRONENBERG, F., ANKER, S. D., HORWICH, T. B. & FONAROW, G. C. 2006. Epidemiology of dialysis patients and heart failure patients. *Semin Nephrol*, 26, 118-33.
- KANNEL, W. B. 2000. Risk stratification in hypertension: new insights from the Framingham Study. *Am J Hypertens*, 13, 3s-10s.
- KANNEL, W. B. & MCGEE, D. L. 1979. Diabetes and cardiovascular disease. The Framingham study. *Jama*, 241, 2035-8.
- KARANICOLAS, P. J., FARROKHVAR, F. & BHANDARI, M. 2010. Blinding: Who, what, when, why, how? *Can J Surg*, 53, 345-8.
- KATZ, A., NAMBI, S. S., MATHER, K., BARON, A. D., FOLLMANN, D. A., SULLIVAN, G. & QUON, M. J. 2000. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *J Clin Endocrinol Metab*, 85, 2402-10.

- KELLEY, D. E. 2005. Skeletal muscle fat oxidation: timing and flexibility are everything. *J Clin Invest*, 115, 1699-702.
- KELLY, S. A., HARTLEY, L., LOVEMAN, E., COLQUITT, J. L., JONES, H. M., AL-KHUDAIRY, L., CLAR, C., GERMANO, R., LUNN, H. R., FROST, G. & REES, K. 2017. Whole grain cereals for the primary or secondary prevention of cardiovascular disease. *Cochrane Database Syst Rev*, 8, 1-100.
- KENGNE, A. P., PATEL, A., COLAGIURI, S., HELLER, S., HAMET, P., MARRE, M., PAN, C. Y., ZOUNGAS, S., GROBBEE, D. E., NEAL, B., CHALMERS, J. & WOODWARD, M. 2010. The Framingham and UK Prospective Diabetes Study (UKPDS) risk equations do not reliably estimate the probability of cardiovascular events in a large ethnically diverse sample of patients with diabetes: the Action in Diabetes and Vascular Disease: Preterax and Diamicron-MR Controlled Evaluation (ADVANCE) Study. *Diabetologia*, 53, 821-31.
- KERNAN, W. N., INZUCCHI, S. E., VISCOLI, C. M., BRASS, L. M., BRAVATA, D. M., SHULMAN, G. I., MCVEETY, J. C. & HORWITZ, R. I. 2003. Pioglitazone Improves Insulin Sensitivity Among Nondiabetic Patients With a Recent Transient Ischemic Attack or Ischemic Stroke. *Stroke*, 34, 1431-1436.
- KIM, G. A. C., S. 2015. Type 2 diabetes in obese adolescents: pathophysiology and treatment *In: DEFRONZO, R. A., FERRANNINI, E., ZIMMIT, P., ALBERTI, K.G.M.M. (ed.) International Textbook of Diabetes Mellitus*. Fourth ed. United Kingdom: John Wiley & Son Ltd.
- KIMMONS, J. E., BLANCK, H. M., TOHILL, B. C., ZHANG, J. & KHAN, L. K. 2006. Associations between body mass index and the prevalence of low micronutrient levels among US adults. *MedGenMed*, 8, 59.
- KISSEBAH, A. H. 1987. Low density lipoprotein metabolism in non-insulin-dependent diabetes mellitus. *Diabetes Metab Rev*, 3, 619-51.
- KLEIN, S. 2004. The case of visceral fat: argument for the defense. *J Clin Invest*, 113, 1530-1532.
- KLEIN, S., ALLISON, D. B., HEYMSFIELD, S. B., KELLEY, D. E., LEIBEL, R. L., NONAS, C. & KAHN, R. 2007. Waist Circumference and Cardiometabolic Risk. *A Consensus Statement from Shaping America's Health: Association for Weight Management and Obesity Prevention; NAASO, The Obesity Society; the American Society for Nutrition; and the American Diabetes Association*, 30, 1647-1652.
- KNOWLER, W. C., BARRETT-CONNOR, E., FOWLER, S. E., HAMMAN, R. F., LACHIN, J. M., WALKER, E. A. & NATHAN, D. M. 2002. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med*, 346, 393-403.
- KNUTSON, K. L., RYDEN, A. M., MANDER, B. A. & VAN CAUTER, E. 2006. Role of sleep duration and quality in the risk and severity of type 2 diabetes mellitus. *Arch Intern Med*, 166, 1768-74.
- KOLOTKIN, R. L., METER, K. & WILLIAMS, G. R. 2001. Quality of life and obesity. *Obes Rev*, 2, 219-29.
- KORYTKOWSKI, M. 2002. When oral agents fail: practical barriers to starting insulin. *Int J Obes Relat Metab Disord*, 26 Suppl 3, S18-24.
- KOSAKA, K., KUZUYA, T., HAGURA, R. & YOSHINAGA, H. 1996. Insulin response to oral glucose load is consistently decreased in established non-insulin-dependent diabetes mellitus: the usefulness of decreased early insulin response as a predictor of non-insulin-dependent diabetes mellitus. *Diabet Med*, 13, S109-19.
- KOYAMA, Y. & BRENNER, D. A. 2017. Liver inflammation and fibrosis. *J Clin Invest*, 127, 55-64.

- KROOK, A., BJORNHOLM, M., GALUSKA, D., JIANG, X. J., FAHLMAN, R., MYERS, M. G., JR., WALLBERG-HENRIKSSON, H. & ZIERATH, J. R. 2000. Characterization of signal transduction and glucose transport in skeletal muscle from type 2 diabetic patients. *Diabetes*, 49, 284-92.
- LACKEY, D. E. & OLEFSKY, J. M. 2016. Regulation of metabolism by the innate immune system. *Nat Rev Endocrinol*, 12, 15-28.
- LAMOND, N., TIGGEMANN, M. & DAWSON, D. 2000. Factors predicting sleep disruption in Type II diabetes. *Sleep*, 23, 415-6.
- LANGENBERG, C., SHARP, S. J., FRANKS, P. W., SCOTT, R. A., DELOUKAS, P., FOROUHI, N. G., FROGUEL, P., GROOP, L. C., HANSEN, T., PALLA, L., PEDERSEN, O., SCHULZE, M. B., TORMO, M. J., WHEELER, E., AGNOLI, C., ARRIOLA, L., BARRICARTE, A., BOEING, H., CLARKE, G. M., CLAVEL-CHAPELON, F., DUELL, E. J., FAGHERAZZI, G., KAAKS, R., KERRISON, N. D., KEY, T. J., KHAW, K. T., KROGER, J., LAJOUS, M., MORRIS, A. P., NAVARRO, C., NILSSON, P. M., OVERVAD, K., PALLI, D., PANICO, S., QUIROS, J. R., ROLANDSSON, O., SACERDOTE, C., SANCHEZ, M. J., SLIMANI, N., SPIJKERMAN, A. M., TUMINO, R., VAN DER, A. D., VAN DER SCHOUW, Y. T., BARROSO, I., MCCARTHY, M. I., RIBOLI, E. & WAREHAM, N. J. 2014. Gene-lifestyle interaction and type 2 diabetes: the EPIC interact case-cohort study. *PLoS Med*, 11, 1-14.
- LARGER, E., RUFAT, P., DUBOIS-LAFORGUE, D. & LEDOUX, S. 2001. Insulin therapy does not itself induce weight gain in patients with type 2 diabetes. *Diabetes Care*, 24, 1849-50.
- LARSEN, C. M., FAULENBACH, M., VAAG, A., VOLUND, A., EHSES, J. A., SEIFERT, B., MANDRUP-POULSEN, T. & DONATH, M. Y. 2007. Interleukin-1-receptor antagonist in type 2 diabetes mellitus. *N Engl J Med*, 356, 1517-26.
- LARSEN, R. N., MANN, N. J., MACLEAN, E. & SHAW, J. E. 2011. The effect of high-protein, low-carbohydrate diets in the treatment of type 2 diabetes: a 12 month randomised controlled trial. *Diabetologia*, 54, 731-40.
- LARSON-MEYER, D. E., HEILBRONN, L. K., REDMAN, L. M., NEWCOMER, B. R., FRISARD, M. I., ANTON, S., SMITH, S. R., ALFONSO, A. & RAVUSSIN, E. 2006. Effect of calorie restriction with or without exercise on insulin sensitivity, beta-cell function, fat cell size, and ectopic lipid in overweight subjects. *Diabetes Care*, 29, 1337-44.
- LAVILLE, M. & ANDREELLI, F. 2000. [Mechanisms for weight gain during blood glucose normalization]. *Diabetes Metab*, 26 Suppl 3, 42-5.
- LAWS, R. 2004. A new evidence-based model for weight management in primary care: the Counterweight Programme. *J Hum Nutr Diet*, 17, 191-208.
- LE FLOCH, J. P. 2010. Critical appraisal of the safety and efficacy of insulin detemir in glycemic control and cardiovascular risk management in diabetics. *Diabetes Metab Syndr Obes*, 3, 197-213.
- LEAN, M. 2011. VLED and formula LED in the management of type 2 diabetes: defining the clinical need and research requirements. *Clin Obes*, 1, 41-9.
- LEAN, M., BROSNAHAN, N., MCLOONE, P., MCCOMBIE, L., HIGGS, A. B., ROSS, H., MACKENZIE, M., GRIEVE, E., FINER, N., RECKLESS, J., HASLAM, D., SLOAN, B. & MORRISON, D. 2013. Feasibility and indicative results from a 12-month low-energy liquid diet treatment and maintenance programme for severe obesity. *Br J Gen Pract*, 63, e115-24.
- LEAN, M. E. & JAMES, W. P. 1986. Prescription of diabetic diets in the 1980s. *Lancet*, 1, 723-5.

