

**The Influence of Exercise on Ulcer Healing in Patients with Chronic
Venous Insufficiency**

by

Mr. Omar Mutlak
M.B.Ch.B, DIC, MSc, MRCS, FEBS, FLBS

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Department of Surgery
Division of Surgery and Cancer
Imperial College London

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Abstract

Venous leg ulcer (VLU) is a common chronic clinical problem and it is the most serious complication of chronic venous insufficiency. Although venous ulcer has a huge burden on individuals and states, the standard type of treatment for this debilitating condition, compression therapy, has not changed in the last 2000 years.

There is growing evidence that exercise could help in venous ulcer management. This study investigated the effect of exercise on 80 VLU patients. These patients were randomised into four groups; a control group, a compression therapy only group, an exercise only group and a compression and exercise group. Non-invasive measurements were performed at the beginning of the 3 month period of regular exercise, and again at the end of the exercise period. This was necessary in order to evaluate the effect of exercise on VLU healing parameters, which include; tcPO₂ level, laser Doppler measurements, resting skin flux (RF), range of ankle joint movement (ROM) and ulcer size. The exercise composed of 10 dorsiflexions each hour, while the participant was awake over a period of 3 months.

Findings included, an increase in the tcPO₂ level and range of ankle joint movement in individuals who had performed the exercise compared to those who did not ($p < 0.001$). Moreover tcPO₂ measurements were higher in the exercise and compression group than those seen in the exercise only group. Ulcer size and RF measurements decreased in patients who had performed exercise compared to those who did not, groups 3 and 4 showed a significant

decrease in ulcer size with p values = 0.001 and < 0.001 respectively. With RF measurements, only groups 3 and 4 showed significant decrease, $p < 0.001$.

The findings indicated that there were changes in the VLU parameters in response to the 3 months period of regular exercise. Regular unsupervised exercise may be included as an integral part of the leg ulcer management.

Author's Declaration

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1. The International Union of Angiology IUA, XXII Congress, Budapest, Hungary, 2015.
2. Annual Scientific Meeting, Vascular Society ASM, Bournemouth, UK, 2015.
3. Controversies and Updates in Vascular Surgery CACVS, Paris, France, 2016.
4. The 27th World Congress of the International Union of Angiology and 15th Congress of the French Society of Vascular Medicine, Lyon, France, 2016.

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Signed: Omar Mutlak Date: 20/12/2016

Dedication

Dedicated to the memories of my parents for the uncompromising principles that guided their short life and for leading their children into intellectual pursuits, and to my brother Hamid, for his support and love during my growing up years.

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List of abbreviations

ABPI Ankle brachial pressure index

ATP Adenosine triphosphate

ADP Adenosine diphosphate

AMP Adenosine monophosphate

CHD Congestive heart disease

CVD Cardiovascular disease

Chb Haemoglobin concentration

CVI Chronic venous insufficiency

CVP Central venous pressure

CVS Cardiovascular system

DVT Deep venous thrombosis

DVS Deep vein surgery

ECG Electrocardiogram

ETC	Electron transport chain
HBOT	Hyperbaric oxygen therapy
IVC	Inferior vena cava
INR	International normalized ratio
IPC	Intermittent pneumatic compression
LDH	Lactate dehydrogenase
LDF	Laser Doppler flowmetry
LMWH	Low molecular weight heparin
NPWT	Negative pressure wound therapy
PDGF	Platelet derived growth factor
PCr	Phosphocreatine
PO ₂	Oxygen partial pressure
PVD	Peripheral vascular disease
PE	Pulmonary embolism
RF	Resting flux

ROM	Range of ankle joint movement
RDVS	Reconstructive deep venous surgery
Shb	Haemoglobin oxygen saturation
SEPS	Subfascial endoscopic surgery
tcPO2	Transcutaneous oxygen pressure
TRC	Transcutaneous Oximetry
UFH	Unfractionated heparin
VPPG	Venous photoplethysmography
VAR	Venoarteriolar response
VI	Venous insufficiency
VTE	Venous thromboembolism
VT	Venous thrombosis
VLU	Venous leg ulcer

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1 CHAPTER ONE: INTRODUCTION AND REVIEW OF THE LITERATURE

1.1 Introduction

A venous leg ulcer (VLU) is a complicated type of ulcer and common clinical problem that affects approximately 2% of the population worldwide. It is a chronic ulcer that takes months to heal, and even after healing, there is a significant recurrence rate that leads to a vicious cycle. VLU affects certain groups of patients who are often not able to cope with the associated problems it can cause. Moreover, a venous leg ulcer places a significant socioeconomic burden on both individuals and society. VLU is a difficult and common clinical condition, where effective treatments are unfortunately limited.

A venous leg ulcer occurs on the gaiter area of the lower limb between the ankle joint and knee joint and usually affects the full thickness of the skin. Some studies have defined venous leg ulcers as “a break in the skin below the knee that has not healed within two weeks or a healed venous leg ulcer” (Shami, Sarin, & Scurr, 1997). Others have defined them as a “loss of skin on the leg or foot that takes more than 6 weeks to heal” (Brem, Kirsner, & Falanga, 2004). Some studies have recorded a shorter VLU healing time, such as that by Cheryl et al, which reported 2 to 5 days as a period of healing for small venous ulcers (Cheryl, Michael, Hughes, & Robert, 2006). A venous ulcer is the end result of venous insufficiency (VI), which occurs as a result of a defect in the venous system due to either venous valve damage or the obstruction of veins. VI can lead to venous hypertension, oedema, lipodermatosclerosis and inflammatory changes, and eventually a venous leg ulcer (Abbade & Lastoria, 2005), (Lazarus, Mahmud, Umair, & Nisa, 2014).