- LEAN, M. E., LESLIE, W. S., BARNES, A. C., BROSNAN, N., THOM, G., MCCOMBIE, L., PETERS, C., ZHYZHNEUSKAYA, S., AL-MRABEH, A., HOLLINGSWORTH, K. G., RODRIGUES, A. M., REHACKOVA, L., ADAMSON, A. J., SNIEHOTTA, F. F., MATHERS, J. C., ROSS, H. M., MCILVENNA, Y., STEFANETTI, R., TRENELL, M., WELSH, P., KEAN, S., FORD, I., MCCONNACHIE, A., SATTAR, N. & TAYLOR, R. 2017. Primary care-led weight management for remission of type 2 diabetes (DiRECT): an open-label, cluster-randomised trial. *Lancet*, 391, 541-551.
- LECUBE, A., SÁNCHEZ, E., GÓMEZ-PERALTA, F., ABREU, C., VALLS, J., MESTRE, O., ROMERO, O., MARTÍNEZ, M. D., SAMPOL, G., CIUDIN, A., HERNÁNDEZ, C. & SIMÓ, R. 2016. Global Assessment of the Impact of Type 2 Diabetes on Sleep through Specific Questionnaires. A Case-Control Study. *PLoS One*, 11.
- LEE, Y., HIROSE, H., OHNEDA, M., JOHNSON, J. H., MCGARRY, J. D. & UNGER, R. H. 1994. Beta-cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte-beta-cell relationships. *Proc Natl Acad Sci U S A*, 91, 10878-82.
- LEE, Y., LINGVAY, I., SZCZEPANIAK, L. S., RAVAZZOLA, M., ORCI, L. & UNGER, R. H. 2010. Pancreatic steatosis: harbinger of type 2 diabetes in obese rodents. *Int J Obes (Lond)*, 34, 396-400.
- LEEDS, A. R. 2014. Formula food-reducing diets: A new evidence-based addition to the weight management tool box. *Nutr Bull*, 39, 238-246.
- LEIBEL, R. L. & HIRSCH, J. 1984. Diminished energy requirements in reduced-obese patients. *Metabolism*, 33, 164-70.
- LEIBEL, R. L., ROSENBAUM, M. & HIRSCH, J. 1995. Changes in energy expenditure resulting from altered body weight. *N Engl J Med*, 332, 621-8.
- LEJEUNE, M. P., KOVACS, E. M. & WESTERTERP-PLANTENGA, M. S. 2005. Additional protein intake limits weight regain after weight loss in humans. *Br J Nutr*, 93, 281-9.
- LEMIEUX, I., LAMARCHE, B., COUILLARD, C., PASCOT, A., CANTIN, B., BERGERON, J., DAGENAIS, G. R. & DESPRES, J. P. 2001. Total cholesterol/HDL cholesterol ratio vs LDL cholesterol/HDL cholesterol ratio as indices of ischemic heart disease risk in men: the Quebec Cardiovascular Study. *Arch Intern Med*, 161, 2685-92.
- LEONG, W. B., TAHERI, S. 2012. The role of bariatric surgery in the treatment of type 2 diabetes mellitus. *J R Coll Physicians Edinb*, 42, 194-198.
- LEROITH, D. 2002. Beta-cell dysfunction and insulin resistance in type 2 diabetes: role of metabolic and genetic abnormalities. *Am J Med*, 113 Suppl 6A, 3s-11s.
- LEVEY, A. S., BOSCH, J. P., LEWIS, J. B., GREENE, T., ROGERS, N. & ROTH, D. 1999. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med*, 130, 461-70.
- LEWIS, M. C., PHILLIPS, M. L., SLAVOTINEK, J. P., KOW, L., THOMPSON, C. H. & TOOULI, J. 2006. Change in liver size and fat content after treatment with Optifast very low calorie diet. *Obes Surg*, 16, 697-701.
- LICHTMAN, S. W., PISARSKA, K., BERMAN, E. R., PESTONE, M., DOWLING, H., OFFENBACHER, E., WEISEL, H., HESHKA, S., MATTHEWS, D. E. & HEYMSFIELD, S. B. 1992. Discrepancy between self-reported and actual caloric intake and exercise in obese subjects. *N Engl J Med*, 327, 1893-8.
- LIM, E. L., HOLLINGSWORTH, K. G., ARIBISALA, B. S., CHEN, M. J., MATHERS, J. C. & TAYLOR, R. 2011. Reversal of type 2 diabetes: normalisation of beta cell

- function in association with decreased pancreas and liver triacylglycerol. *Diabetologia*, 54, 2506-14.
- LINDBERG, E., THEORELL-HAGLOW, J., SVENSSON, M., GISLASON, T., BERNE, C. & JANSON, C. 2012. Sleep apnea and glucose metabolism: a long-term follow-up in a community-based sample. *Chest*, 142, 935-942.
- LINDHOLM, A. 2002. New insulins in the treatment of diabetes mellitus. *Best Pract Res Clin Gastroenterol*, 16, 475-92.
- LINGVAY, I., ESSER, V., LEGENDRE, J. L., PRICE, A. L., WERTZ, K. M., ADAMS-HUET, B., ZHANG, S., UNGER, R. H. & SZCZEPANIAK, L. S. 2009. Noninvasive quantification of pancreatic fat in humans. *J Clin Endocrinol Metab*, 94, 4070-6.
- LIPS, M. A., DE GROOT, G. H., VAN KLINKEN, J. B., AARTS, E., BERENDS, F. J., JANSSEN, I. M., VAN RAMSHORST, B., VAN WAGENSVELD, B. A., SWANK, D. J., VAN DIELEN, F., WILLEMS VAN DIJK, K. & PIJL, H. 2014. Calorie restriction is a major determinant of the short-term metabolic effects of gastric bypass surgery in obese type 2 diabetic patients. *Clin Endocrinol (Oxf)*, 80, 834-42.
- LISSNER, L., ODELL, P. M., D'AGOSTINO, R. B., STOKES, J., 3RD, KREGER, B. E., BELANGER, A. J. & BROWNELL, K. D. 1991. Variability of body weight and health outcomes in the Framingham population. *N Engl J Med*, 324, 1839-44.
- LOOKER, H. C., KNOWLER, W. C. & HANSON, R. L. 2001. Changes in BMI and weight before and after the development of type 2 diabetes. *Diabetes Care*, 24, 1917-22.
- LOWE, M. R. & BUTRYN, M. L. 2007. Hedonic hunger: a new dimension of appetite? *Physiol Behav*, 91, 432-9.
- LUPI, R., DOTTA, F., MARSELLI, L., DEL GUERRA, S., MASINI, M., SANTANGELO, C., PATANÉ, G., BOGGI, U., PIRO, S., ANELLO, M., BERGAMINI, E., MOSCA, F., DI MARIO, U., DEL PRATO, S. & MARCHETTI, P. 2002. Prolonged Exposure to Free Fatty Acids Has Cytostatic and Pro-Apoptotic Effects on Human Pancreatic Islets. *Evidence that β -Cell Death Is Caspase Mediated, Partially Dependent on Ceramide Pathway, and Bcl-2 Regulated*, 51, 1437-1442.
- LUZI, L. & DEFRONZO, R. A. 1989. Effect of loss of first-phase insulin secretion on hepatic glucose production and tissue glucose disposal in humans. *Am J Physiol*, 257, E241-6.
- MALANDRUCCO, I., PASQUALETTI, P., GIORDANI, I., MANFELLOTTI, D., DE MARCO, F., ALEGIANI, F., SIDOTI, A. M., PICCONI, F., DI FLAVIANI, A., FRAJESE, G., BONADONNA, R. C. & FRONTONI, S. 2012. Very-low-calorie diet: a quick therapeutic tool to improve beta cell function in morbidly obese patients with type 2 diabetes. *Am J Clin Nutr*, 95, 609-13.
- MANLEY, S. E., STRATTON, I.M., CLARK, P.M. AND LUZIO, S.D. 2007. Comparison of 11 Human Insulin Assays: Implications for Clinical Investigation and Research. *Clinical Chemistry*, 53, 922-932.
- MARIC-BILKAN, C. 2013. Obesity and Diabetic Kidney Disease. *Med Clin North Am*, 97, 59-74.
- MARIN, J. M., CARRIZO, S. J., VICENTE, E. & AGUSTI, A. G. 2005. Long-term cardiovascular outcomes in men with obstructive sleep apnoea-hypopnoea with or without treatment with continuous positive airway pressure: an observational study. *Lancet*, 365, 1046-53.
- MARSHALL, N. S., WONG, K. K., LIU, P. Y., CULLEN, S. R., KNUIMAN, M. W. & GRUNSTEIN, R. R. 2008. Sleep apnea as an independent risk factor for all-cause mortality: the Busselton Health Study. *Sleep*, 31, 1079-85.
- MATHER, K. J., HUNT, A. E., STEINBERG, H. O., PARADISI, G., HOOK, G., KATZ, A., QUON, M. J. & BARON, A. D. 2001. Repeatability characteristics of simple indices

- of insulin resistance: implications for research applications. *J Clin Endocrinol Metab*, 86, 5457-64.
- MATSUDA, M. & DEFRONZO, R. A. 1999. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care*, 22, 1462-70.
- MATTHEWS, D. R., CULL, C. A., STRATTON, I. M., HOLMAN, R. R. & TURNER, R. C. 1998. UKPDS 26: Sulphonylurea failure in non-insulin-dependent diabetic patients over six years. UK Prospective Diabetes Study (UKPDS) Group. *Diabet Med*, 15, 297-303.
- MATTHEWS, D. R., HOSKER, J. P., RUDENSKI, A. S., NAYLOR, B. A., TREACHER, D. F. & TURNER, R. C. 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*, 28, 412-9.
- MATTOCK, M. B., MORRISH, N. J., VIBERTI, G., KEEN, H., FITZGERALD, A. P. & JACKSON, G. 1992. Prospective study of microalbuminuria as predictor of mortality in NIDDM. *Diabetes*, 41, 736-41.
- MCNICHOLAS, W. T. 2008. Diagnosis of obstructive sleep apnea in adults. *Proc Am Thorac Soc*, 5, 154-60.
- MEHTA, T., SMITH, D. L., JR., MUHAMMAD, J. & CASAZZA, K. 2014. Impact of weight cycling on risk of morbidity and mortality. *Obes Rev*, 15, 870-81.
- MEIER, J. J. 2012. GLP-1 receptor agonists for individualized treatment of type 2 diabetes mellitus. *Nat Rev Endocrinol*, 8, 728-42.
- MEIGS, J. B., CUPPLES, L. A. & WILSON, P. W. 2000. Parental transmission of type 2 diabetes: the Framingham Offspring Study. *Diabetes*, 49, 2201-7.
- MEISINGER, C., DORING, A., THORAND, B., HEIER, M. & LOWEL, H. 2006. Body fat distribution and risk of type 2 diabetes in the general population: are there differences between men and women? The MONICA/KORA Augsburg cohort study. *Am J Clin Nutr*, 84, 483-9.
- MELIN, I., KARLSTROM, B., LAPPALAINEN, R., BERGLUND, L., MOHSEN, R. & VESSBY, B. 2003. A programme of behaviour modification and nutrition counselling in the treatment of obesity: a randomised 2-y clinical trial. *Int J Obes Relat Metab Disord*, 27, 1127-35.
- MENEGHINI, L. F. 2009. Early insulin treatment in type 2 diabetes: what are the pros? *Diabetes Care*, 32 Suppl 2, S266-9.
- MERCOLINI, L., MUSENGA, A., SALADINI, B., BIGUCCI, F., LUPPI, B., ZECCHI, V. & RAGGI, M. A. 2008. Determination of insulin in innovative formulations by means of LC coupled to fluorescence detection. *J Pharm Biomed Anal*, 48, 1303-9.
- MIFFLIN, M. D., ST JEOR, S. T., HILL, L. A., SCOTT, B. J., DAUGHERTY, S. A. & KOH, Y. O. 1990. A new predictive equation for resting energy expenditure in healthy individuals. *Am J Clin Nutr*, 51, 241-7.
- MOGENSEN, U. M., ANDERSSON, C., FOSBOL, E. L., SCHRAMM, T. K., VAAG, A., SCHELLER, N. M., TORP-PEDERSEN, C., GISLASON, G. & KOBER, L. 2014. Cardiovascular safety of combination therapies with incretin-based drugs and metformin compared with a combination of metformin and sulphonylurea in type 2 diabetes mellitus - a retrospective nationwide study. *Diabetes Obes Metab*, 16, 1001-8.
- MORAES-VIEIRA, P. M., SAGHATELIAN, A. & KAHN, B. B. 2016. GLUT4 Expression in Adipocytes Regulates De Novo Lipogenesis and Levels of a Novel Class of Lipids With Antidiabetic and Anti-inflammatory Effects. *Diabetes*, 65, 1808-1815.

- MORAN, L., TSAGARELI, V., NORMAN, R. & NOAKES, M. 2011. Diet and IVF pilot study: short-term weight loss improves pregnancy rates in overweight/obese women undertaking IVF. *Aust N Z J Obstet Gynaecol*, 51, 455-9.
- MORGAN, C. L., JENKINS-JONES, S., EVANS, M., BARNETT, A. H., POOLE, C. D. & CURRIE, C. J. 2012. Weight change in people with type 2 diabetes: secular trends and the impact of alternative antihyperglycaemic drugs. *Diabetes Obes Metab*, 14, 424-32.
- MORINAGA, H., MAYORAL, R., HEINRICHS-DORFF, J., OSBORN, O., FRANCK, N., HAH, N., WALENTA, E., BANDYOPADHYAY, G., PESSENTHEINER, A. R., CHI, T. J., CHUNG, H., BOGNER-STRAUSS, J. G., EVANS, R. M., OLEFSKY, J. M. & OH, D. Y. 2015. Characterization of distinct subpopulations of hepatic macrophages in HFD/obese mice. *Diabetes*, 64, 1120-30.
- MORINO, K., PETERSEN, K. F. & SHULMAN, G. I. 2006. Molecular mechanisms of insulin resistance in humans and their potential links with mitochondrial dysfunction. *Diabetes*, 55 Suppl 2, S9-s15.
- MULHOLLAND, Y., NICOKAVOURA, E., BROOM, J. & ROLLAND, C. 2012. Very-low-energy diets and morbidity: a systematic review of longer-term evidence. *Br J Nutr*, 108, 832-51.
- MURPHY, K. G. & BLOOM, S. R. 2004. Gut hormones in the control of appetite. *Exp Physiol*, 89, 507-16.
- NAKAR, S., YITZHAKI, G., ROSENBERG, R. & VINKER, S. 2007. Transition to insulin in Type 2 diabetes: family physicians' misconception of patients' fears contributes to existing barriers. *J Diabetes Complications*, 21, 220-6.
- NATALI, A., DEL PRATO, S. AND MARL A. 2015. Normal β -cell Secretion. In: DEFRONZO, R. A., FERRANNINI, E., ZIMMIT, P., ALBERTI, K.G.M.M. (ed.) *International Textbook of Diabetes Mellitus*. Fourth ed. United Kingdom: John Wiley & Sons Ltd.
- NATIONAL COLLABORATING CENTRE FOR CHRONIC, C. 2008. National Institute for Health and Clinical Excellence: Guidance. *Type 2 Diabetes: National Clinical Guideline for Management in Primary and Secondary Care (Update)*. London: Royal College of Physicians (UK), Royal College of Physicians of London.
- NATIONAL INSTITUTE FOR HEALTH AND CARE EXCELLENCE 2006. Obesity - Guidance on the prevention, identification, assessment and management of overweight and obesity in adults and children CG43. In: NICE (ed.). UK.
- NAVANEETHAN, S. D., YEHNERT, H., MOUSTARAH, F., SCHREIBER, M. J., SCHAUER, P. R. & BEDDHU, S. 2009. Weight loss interventions in chronic kidney disease: a systematic review and meta-analysis. *Clin J Am Soc Nephrol*, 4, 1565-74.
- NERFELDT, P., NILSSON, B. Y., MAYOR, L., UDDEN, J. & FRIBERG, D. 2010. A two-year weight reduction program in obese sleep apnea patients. *J Clin Sleep Med*, 6, 479-86.
- NEWSHOLME, P., KEANE, D., WELTERS, H. J. & MORGAN, N. G. 2007. Life and death decisions of the pancreatic beta-cell: the role of fatty acids. *Clin Sci (Lond)*, 112, 27-42.
- NEWSHOLME, P. & KRAUSE, M. 2012. Nutritional regulation of insulin secretion: implications for diabetes. *Clin Biochem Rev*, 33, 35-47.
- NHMRC 2013. National Health and Medical Research Council Clinical practice guidelines for the management of overweight and obesity in adults, adolescents and children in Australia. Melbourne: National Health and Medical Research Council.

- NICE 2006. Obesity: guidance on the prevention, identification, assessment and management of overweight and obesity in adults and children CG43. *In*: (UK), N. I. F. H. A. C. E. (ed.). London.
- NICE 2012. National Institute for Health and Care Excellence Preventing type 2 diabetes: risk identification and interventions for individuals at high risk.
- NICE 2014a. National Institute for Health and Care Excellence Lipid Modification: Cardiovascular risk assessment and the modification of blood lipids for primary and secondary prevention of cardiovascular disease. CG181. *In*: NICE (ed.). London.
- NICE 2014b. National Institute for Health and Clinical Excellence: Guidance. *Obesity: Identification, Assessment and Management of Overweight and Obesity in Children, Young People and Adults: Partial Update of CG43*. London: National Institute for Health and Care Excellence (UK)
- NICE 2014c. National Institute for Health and Clinical Excellence: Guidance. *Type 2 Diabetes: The Management of Diabetes (Partial Update)*. London: National Institute for Health and Clinical Excellence (UK).
- NICE 2015. National Institute for Health and Clinical Excellence: Guideline NG28 Type 2 diabetes in adults: management. *National Institute for Health and Clinical Excellence: Guideline NG28 Type 2 diabetes in adults: management*. London: National Institute for Health and Care Excellence (UK)
- NIELD, L., MOORE, H. J., HOOPER, L., CRUICKSHANK, J. K., VYAS, A., WHITTAKER, V. & SUMMERBELL, C. D. 2007. Dietary advice for treatment of type 2 diabetes mellitus in adults. *Cochrane Database Syst Rev*, Cd004097.
- NIELD, L., SUMMERBELL, C. D., HOOPER, L., WHITTAKER, V. & MOORE, H. 2008. Dietary advice for the prevention of type 2 diabetes mellitus in adults. *Cochrane Database Syst Rev*, Cd005102.
- NIELSEN, L. V., NIELSEN, M. S., SCHMIDT, J. B., PEDERSEN, S. D. & SJODIN, A. 2016. Efficacy of a liquid low-energy formula diet in achieving preoperative target weight loss before bariatric surgery. *J Nutr Sci*, 5, e22.
- NIELSEN, S., GUO, Z., JOHNSON, C. M., HENSRUD, D. D. & JENSEN, M. D. 2004. Splanchnic lipolysis in human obesity. *J Clin Invest*, 113, 1582-8.
- NOF. 2010. National Obesity Forum Position statement on the application of very low energy diets in achieving weight loss in the management of obesity. . [Accessed 20 July 2015].
- NORRIS, S. L., ZHANG, X., AVENELL, A., GREGG, E., BROWN, T. J., SCHMID, C. H. & LAU, J. 2005. Long-term non-pharmacologic weight loss interventions for adults with type 2 diabetes. *Cochrane Database Syst Rev*, 1-92.
- NOVOTNY, L. & VITEK, L. 2003. Inverse relationship between serum bilirubin and atherosclerosis in men: a meta-analysis of published studies. *Exp Biol Med (Maywood)*, 228, 568-71.
- NUTTALL, F. Q., MOORADIAN, A. D., GANNON, M. C., BILLINGTON, C. & KREZOWSKI, P. 1984. Effect of protein ingestion on the glucose and insulin response to a standardized oral glucose load. *Diabetes Care*, 7, 465-70.
- O'DONOVAN, G., BLAZEVOICH, A. J., BOREHAM, C., COOPER, A. R., CRANK, H., EKELUND, U., FOX, K. R., GATELY, P., GILES-CORTI, B., GILL, J. M., HAMER, M., MCDERMOTT, I., MURPHY, M., MUTRIE, N., REILLY, J. J., SAXTON, J. M. & STAMATAKIS, E. 2010. The ABC of Physical Activity for Health: a consensus statement from the British Association of Sport and Exercise Sciences. *J Sports Sci*, 28, 573-91.

- OECD. 2017. *Organisation for Economic Co-operation Development Obesity Update 2017* [Online]. OECD Publishing. Available: <http://www.oecd.org/health/obesity-update.htm> [Accessed 23 November 2017].
- OHLSON, L.-O., LARSSON, B., SVÄRDSUDD, K., WELIN, L., ERIKSSON, H., WILHELMSSEN, L., BJÖRNTORP, P. & TIBBLIN, G. 1985. The Influence of Body Fat Distribution on the Incidence of Diabetes Mellitus: 13.5 Years of Follow-up of the Participants in the Study of Men Born in 1913. *Diabetes*, 34, 1055-1058.
- OKITA, K., IWAHASHI, H., KOZAWA, J., OKAUCHI, Y., FUNAHASHI, T., IMAGAWA, A. & SHIMOMURA, I. 2013. Homeostasis model assessment of insulin resistance for evaluating insulin sensitivity in patients with type 2 diabetes on insulin therapy. *Endocr J*, 60, 283-90.
- OWENS, D. R., ZINMAN, B. & BOLLI, G. B. 2001. Insulins today and beyond. *Lancet*, 358, 739-46.
- PACKIANATHAN, I. C., FULLER, N. J., PETERSON, D. B., WRIGHT, A., COWARD, W. A. & FINER, N. 2005. Use of a reference four-component model to define the effects of insulin treatment on body composition in type 2 diabetes: the 'Darwin study'. *Diabetologia*, 48, 222-9.
- PAISEY, R. B., FROST, J., HARVEY, P., PAISEY, A., BOWER, L., PAISEY, R. M., TAYLOR, P. & BELKA, I. 2002. Five year results of a prospective very low calorie diet or conventional weight loss programme in type 2 diabetes. *J Hum Nutr Diet*, 15, 121-7.
- PAMIDI, S. & TASALI, E. 2012. Obstructive Sleep Apnea and Type 2 Diabetes: Is There a Link? *Front Neurol*, 3, 1-13.
- PAN, X. R., LI, G. W., HU, Y. H., WANG, J. X., YANG, W. Y., AN, Z. X., HU, Z. X., LIN, J., XIAO, J. Z., CAO, H. B., LIU, P. A., JIANG, X. G., JIANG, Y. Y., WANG, J. P., ZHENG, H., ZHANG, H., BENNETT, P. H. & HOWARD, B. V. 1997. Effects of diet and exercise in preventing NIDDM in people with impaired glucose tolerance. The Da Qing IGT and Diabetes Study. *Diabetes Care*, 20, 537-44.
- PARRETTI, H. M., JEBB, S. A., JOHNS, D. J., LEWIS, A. L., CHRISTIAN-BROWN, A. M. & AVEYARD, P. 2016. Clinical effectiveness of very-low-energy diets in the management of weight loss: a systematic review and meta-analysis of randomized controlled trials. *Obes Rev*, 17, 225-34.
- PATEL, A., MACMAHON, S., CHALMERS, J., NEAL, B., BILLOT, L., WOODWARD, M., MARRE, M., COOPER, M., GLASZIOU, P., GROBBEE, D., HAMET, P., HARRAP, S., HELLER, S., LIU, L., MANCIA, G., MOGENSEN, C. E., PAN, C., POULTER, N., RODGERS, A., WILLIAMS, B., BOMPOINT, S., DE GALAN, B. E., JOSHI, R. & TRAVERT, F. 2008. Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. *N Engl J Med*, 358, 2560-72.
- PATEYJOHNS, I. R., BRINKWORTH, G. D., BUCKLEY, J. D., NOAKES, M. & CLIFTON, P. M. 2006. Comparison of three bioelectrical impedance methods with DXA in overweight and obese men. *Obesity (Silver Spring)*, 14, 2064-70.
- PAUL, S. K., SHAW, J. E., MONTVIDA, O. & KLEIN, K. 2016. Weight gain in insulin-treated patients by body mass index category at treatment initiation: new evidence from real-world data in patients with type 2 diabetes. *Diabetes Obes Metab*, 18, 1244-1252.
- PEDERSEN, L. R., OLSEN, R. H., JURSS, A., ASTRUP, A., CHABANOVA, E., SIMONSEN, L., WISLOFF, U., HAUGAARD, S. B. & PRESCOTT, E. 2014. A randomised trial comparing weight loss with aerobic exercise in overweight individuals with coronary artery disease: The CUT-IT trial. *Eur J Prev Cardiol*.

- PEDERSEN, L. R., OLSEN, R. H., JURIS, A., ASTRUP, A., CHABANOVA, E., SIMONSEN, L., WISLOFF, U., HAUGAARD, S. B. & PRESCOTT, E. 2015. A randomised trial comparing weight loss with aerobic exercise in overweight individuals with coronary artery disease: The CUT-IT trial. *Eur J Prev Cardiol*, 22, 1009-17.
- PEPPARD, P. E., YOUNG, T., PALTA, M., DEMPSEY, J. & SKATRUD, J. 2000. Longitudinal study of moderate weight change and sleep-disordered breathing. *Jama*, 284, 3015-21.
- PERLEY, M. J. & KIPNIS, D. M. 1967. Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic subjects. *J Clin Invest*, 46, 1954-62.
- PERLSTEIN, T. S., PANDE, R. L., CREAGER, M. A., WEUVE, J. & BECKMAN, J. A. 2008. Serum total bilirubin level, prevalent stroke, and stroke outcomes: NHANES 1999-2004. *Am J Med*, 121, 781-788.e1.
- PERRY, R. J., PENG, L., CLINE, G. W., WANG, Y., RABIN-COURT, A., SONG, J. D., ZHANG, D., ZHANG, X. M., NOZAKI, Y., DUFOUR, S., PETERSEN, K. F. & SHULMAN, G. I. 2018. Mechanisms by which a Very-Low-Calorie Diet Reverses Hyperglycemia in a Rat Model of Type 2 Diabetes. *Cell Metab*, 27, 210-217.e3.
- PETERSEN, K. F., DUFOUR, S., BEFROY, D., GARCIA, R. & SHULMAN, G. I. 2004. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. *N Engl J Med*, 350, 664-71.
- PETERSEN, K. F., DUFOUR, S. & SHULMAN, G. I. 2005. Decreased insulin-stimulated ATP synthesis and phosphate transport in muscle of insulin-resistant offspring of type 2 diabetic parents. *PLoS Med*, 2, e233.
- PEYROT, M., RUBIN, R. R. & KHUNTI, K. 2010. Addressing barriers to initiation of insulin in patients with type 2 diabetes. *Prim Care Diabetes*, 4 Suppl 1, S11-8.
- PEYROT, M., RUBIN, R. R., LAURITZEN, T., SKOVLUND, S. E., SNOEK, F. J., MATTHEWS, D. R., LANDGRAF, R. & KLEINEBREIL, L. 2005. Resistance to insulin therapy among patients and providers: results of the cross-national Diabetes Attitudes, Wishes, and Needs (DAWN) study. *Diabetes Care*, 28, 2673-9.
- PHILLIPS, D. I., CLARK, P. M., HALES, C. N. & OSMOND, C. 1994. Understanding oral glucose tolerance: comparison of glucose or insulin measurements during the oral glucose tolerance test with specific measurements of insulin resistance and insulin secretion. *Diabet Med*, 11, 286-92.
- PI-SUNYER, F. X. 2009. The impact of weight gain on motivation, compliance, and metabolic control in patients with type 2 diabetes mellitus. *Postgrad Med*, 121, 94-107.
- PI-SUNYER, X., BLACKBURN, G., BRANCATI, F. L., BRAY, G. A., BRIGHT, R., CLARK, J. M., CURTIS, J. M., ESPELAND, M. A., FOREYT, J. P., GRAVES, K., HAFFNER, S. M., HARRISON, B., HILL, J. O., HORTON, E. S., JAKICIC, J., JEFFERY, R. W., JOHNSON, K. C., KAHN, S., KELLEY, D. E., KITABCHI, A. E., KNOWLER, W. C., LEWIS, C. E., MASCHAK-CAREY, B. J., MONTGOMERY, B., NATHAN, D. M., PATRICIO, J., PETERS, A., REDMON, J. B., REEVES, R. S., RYAN, D. H., SAFFORD, M., VAN DORSTEN, B., WADDEN, T. A., WAGENKNECHT, L., WESCHE-THOBABEN, J., WING, R. R. & YANOVSKI, S. Z. 2007. Reduction in weight and cardiovascular disease risk factors in individuals with type 2 diabetes: one-year results of the look AHEAD trial. *Diabetes Care*, 30, 1374-83.
- PIATTI, P. M., MONTI, F., FERMO, I., BARUFFALDI, L., NASSER, R., SANTAMBROGIO, G., LIBRENTI, M. C., GALLI-KIENLE, M., PONTIROLI, A. E. & POZZA, G. 1994. Hypocaloric high-protein diet improves glucose oxidation and

- spares lean body mass: comparison to hypocaloric high-carbohydrate diet. *Metabolism*, 43, 1481-7.
- PLOURDE, C. E., GRENIER-LAROCHE, T., CARON-DORVAL, D., BIRON, S., MARCEAU, S., LEBEL, S., BIERTHO, L., TCHERNOF, A., RICHARD, D. & CARPENTIER, A. C. 2014. Biliopancreatic diversion with duodenal switch improves insulin sensitivity and secretion through caloric restriction. *Obesity (Silver Spring)*, 22, 1838-46.
- POLONSKY, W. H., FISHER, L., GUZMAN, S., VILLA-CABALLERO, L. & EDELMAN, S. V. 2005. Psychological insulin resistance in patients with type 2 diabetes: the scope of the problem. *Diabetes Care*, 28, 2543-5.
- POUWER, F. & HERMANN, N. 2009. Insulin therapy and quality of life. A review. *Diabetes Metab Res Rev*, 25 Suppl 1, S4-s10.
- PRENTICE, A. M., BLACK, A. E., COWARD, W. A., DAVIES, H. L., GOLDBERG, G. R., MURGATROYD, P. R., ASHFORD, J., SAWYER, M. & WHITEHEAD, R. G. 1986. High levels of energy expenditure in obese women. *Br Med J (Clin Res Ed)*, 292, 983-7.
- PROKS, P., ELIASSON, L., AMMALA, C., RORSMAN, P. & ASHCROFT, F. M. 1996. Ca(2+)- and GTP-dependent exocytosis in mouse pancreatic beta-cells involves both common and distinct steps. *J Physiol*, 496 (Pt 1), 255-64.
- PUBLIC HEALTH ENGLAND 2014. Everybody active, every day. An evidence-based approach to physical activity. In: ENGLAND, P. H. (ed.). London.
- PUNJABI, N. M. 2008. The epidemiology of adult obstructive sleep apnea. *Proc Am Thorac Soc*, 5, 136-43.
- PURCELL, K., SUMITHRAN, P., PRENDERGAST, L. A., BOUNIU, C. J., DELBRIDGE, E. & PROIETTO, J. 2014. The effect of rate of weight loss on long-term weight management: a randomised controlled trial. *Lancet Diabetes Endocrinol*, 2, 954-962.
- PURNELL, J. Q., ZINMAN, B. & BRUNZELL, J. D. 2013. The effect of excess weight gain with intensive diabetes mellitus treatment on cardiovascular disease risk factors and atherosclerosis in type 1 diabetes mellitus: results from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study (DCCT/EDIC) study. *Circulation*, 127, 180-7.
- RABA, J. A. M., H.A. 1995. Glucose Oxidase as an Analytical Reagent. *Critical Reviews in Analytical Chemistry*, 25, 1-42.
- RANA, J. S., LIU, J. Y., MOFFET, H. H., JAFFE, M. & KARTER, A. J. 2016. Diabetes and Prior Coronary Heart Disease are Not Necessarily Risk Equivalent for Future Coronary Heart Disease Events. *J Gen Intern Med*, 31, 387-93.
- REAVEN, G. 2004. The metabolic syndrome or the insulin resistance syndrome? Different names, different concepts, and different goals. *Endocrinol Metab Clin North Am*, 33, 283-303.
- REAVEN, G. M. 1985. Beneficial effect of moderate weight loss in older patients with non-insulin-dependent diabetes mellitus poorly controlled with insulin. *J Am Geriatr Soc*, 33, 93-5.
- REHACKOVA, L., ARAUJO-SOARES, V., ADAMSON, A. J., STEVEN, S., TAYLOR, R. & SNIEHOTTA, F. F. 2017. Acceptability of a very-low-energy diet in Type 2 diabetes: patient experiences and behaviour regulation. *Diabet Med*, 34, 1554-1567.
- RETNAKARAN, R., QI, Y., GORAN, M. I. & HAMILTON, J. K. 2009. Evaluation of proposed oral disposition index measures in relation to the actual disposition index. *Diabet Med*, 26, 1198-203.

- RICE, B., JANSSEN, I., HUDSON, R. & ROSS, R. 1999. Effects of aerobic or resistance exercise and/or diet on glucose tolerance and plasma insulin levels in obese men. *Diabetes Care*, 22, 684-91.
- RICHARDSON, S. J., WILLCOX, A., BONE, A. J., FOULIS, A. K. & MORGAN, N. G. 2009. Islet-associated macrophages in type 2 diabetes. *Diabetologia*, 52, 1686-8.
- RIDDERSTRALE, M., GUDBJORNSDOTTIR, S., ELIASSON, B., NILSSON, P. M. & CEDERHOLM, J. 2006. Obesity and cardiovascular risk factors in type 2 diabetes: results from the Swedish National Diabetes Register. *J Intern Med*, 259, 314-22.
- RIDER, O. J., FRANCIS, J. M., ALI, M. K., PETERSEN, S. E., ROBINSON, M., ROBSON, M. D., BYRNE, J. P., CLARKE, K. & NEUBAUER, S. 2009. Beneficial cardiovascular effects of bariatric surgical and dietary weight loss in obesity. *J Am Coll Cardiol*, 54, 718-26.
- RIECKE, B. F., CHRISTENSEN, R., CHRISTENSEN, P., LEEDS, A. R., BOESEN, M., LOHMANDER, L. S., ASTRUP, A. & BLIDDAL, H. 2010. Comparing two low-energy diets for the treatment of knee osteoarthritis symptoms in obese patients: a pragmatic randomized clinical trial. *Osteoarthritis Cartilage*, 18, 746-54.
- RIGALLEAU, V., DELAFAYE, C., BAILLET, L., VERGNOT, V., BRUNOU, P., GATTA, B. & GIN, H. 1999. Composition of insulin-induced body weight gain in diabetic patients: a bio-impedance study. *Diabetes Metab*, 25, 321-8.
- RISSANEN, A., HOWARD, C. P., BOTHA, J. & THUREN, T. 2012. Effect of anti-IL-1beta antibody (canakinumab) on insulin secretion rates in impaired glucose tolerance or type 2 diabetes: results of a randomized, placebo-controlled trial. *Diabetes Obes Metab*, 14, 1088-96.
- RITZ, E., RYCHLIK, I., LOCATELLI, F. & HALIMI, S. 1999. End-stage renal failure in type 2 diabetes: A medical catastrophe of worldwide dimensions. *Am J Kidney Dis*, 34, 795-808.
- RITZEL, R., ROUSSEL, R., BOLLI, G. B., VINET, L., BRULLE-WOHLHUETER, C., GLEZER, S. & YKI-JARVINEN, H. 2015. Patient-level meta-analysis of the EDITION 1, 2 and 3 studies: glycaemic control and hypoglycaemia with new insulin glargine 300 U/ml versus glargine 100 U/ml in people with type 2 diabetes. *Diabetes Obes Metab*, 17, 859-67.
- ROBINSON, J. G., WANG, S., SMITH, B. J. & JACOBSON, T. A. 2009. Meta-analysis of the relationship between non-high-density lipoprotein cholesterol reduction and coronary heart disease risk. *J Am Coll Cardiol*, 53, 316-22.
- RODEN, M., PERSEGHIN, G., PETERSEN, K. F., HWANG, J. H., CLINE, G. W., GEROW, K., ROTHMAN, D. L. & SHULMAN, G. I. 1996. The roles of insulin and glucagon in the regulation of hepatic glycogen synthesis and turnover in humans. *J Clin Invest*, 97, 642-8.
- ROLLAND, C. & BROOM, I. 2011. The Effects of Very-Low-Calorie Diets on HDL: A Review. *Cholesterol*, 2011, 1-10.
- ROLLAND, C., HESSION, M., MURRAY, S., WISE, A. & BROOM, I. 2009. Randomized clinical trial of standard dietary treatment versus a low-carbohydrate/high-protein diet or the LighterLife Programme in the management of obesity*. *J Diabetes*, 1, 207-17.
- ROLLAND, C., JOHNSTON, K. L., LULA, S., MACDONALD, I. & BROOM, J. 2014. Long-term weight loss maintenance and management following a VLCD: a 3-year outcome. *Int J Clin Pract*, 68, 379-87.
- ROLLAND, C., MAVROEIDI, A., JOHNSTON, K. L. & BROOM, J. 2013. The effect of very low-calorie diets on renal and hepatic outcomes: a systematic review. *Diabetes Metab Syndr Obes*, 6, 393-401.

- ROUBENOFF, R. 1996. Applications of bioelectrical impedance analysis for body composition to epidemiologic studies. *Am J Clin Nutr*, 64, 459s-462s.
- ROUMIE, C. L., GREEVY, R. A., GRIJALVA, C. G., HUNG, A. M., LIU, X., MURFF, H. J., ELASY, T. A. & GRIFFIN, M. R. 2014. Association between intensification of metformin treatment with insulin vs sulfonylureas and cardiovascular events and all-cause mortality among patients with diabetes. *Jama*, 311, 2288-96.
- RUBIN, R. R. & PEYROT, M. 1999. Quality of life and diabetes. *Diabetes Metab Res Rev*, 15, 205-18.
- RUSSELL-JONES, D. & KHAN, R. 2007. Insulin-associated weight gain in diabetes--causes, effects and coping strategies. *Diabetes Obes Metab*, 9, 799-812.
- RUSSELL-JONES, D., POWWER, F. & KHUNTI, K. 2018. Identification of barriers to insulin therapy and approaches to overcoming them. *Diabetes Obes Metab*, 20, 488-496.
- RYE, K. A. & BARTER, P. J. 2014. Cardioprotective functions of HDLs. *J Lipid Res*, 55, 168-79.
- SAIKI, A., NAGAYAMA, D., OHHIRA, M., ENDOH, K., OHTSUKA, M., KOIDE, N., OYAMA, T., MIYASHITA, Y. & SHIRAI, K. 2005. Effect of weight loss using formula diet on renal function in obese patients with diabetic nephropathy. *Int J Obes (Lond)*, 29, 1115-20.
- SALLE, A., GUILLLOTEAU, G., RYAN, M., BOUHANICK, B. & RITZ, P. 2004. Effect of insulin treatment on the body composition of Type 2 diabetic patients. *Diabet Med*, 21, 1298-303.
- SALTIEL, A. R. & OLEFSKY, J. M. 2017. Inflammatory mechanisms linking obesity and metabolic disease. *J Clin Invest*, 127, 1-4.
- SARIS, W. H. 2001. Very-low-calorie diets and sustained weight loss. *Obes Res*, 9 Suppl 4, 295s-301s.
- SARKHOSH, K., SWITZER, N. J., EL-HADI, M., BIRCH, D. W., SHI, X. & KARMALI, S. 2013. The impact of bariatric surgery on obstructive sleep apnea: a systematic review. *Obes Surg*, 23, 414-23.
- SATTAR, N., PETRIE, J. R. & JAAP, A. J. 1998. The atherogenic lipoprotein phenotype and vascular endothelial dysfunction. *Atherosclerosis*, 138, 229-35.
- SATTAR, N., WELSH, P. AND PRESS, D. 2015. The insulin resistance syndrome. In: DEFRONZO, R. A., FERRANNINI, E., ZIMMIT, P., ALBERTI, K.G.M.M. (ed.) *International Textbook of Diabetes Mellitus*. Fourth ed. United Kingdom: John Wiley & Sons Ltd.
- SCHAUER, P. R., BHATT, D. L., KIRWAN, J. P., WOLSKI, K., AMINIAN, A., BRETHAUER, S. A., NAVANEETHAN, S. D., SINGH, R. P., POTHIER, C. E., NISSEN, S. E. & KASHYAP, S. R. 2017a. Bariatric Surgery versus Intensive Medical Therapy for Diabetes - 5-Year Outcomes. *N Engl J Med*, 376, 641-651.
- SCHAUER, P. R., BHATT, D. L., KIRWAN, J. P., WOLSKI, K., AMINIAN, A., BRETHAUER, S. A., NAVANEETHAN, S. D., SINGH, R. P., POTHIER, C. E., NISSEN, S. E. & KASHYAP, S. R. 2017b. Bariatric Surgery versus Intensive Medical Therapy for Diabetes — 5-Year Outcomes. *New England Journal of Medicine*, 376, 641-651.
- SCHAUER, P. R., BHATT, D. L., KIRWAN, J. P., WOLSKI, K., BRETHAUER, S. A., NAVANEETHAN, S. D., AMINIAN, A., POTHIER, C. E., KIM, E. S., NISSEN, S. E. & KASHYAP, S. R. 2014. Bariatric surgery versus intensive medical therapy for diabetes--3-year outcomes. *N Engl J Med*, 370, 2002-13.
- SCHAUER, P. R., KASHYAP, S. R., WOLSKI, K., BRETHAUER, S. A., KIRWAN, J. P., POTHIER, C. E., THOMAS, S., ABOOD, B., NISSEN, S. E. & BHATT, D. L. 2012.

- Bariatric surgery versus intensive medical therapy in obese patients with diabetes. *N Engl J Med*, 366, 1567-76.
- SCHWARZ, J. M., LINFOOT, P., DARE, D. & AGHAJANIAN, K. 2003. Hepatic de novo lipogenesis in normoinsulinemic and hyperinsulinemic subjects consuming high-fat, low-carbohydrate and low-fat, high-carbohydrate isoenergetic diets. *Am J Clin Nutr*, 77, 43-50.
- SCOTT, N. W., MCPHERSON, G. C., RAMSAY, C. R. & CAMPBELL, M. K. 2002. The method of minimization for allocation to clinical trials. a review. *Control Clin Trials*, 23, 662-74.
- SCOTT, R. A., LANGENBERG, C., SHARP, S. J., FRANKS, P. W., ROLANDSSON, O., DROGAN, D., VAN DER SCHOUW, Y. T., EKELUND, U., KERRISON, N. D., ARDANAZ, E., ARRIOLA, L., BALKAU, B., BARRICARTE, A., BARROSO, I., BENDINELLI, B., BEULENS, J. W., BOEING, H., DE LAUZON-GUILLAIN, B., DELOUKAS, P., FAGHERAZZI, G., GONZALEZ, C., GRIFFIN, S. J., GROOP, L. C., HALKJAER, J., HUERTA, J. M., KAKS, R., KHAW, K. T., KROGH, V., NILSSON, P. M., NORAT, T., OVERVAD, K., PANICO, S., RODRIGUEZ-SUAREZ, L., ROMAGUERA, D., ROMIEU, I., SACERDOTE, C., SANCHEZ, M. J., SPIJKERMAN, A. M., TEUCHER, B., TJONNELAND, A., TUMINO, R., VAN DER, A. D., WARK, P. A., MCCARTHY, M. I., RIBOLI, E. & WAREHAM, N. J. 2013. The link between family history and risk of type 2 diabetes is not explained by anthropometric, lifestyle or genetic risk factors: the EPIC-InterAct study. *Diabetologia*, 56, 60-9.
- SEAGLE, H. M., STRAIN, G. W., MAKRIS, A. & REEVES, R. S. 2009. Position of the American Dietetic Association: weight management. *J Am Diet Assoc*, 109, 330-46.
- SEINO, S., SHIBASAKI, T. AND MINAMI, K. 2015. β -cell biology of insulin secretion. In: DEFRONZO, R. A., FERRANNINI, E., ZIMMIT, P., ALBERTI, K.G.M.M. (ed.) *International Textbook of Diabetes Mellitus*. Fourth ed. UK: John Willet & Sons Ltd.
- SELTZER, H. S., ALLEN, E. W., HERRON, A. L., JR. & BRENNAN, M. T. 1967. Insulin secretion in response to glycemic stimulus: relation of delayed initial release to carbohydrate intolerance in mild diabetes mellitus. *J Clin Invest*, 46, 323-35.
- SELVIN, E., PAYNTER, N. P. & ERLINGER, T. P. 2007. The effect of weight loss on C-reactive protein: a systematic review. *Arch Intern Med*, 167, 31-9.
- SENARATNA, C. V., PERRET, J. L., LODGE, C. J., LOWE, A. J., CAMPBELL, B. E., MATHESON, M. C., HAMILTON, G. S. & DHARMAGE, S. C. 2017. Prevalence of obstructive sleep apnea in the general population: A systematic review. *Sleep Med Rev*, 34, 70-81.
- SHAH, P. K. 2011. Effects of intensive insulin therapy alone and in combination with pioglitazone on body weight, composition, distribution and liver fat content in patients with type 2 diabetes. 13, 505-10.
- SHARRETT, A. R., BALLANTYNE, C. M., COADY, S. A., HEISS, G., SORLIE, P. D., CATELLIER, D. & PATSCH, W. 2001. Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein(a), apolipoproteins A-I and B, and HDL density subfractions: The Atherosclerosis Risk in Communities (ARIC) Study. *Circulation*, 104, 1108-13.
- SHEPARD, J. W., JR. 1992. Hypertension, cardiac arrhythmias, myocardial infarction, and stroke in relation to obstructive sleep apnea. *Clin Chest Med*, 13, 437-58.
- SHEPHERD, P. R. & KAHN, B. B. 1999. Glucose transporters and insulin action--implications for insulin resistance and diabetes mellitus. *N Engl J Med*, 341, 248-57.

- SHIMABUKURO, M., ZHOU, Y. T., LEVI, M. & UNGER, R. H. 1998. Fatty acid-induced beta cell apoptosis: a link between obesity and diabetes. *Proc Natl Acad Sci U S A*, 95, 2498-502.
- SIGN 2010a. Scottish Intercollegiate Guidelines Network 115 Management of Obesity: a national clinical guideline. In: SCOTLAND, N. (ed.). Edinburgh.
- SIGN 2010b. Scottish Intercollegiate Guidelines Network Management of Diabetes: A national clinical guideline, . Edinburgh, UK.
- SIMMONS, R. K., COLEMAN, R. L., PRICE, H. C., HOLMAN, R. R., KHAW, K. T., WAREHAM, N. J. & GRIFFIN, S. J. 2009. Performance of the UK Prospective Diabetes Study Risk Engine and the Framingham Risk Equations in Estimating Cardiovascular Disease in the EPIC- Norfolk Cohort. *Diabetes Care*, 32, 708-13.
- SIMON, C. & BRANDENBERGER, G. 2002. Ultradian oscillations of insulin secretion in humans. *Diabetes*, 51 Suppl 1, S258-61.
- SINCLAIR, A. J., BURDON, M. A., NIGHTINGALE, P. G., BALL, A. K., GOOD, P., MATTHEWS, T. D., JACKS, A., LAWDEN, M., CLARKE, C. E., STEWART, P. M., WALKER, E. A., TOMLINSON, J. W. & RAUZ, S. 2010. Low energy diet and intracranial pressure in women with idiopathic intracranial hypertension: prospective cohort study. *British Medical Journal*, 341.
- SINGH, G. M., DANAEI, G., FARZADFAR, F., STEVENS, G. A., WOODWARD, M., WORMSER, D., KAPTOGE, S., WHITLOCK, G., QIAO, Q., LEWINGTON, S., DI ANGELANTONIO, E., VANDER HOORN, S., LAWES, C. M., ALI, M. K., MOZAFFARIAN, D. & EZZATI, M. 2013. The age-specific quantitative effects of metabolic risk factors on cardiovascular diseases and diabetes: a pooled analysis. *PLoS One*, 8, 1-10.
- SINHA, A., FORMICA, C., TSALAMANDRIS, C., PANAGIOTOPOULOS, S., HENDRICH, E., DELUISE, M., SEEMAN, E. & JERUMS, G. 1996. Effects of insulin on body composition in patients with insulin-dependent and non-insulin-dependent diabetes. *Diabet Med*, 13, 40-6.
- SJOSTROM, C. D., LISSNER, L. & SJOSTROM, L. 1997. Relationships between changes in body composition and changes in cardiovascular risk factors: the SOS Intervention Study. Swedish Obese Subjects. *Obes Res*, 5, 519-30.
- SJOSTROM, C. D., LYSTIG, T. & LINDROOS, A. K. 2011. Impact of weight change, secular trends and ageing on cardiovascular risk factors: 10-year experiences from the SOS study. *Int J Obes (Lond)*, 35, 1413-20.
- SJOSTROM, L. 2013. Review of the key results from the Swedish Obese Subjects (SOS) trial - a prospective controlled intervention study of bariatric surgery. *J Intern Med*, 273, 219-34.
- SJOSTROM, L., LINDROOS, A. K., PELTONEN, M., TORGERSO, J., BOUCHAR, C., CARLSSON, B., DAHLGREN, S., LARSSON, B., NARBRO, K., SJOSTROM, C. D., SULLIVAN, M. & WEDEL, H. 2004. Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. *N Engl J Med*, 351, 2683-93.
- SJOSTROM, L., PELTONEN, M., JACOBSON, P., AHLIN, S., ANDERSSON-ASSARSSON, J., ANVEDEN, A., BOUCHAR, C., CARLSSON, B., KARASON, K., LONROTH, H., NASLUND, I., SJOSTROM, E., TAUBE, M., WEDEL, H., SVENSSON, P. A., SJOHOLM, K. & CARLSSON, L. M. 2014. Association of bariatric surgery with long-term remission of type 2 diabetes and with microvascular and macrovascular complications. *Jama*, 311, 2297-304.
- SMITH-PALMER, J., BRANDLE, M., TREVISAN, R., ORSINI FEDERICI, M., LIABAT, S. & VALENTINE, W. 2014. Assessment of the association between glycemic

- variability and diabetes-related complications in type 1 and type 2 diabetes. *Diabetes Res Clin Pract*, 105, 273-84.
- SNEL, M., GASTALDELLI, A., OUWENS, D. M., HESSELINK, M. K., SCHAART, G., BUZZIGOLI, E., FROLICH, M., ROMIJN, J. A., PIJL, H., MEINDERS, A. E. & JAZET, I. M. 2012a. Effects of adding exercise to a 16-week very low-calorie diet in obese, insulin-dependent type 2 diabetes mellitus patients. *J Clin Endocrinol Metab*, 97, 2512-20.
- SNEL, M., JONKER, J. T., HAMMER, S., KERPERSHOEK, G., LAMB, H. J., MEINDERS, A. E., PIJL, H., DE ROOS, A., ROMIJN, J. A., SMIT, J. W. & JAZET, I. M. 2012b. Long-term beneficial effect of a 16-week very low calorie diet on pericardial fat in obese type 2 diabetes mellitus patients. *Obesity (Silver Spring)*, 20, 1572-6.
- SNEL, M., JONKER, J. T., SCHOONES, J., LAMB, H., DE ROOS, A., PIJL, H., SMIT, J. W., MEINDERS, A. E. & JAZET, I. M. 2012c. Ectopic fat and insulin resistance: pathophysiology and effect of diet and lifestyle interventions. *Int J Endocrinol*, 2012, 1-19.
- SNEL, M., SLEDDERING, M. A., VD PEIJL, I. D., ROMIJN, J. A., PIJL, H., MEINDERS, A. E. & JAZET, I. M. 2012d. Quality of life in type 2 diabetes mellitus after a very low calorie diet and exercise. *Eur J Intern Med*, 23, 143-9.
- SNEL, M., VAN DIEPEN, J. A., STIJNEN, T., PIJL, H., ROMIJN, J. A., MEINDERS, A. E., VOSHOL, P. & JAZET, I. M. 2011. Immediate and long-term effects of addition of exercise to a 16-week very low calorie diet on low-grade inflammation in obese, insulin-dependent type 2 diabetic patients. *Food Chem Toxicol*, 49, 3104-11.
- SNIDERMAN, A. D., WILLIAMS, K., CONTOIS, J. H., MONROE, H. M., MCQUEEN, M. J., DE GRAAF, J. & FURBERG, C. D. 2011. A meta-analysis of low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, and apolipoprotein B as markers of cardiovascular risk. *Circ Cardiovasc Qual Outcomes*, 4, 337-45.
- SO, W. Y., KONG, A. P., MA, R. C., OZAKI, R., SZETO, C. C., CHAN, N. N., NG, V., HO, C. S., LAM, C. W., CHOW, C. C., COCKRAM, C. S., CHAN, J. C. & TONG, P. C. 2006. Glomerular filtration rate, cardiorenal end points, and all-cause mortality in type 2 diabetic patients. *Diabetes Care*, 29, 2046-52.
- SOMERS, V. K., WHITE, D. P., AMIN, R., ABRAHAM, W. T., COSTA, F., CULEBRAS, A., DANIELS, S., FLORAS, J. S., HUNT, C. E., OLSON, L. J., PICKERING, T. G., RUSSELL, R., WOO, M. & YOUNG, T. 2008. Sleep apnea and cardiovascular disease: an American Heart Association/american College Of Cardiology Foundation Scientific Statement from the American Heart Association Council for High Blood Pressure Research Professional Education Committee, Council on Clinical Cardiology, Stroke Council, and Council On Cardiovascular Nursing. In collaboration with the National Heart, Lung, and Blood Institute National Center on Sleep Disorders Research (National Institutes of Health). *Circulation*, 118, 1080-111.
- SORLI, C. & HEILE, M. K. 2014. Identifying and meeting the challenges of insulin therapy in type 2 diabetes. *J Multidiscip Healthc*, 7, 267-82.
- SOUZA, C. T. D., ARAUJO, E. P., BORDIN, S., ASHIMINE, R., ZOLLNER, R. L., BOSCHERO, A. C., SAAD, M. J. A. & VELLOSO, L. A. 2005. Consumption of a Fat-Rich Diet Activates a Proinflammatory Response and Induces Insulin Resistance in the Hypothalamus. *Endocrinology*, 146, 4192-4199.
- SOWERS, J. R., EPSTEIN, M. & FROHLICH, E. D. 2001. Diabetes, Hypertension, and Cardiovascular Disease. *An Update*, 37, 1053-1059.

- STAMLER, J., VACCARO, O., NEATON, J. D. & WENTWORTH, D. 1993. Diabetes, Other Risk Factors, and 12-Yr Cardiovascular Mortality for Men Screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care*, 16, 434-444.
- STATISTICS, O. O. N. 2018. Avoidable mortality in the UK: 2016. In: STATISTICS, O. O. N. (ed.).
- STEVEN, S., HOLLINGSWORTH, K. G., AL-MRABEH, A., AVERY, L., ARIBISALA, B., CASLAKE, M. & TAYLOR, R. 2016a. Very-Low-Calorie Diet and 6 Months of Weight Stability in Type 2 Diabetes: Pathophysiologic Changes in Responders and Nonresponders. *Diabetes Care*, 39, 808-15.
- STEVEN, S., HOLLINGSWORTH, K. G., SMALL, P. K., WOODCOCK, S. A., PUCCI, A., ARIBASALA, B., AL-MRABEH, A., BATTERHAM, R. L. & TAYLOR, R. 2016b. Calorie restriction and not glucagon-like peptide-1 explains the acute improvement in glucose control after gastric bypass in Type 2 diabetes. *Diabet Med*, 33, 1723-1731.
- STEVEN, S. & TAYLOR, R. 2015. Restoring normoglycaemia by use of a very low calorie diet in long- and short-duration Type 2 diabetes. *Diabet Med*, 32, 1149-55.
- STEVENS, G. A., SINGH, G. M., LU, Y., DANAEI, G., LIN, J. K., FINUCANE, M. M., BAHALIM, A. N., MCINTIRE, R. K., GUTIERREZ, H. R., COWAN, M., PACIOREK, C. J., FARZADFAR, F., RILEY, L. & EZZATI, M. 2012. National, regional, and global trends in adult overweight and obesity prevalences. *Popul Health Metr*, 10, 22.
- STOCKER, R., YAMAMOTO, Y., MCDONAGH, A. F., GLAZER, A. N. & AMES, B. N. 1987. Bilirubin is an antioxidant of possible physiological importance. *Science*, 235, 1043-6.
- STOKES, J., 3RD, KANNEL, W. B., WOLF, P. A., D'AGOSTINO, R. B. & CUPPLES, L. A. 1989. Blood pressure as a risk factor for cardiovascular disease. The Framingham Study--30 years of follow-up. *Hypertension*, 13, 113-8.
- STRADLING, J. R. & CROSBY, J. H. 1991. Predictors and prevalence of obstructive sleep apnoea and snoring in 1001 middle aged men. *Thorax*, 46, 85-90.
- STRATTON, I. M., ADLER, A. I., NEIL, H. A., MATTHEWS, D. R., MANLEY, S. E., CULL, C. A., HADDEN, D., TURNER, R. C. & HOLMAN, R. R. 2000. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *Bmj*, 321, 405-12.
- STRAUB, S. G. & SHARP, G. W. 2002. Glucose-stimulated signaling pathways in biphasic insulin secretion. *Diabetes Metab Res Rev*, 18, 451-63.
- SUMITHRAN, P., PRENDERGAST, L. A., DELBRIDGE, E., PURCELL, K., SHULKES, A., KRIKETOS, A. & PROIETTO, J. 2011. Long-term persistence of hormonal adaptations to weight loss. *N Engl J Med*, 365, 1597-604.
- SWIFT, D. L., JOHANNSEN, N. M., LAVIE, C. J., EARNEST, C. P. & CHURCH, T. S. 2014. The Role of Exercise and Physical Activity in Weight Loss and Maintenance. *Prog Cardiovasc Dis*, 56, 441-7.
- SWINBURN, B. A., SACKS, G., HALL, K. D., MCPHERSON, K., FINEGOOD, D. T., MOODIE, M. L. & GORTMAKER, S. L. 2011. The global obesity pandemic: shaped by global drivers and local environments. *Lancet*, 378, 804-14.
- TASALI, E. & IP, M. S. 2008. Obstructive sleep apnea and metabolic syndrome: alterations in glucose metabolism and inflammation. *Proc Am Thorac Soc*, 5, 207-17.
- TAYLOR, R. 2008. Pathogenesis of type 2 diabetes: tracing the reverse route from cure to cause. *Diabetologia*, 51, 1781-9.
- TAYLOR, R. 2013. Type 2 diabetes: etiology and reversibility. *Diabetes Care*, 36, 1047-55.

- THE LOOK AHEAD RESEARCH GROUP 2013. Cardiovascular Effects of Intensive Lifestyle Intervention in Type 2 Diabetes. *New England Journal of Medicine*, 369, 145-154.
- THIEBAUD, D., JACOT, E., DEFRONZO, R. A., MAEDER, E., JEQUIER, E. & FELBER, J. P. 1982. The effect of graded doses of insulin on total glucose uptake, glucose oxidation, and glucose storage in man. *Diabetes*, 31, 957-63.
- TOMASZEWSKI, M., CHARCHAR, F. J., MARIC, C., MCCLURE, J., CRAWFORD, L., GRZESZCZAK, W., SATTAR, N., ZUKOWSKA-SZCZECHOWSKA, E. & DOMINICZAK, A. F. 2007. Glomerular hyperfiltration: a new marker of metabolic risk. *Kidney Int*, 71, 816-21.
- TRIPLITT, C. & CHIQUETTE, E. 2006. Exenatide: from the Gila monster to the pharmacy. *J Am Pharm Assoc (2003)*, 46, 44-52; quiz 53-5.
- TSAI, A. G. & WADDEN, T. A. 2006. The evolution of very-low-calorie diets: an update and meta-analysis. *Obesity (Silver Spring)*, 14, 1283-93.
- TSAI, Y. W., KANN, N. H., TUNG, T. H., CHAO, Y. J., LIN, C. J., CHANG, K. C., CHANG, S. S. & CHEN, J. Y. 2012. Impact of subjective sleep quality on glycemic control in type 2 diabetes mellitus. *Fam Pract*, 29, 30-5.
- TUDOR-LOCKE, C., CRAIG, C. L., AOYAGI, Y., BELL, R. C., CROTEAU, K. A., DE BOURDEAUDHUIJ, I., EWALD, B., GARDNER, A. W., HATANO, Y., LUTES, L. D., MATSUDO, S. M., RAMIREZ-MARRERO, F. A., ROGERS, L. Q., ROWE, D. A., SCHMIDT, M. D., TULLY, M. A. & BLAIR, S. N. 2011a. How many steps/day are enough? For older adults and special populations. *Int J Behav Nutr Phys Act*, 8, 1-19.
- TUDOR-LOCKE, C., CRAIG, C. L., BROWN, W. J., CLEMES, S. A., DE COCKER, K., GILES-CORTI, B., HATANO, Y., INOUE, S., MATSUDO, S. M., MUTRIE, N., OPPERT, J. M., ROWE, D. A., SCHMIDT, M. D., SCHOFIELD, G. M., SPENCE, J. C., TEIXEIRA, P. J., TULLY, M. A. & BLAIR, S. N. 2011b. How many steps/day are enough? For adults. *Int J Behav Nutr Phys Act*, 8, 1-17.
- TUOMILEHTO, H. P., SEPPA, J. M., PARTINEN, M. M., PELTONEN, M., GYLLING, H., TUOMILEHTO, J. O., VANNINEN, E. J., KOKKARINEN, J., SAHLMAN, J. K., MARTIKAINEN, T., SOINI, E. J., RANDELL, J., TUKIAINEN, H. & UUSITUPA, M. 2009. Lifestyle intervention with weight reduction: first-line treatment in mild obstructive sleep apnea. *Am J Respir Crit Care Med*, 179, 320-7.
- TUOMILEHTO, J., LINDSTROM, J., ERIKSSON, J. G., VALLE, T. T., HAMALAINEN, H., ILANNE-PARIKKA, P., KEINANEN-KIUKAANNIEMI, S., LAAKSO, M., LOUHERANTA, A., RASTAS, M., SALMINEN, V. & UUSITUPA, M. 2001. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med*, 344, 1343-50.
- TURKOGLU, C., DUMAN, B. S., GUNAY, D., CAGATAY, P., OZCAN, R. & BUYUKDEVIRIM, A. S. 2003. Effect of abdominal obesity on insulin resistance and the components of the metabolic syndrome: evidence supporting obesity as the central feature. *Obes Surg*, 13, 699-705.
- TURNER, R. C., CULL, C. A., FRIGHI, V. & HOLMAN, R. R. 1999. Glycemic control with diet, sulfonylurea, metformin, or insulin in patients with type 2 diabetes mellitus: progressive requirement for multiple therapies (UKPDS 49). UK Prospective Diabetes Study (UKPDS) Group. *Jama*, 281, 2005-12.
- TUSHUIZEN, M. E., BUNCK, M. C., POWWELS, P. J., BONTEMPS, S., VAN WAESBERGHE, J. H., SCHINDHELM, R. K., MARI, A., HEINE, R. J. & DIAMANT, M. 2007. Pancreatic fat content and beta-cell function in men with and without type 2 diabetes. *Diabetes Care*, 30, 2916-21.

- TUTTLE, K. R., BAKRIS, G. L., BILOUS, R. W., CHIANG, J. L., DE BOER, I. H., GOLDSTEIN-FUCHS, J., HIRSCH, I. B., KALANTAR-ZADEH, K., NARVA, A. S., NAVANEETHAN, S. D., NEUMILLER, J. J., PATEL, U. D., RATNER, R. E., WHALEY-CONNELL, A. T. & MOLITCH, M. E. 2014. Diabetic Kidney Disease: A Report From an ADA Consensus Conference. *Diabetes Care*, 37, 2864-2883.
- UKPDS STUDY GROUP 1990. UK Prospective Diabetes Study 6. Complications in newly diagnosed type 2 diabetic patients and their association with different clinical and biochemical risk factors. *Diabetes Res*, 13, 1-11.
- UKPDS STUDY GROUP 1995. U.K. prospective diabetes study 16. Overview of 6 years' therapy of type II diabetes: a progressive disease. U.K. Prospective Diabetes Study Group. *Diabetes*, 44, 1249-58.
- UKPDS STUDY GROUP 1998. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK Prospective Diabetes Study (UKPDS) Group. *Lancet*, 352, 837-53.
- UNGER, R. H. 1995. Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. *Diabetes*, 44, 863-70.
- UTZSCHNEIDER, K. M. & KAHN, S. E. 2006. Review: The role of insulin resistance in nonalcoholic fatty liver disease. *J Clin Endocrinol Metab*, 91, 4753-61.
- UTZSCHNEIDER, K. M., PRIGEON, R. L., FAULENBACH, M. V., TONG, J., CARR, D. B., BOYKO, E. J., LEONETTI, D. L., MCNEELY, M. J., FUJIMOTO, W. Y. & KAHN, S. E. 2009. Oral disposition index predicts the development of future diabetes above and beyond fasting and 2-h glucose levels. *Diabetes Care*, 32, 335-41.
- VALMADRID, C. T., KLEIN, R., MOSS, S. E. & KLEIN, B. E. 2000. The risk of cardiovascular disease mortality associated with microalbuminuria and gross proteinuria in persons with older-onset diabetes mellitus. *Arch Intern Med*, 160, 1093-100.
- VAN LOON, L. J. & GOODPASTER, B. H. 2006. Increased intramuscular lipid storage in the insulin-resistant and endurance-trained state. *Pflugers Arch*, 451, 606-16.
- VAN STRIEN, T., FRIJTERS, J. E. R., BERGERS, G. P. A. & DEFARES, P. B. 1986. The Dutch Eating Behavior Questionnaire (DEBQ) for Assessment of Restrained Emotional, and External Eating Behavior. *International Journal of Eating Disorders*, 5, 295-315.
- VASQUEZ, B., FLOCK, E. V., SAVAGE, P. J., NAGULESPARAN, M., BENNION, L. J., BAIRD, H. R. & BENNETT, P. H. 1984. Sustained reduction of proteinuria in type 2 (non-insulin-dependent) diabetes following diet-induced reduction of hyperglycaemia. *Diabetologia*, 26, 127-33.
- VENIANT, M. M., SULLIVAN, M. A., KIM, S. K., AMBROZIAK, P., CHU, A., WILSON, M. D., HELLERSTEIN, M. K., RUDEL, L. L., WALZEM, R. L. & YOUNG, S. G. 2000. Defining the atherogenicity of large and small lipoproteins containing apolipoprotein B100. *J Clin Invest*, 106, 1501-10.
- VORA, J. & HEISE, T. 2013. Variability of glucose-lowering effect as a limiting factor in optimizing basal insulin therapy: a review. *Diabetes Obes Metab*, 15, 701-12.
- WADDEN, T. A., BUTRYN, M. L. & BYRNE, K. J. 2004. Efficacy of lifestyle modification for long-term weight control. *Obes Res*, 12 Suppl, 151s-62s.
- WADDEN, T. A., NEIBERG, R. H., WING, R. R., CLARK, J. M., DELAHANTY, L. M., HILL, J. O., KRAKOFF, J., OTTO, A., RYAN, D. H. & VITOLINS, M. Z. 2011. Four-year weight losses in the Look AHEAD study: factors associated with long-term success. *Obesity (Silver Spring)*, 19, 1987-98.

- WADDEN, T. A., STUNKARD, A. J. & BROWNELL, K. D. 1983. Very low calorie diets: their efficacy, safety, and future. *Ann Intern Med*, 99, 675-84.
- WALLACE, T. M., LEVY, J. C. & MATTHEWS, D. R. 2004. Use and Abuse of HOMA Modeling. *Diabetes Care*, 27, 1487-1495.
- WANNAMETHEE, S. G., SHAPER, A. G., WHINCUP, P. H., LENNON, L. & SATTAR, N. 2011. Impact of diabetes on cardiovascular disease risk and all-cause mortality in older men: influence of age at onset, diabetes duration, and established and novel risk factors. *Arch Intern Med*, 171, 404-10.
- WARD, W. K., BOLGIANO, D. C., MCKNIGHT, B., HALTER, J. B. & PORTE, D., JR. 1984. Diminished B cell secretory capacity in patients with noninsulin-dependent diabetes mellitus. *J Clin Invest*, 74, 1318-28.
- WEBSTER, J. D., HESP, R. & GARROW, J. S. 1984. The composition of excess weight in obese women estimated by body density, total body water and total body potassium. *Hum Nutr Clin Nutr*, 38, 299-306.
- WEST, S. D., NICOLL, D. J. & STRADLING, J. R. 2006. Prevalence of obstructive sleep apnoea in men with type 2 diabetes. *Thorax*, 61, 945-950.
- WESTERTERP-PLANTENGA, M. S., LEJEUNE, M. P., NIJS, I., VAN OOIJEN, M. & KOVACS, E. M. 2004. High protein intake sustains weight maintenance after body weight loss in humans. *Int J Obes Relat Metab Disord*, 28, 57-64.
- WHITLOCK, G., LEWINGTON, S., SHERLIKER, P., CLARKE, R., EMBERSON, J., HALSEY, J., QIZILBASH, N., COLLINS, R. & PETO, R. 2009. Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies. *Lancet*, 373, 1083-96.
- WHO & IDF 2006. Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia: report of a WHO/IDF consultation *In: FEDERATION, W. H. O. I. D.* (ed.). Geneva, Switzerland: WHO Press.
- WHO, W. H. O. E. C. 2004. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet*, 363, 157-63.
- WILCOX, G. 2005. Insulin and Insulin Resistance. *Clin Biochem Rev*, 26, 19-39.
- WILDING, J. P. 2014. The importance of weight management in type 2 diabetes mellitus. *Int J Clin Pract*, 68, 682-91.
- WILLIAMS, C. D., STENGEL, J., ASIKE, M. I., TORRES, D. M., SHAW, J., CONTRERAS, M., LANDT, C. L. & HARRISON, S. A. 2011. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology*, 140, 124-31.
- WILLIAMSON, D. F., THOMPSON, T. J., THUN, M., FLANDERS, D., PAMUK, E. & BYERS, T. 2000. Intentional weight loss and mortality among overweight individuals with diabetes. *Diabetes Care*, 23, 1499-504.
- WILSON, P. W., D'AGOSTINO, R. B., LEVY, D., BELANGER, A. M., SILBERSCHATZ, H. & KANNEL, W. B. 1998. Prediction of coronary heart disease using risk factor categories. *Circulation*, 97, 1837-47.
- WING, R. R. & HILL, J. O. 2001. Successful weight loss maintenance. *Annu Rev Nutr*, 21, 323-41.
- WING, R. R., LANG, W., WADDEN, T. A., SAFFORD, M., KNOWLER, W. C., BERTONI, A. G., HILL, J. O., BRANCATI, F. L., PETERS, A. & WAGENKNECHT, L. 2011. Benefits of Modest Weight Loss in Improving Cardiovascular Risk Factors in Overweight and Obese Individuals With Type 2 Diabetes. *Diabetes Care*, 34, 1481-1486.

- WING, R. R., MARCUS, M. D., SALATA, R., EPSTEIN, L. H., MIASKIEWICZ, S. & BLAIR, E. H. 1991. Effects of a very-low-calorie diet on long-term glycemic control in obese type 2 diabetic subjects. *Archives of internal medicine*, 151, 1334-40.
- WING, R. R., PAPANDONATOS, G., FAVA, J. L., GORIN, A. A., PHELAN, S., MCCAFFERY, J. & TATE, D. F. 2008. Maintaining large weight losses: the role of behavioral and psychological factors. *J Consult Clin Psychol*, 76, 1015-21.
- WING, R. R. & PHELAN, S. 2005. Long-term weight loss maintenance. *Am J Clin Nutr*, 82, 222s-225s.
- WOODEND, D. M. & ANDERSON, G. H. 2001. Effect of sucrose and safflower oil preloads on short term appetite and food intake of young men. *Appetite*, 37, 185-95.
- WORMSER, D., KAPTOGE, S., DI ANGELANTONIO, E., WOOD, A. M., PENNELLS, L., THOMPSON, A., SARWAR, N., KIZER, J. R., LAWLOR, D. A., NORDESTGAARD, B. G., RIDKER, P., SALOMAA, V., STEVENS, J., WOODWARD, M., SATTAR, N., COLLINS, R., THOMPSON, S. G., WHITLOCK, G. & DANESH, J. 2011. Separate and combined associations of body-mass index and abdominal adiposity with cardiovascular disease: collaborative analysis of 58 prospective studies. *Lancet*, 377, 1085-95.
- YKI-JARVINEN, H., RYYSY, L., KAUPPILA, M., KUJANSUU, E., LAHTI, J., MARJANEN, T., NISKANEN, L., RAJALA, S., SALO, S., SEPPALA, P., TULOKAS, T., VIIKARI, J. & TASKINEN, M. R. 1997. Effect of obesity on the response to insulin therapy in noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab*, 82, 4037-43.
- YOUNG, T., PEPPARD, P. E. & TAHERI, S. 2005. Excess weight and sleep-disordered breathing. *J Appl Physiol* (1985), 99, 1592-9.
- YOUNG, T., SKATRUD, J. & PEPPARD, P. E. 2004. Risk factors for obstructive sleep apnea in adults. *Jama*, 291, 2013-6.
- YU, C., CHEN, Y., CLINE, G. W., ZHANG, D., ZONG, H., WANG, Y., BERGERON, R., KIM, J. K., CUSHMAN, S. W., COONEY, G. J., ATCHESON, B., WHITE, M. F., KRAEGEN, E. W. & SHULMAN, G. I. 2002. Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. *J Biol Chem*, 277, 50230-6.
- YUSUF, S., HAWKEN, S., OUNPUU, S., DANS, T., AVEZUM, A., LANAS, F., MCQUEEN, M., BUDAJ, A., PAIS, P., VARIGOS, J. & LISHENG, L. 2004. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet*, 364, 937-52.
- ZIGMOND, E., SAMIA-GRINBERG, S., PASMANIK-CHOR, M., BRAZOWSKI, E., SHIBOLET, O., HALPERN, Z. & VAROL, C. 2014. Infiltrating monocyte-derived macrophages and resident kupffer cells display different ontogeny and functions in acute liver injury. *J Immunol*, 193, 344-53.
- ZINMAN, B., PHILIS-TSIMIKAS, A., CARIOU, B., HANDELSMAN, Y., RODBARD, H. W., JOHANSEN, T., ENDAHL, L. & MATHIEU, C. 2012. Insulin degludec versus insulin glargine in insulin-naïve patients with type 2 diabetes: a 1-year, randomized, treat-to-target trial (BEGIN Once Long). *Diabetes Care*, 35, 2464-71.