Several studies have reported that 40–50% of adults present with chronic venous insufficiency and 1–2% of adults suffer from venous ulceration (Yarwood-Ross & Haigh,

2012) (Persoon, et al., 2004). Due to a lack of research and underestimation in the developing world, the actual prevalence of VLU may be much higher than expected. In the United Kingdom (UK), the prevalence of venous leg ulcer disease is between 1.5 and 3.0/1000 people. In North America, chronic venous leg ulcers affect between 500,000 and 2 million individuals per year, and more than 50% of leg ulcers are considered to be venous leg ulcers (Robertson, Eans, & Fowkes, 2008). The incidences of VLU are likely to increase with an ageing population and this will, due to the associated risk factors, place an even greater burden on health services (Finlayson, Edwards, & Courtney, 2011).

The socioeconomic impact of VLU is dramatic because patients affected by this condition lack the ability to engage in occupational and social activities, also VLU impairs the quality of life and creates financial difficulties for the patient. The impact of VLU on individuals and society due to pain, reduced quality of life, limitation of movement, lack of functional ability and social isolation has been widely reported (Van Gent, Wilschut, & Wittens, 2010). Disabilities associated with VLU lead to a loss of productivity, which is estimated to cost around two million workdays annually, and >12% of workers suffering from VLU are forced to retire early (Eberhardt & Faffetto, 2005) (Eberhardt & Raffetto, 2014).

Regarding the financial burden placed on society, the estimated cost of VLU is approximately 2–3% of the total healthcare expenditure in the western world (Phillips, Stanton, Provan, & Lew, 1994). The annual cost to the NHS in the UK has been estimated at £300 million, which does not include the loss of productivity due to associated venous ulcer illness (Schmid-Schonbein, et al., 2006). In the United States, venous ulcer treatment costs \$1.0-1.6 billion

for more than 6 million patients each year (Franzeck, Haselbach, Speiser, & Bollinger, 1993) (Gothgen & Jacobsen, 1978).

Treating a VLU is often a difficult and lengthy process that is further complicated by additional factors. VLU patients suffer from significant comorbidities, such as cardiovascular disease (CVD), osteoarthritis, respiratory problems and limitations of movement. In addition, VLU patients are often debilitated, elderly often suffering from isolation with have little or no support. As a result, a VLU can become a lifelong chronic condition that imposes a huge burden on both individuals and society.

Although a VLU is a challenging clinical problem and causes significant stress to both individuals and society, the management of these ulcers has not changed significantly over the years. Not surprisingly, a chronic problem as common as a VLU has led to the development of different modalities of treatment, many of which are unproven. Compression therapy has been used since 3000 BC, and interestingly, this technique is still the accepted “gold-standard” treatment of VLU worldwide. Recently, there is growing evidence that exercise could help in the management of VLU (Heinen, et al., 2012) (Nicholas, Andrea, & Alejandra, 2013).

In chronic venous insufficiency, deoxygenated venous blood accumulates in the microcirculation at skin level and leads to venous hypervolemia and peripheral oedema. This phenomenon prevents the regular and normally smooth process of exchange between venous blood and arterial blood. Instead, the accumulation of deoxygenated blood stagnates in the dermis, leading to microvascular venous ischemia. Regular exercise may break this vicious cycle through the action of calf muscle pumping. This will allow continuous evacuation of

deoxygenated venous blood which would be replaced with oxygenated blood. Eventually oxygen level in the skin will improve resulting in the venous ulcer healing.

It would be very interesting if such a simple, feasible, available and cost-effective solution could help in such a complicated and difficult clinical condition. It would be valuable to investigate the effects of exercise on VLU by measuring the changes in physiological parameters. In this study we are investigating the effect of regular exercise on venous ulcer patients (80), composed of 10 dorsiflexions every hour. Participants will be awake and the process will be performed over a period of 3 months.

1.2 Hypothesis

The regular domiciliary exercise will improve the tissue oxygenation in patients with chronic venous insufficiency and this would lead to consequent improvements in the leg ulcer healing process.

1.3 Aims of the study

This is a prospective study to investigate the relationship between exercise and venous leg ulcer in various groups of patients with chronic venous insufficiency. The specific aims include:

1. Identifying the effect of exercise (Dorsiflexion) on transcutaneous oxygen level, (tcPO₂).
2. Identifying the effect of exercise (Dorsiflexion) on Laser Doppler blood flowmetry.
3. Identifying the effect of exercise (Dorsiflexion) on the range of ankle joint movement (ROM) and ulcer size measurements.

1.4 Historical aspects

It is thought that the papyrus of Ebers (1550 BC) contains the first written studies about veins. Although, Hippocrates is considered to be the first who wrote in more detail on the subject, he was the first to note the association between varicose veins and ulcer (Belcaro G. , 1997). Hippocrates believed that leg ulcer is associated with leg dependency during horse riding and periods of long standing. He suggested “puncturing and bandaging” for varicose veins treatment. Hippocrates also recommended that patients with leg ulcers should avoid hanging their legs or standing for long periods of time (Belcaro G. , 1997). In Roman times bandages were used for venous ulcer treatment. In addition, Aurelius Celsus (25 BC-50 AD) and Galen (130-200 AD) advised the application of wine on leg ulcers.

The Ancient Greek's believed that the human body is composed of four basic elements (humours). Namely; black bile, yellow bile, phlegm and blood (**Figure 1.1**). Those humours have to be in balance and any disturbance in this equilibrium may cause illness. The concept of four humors may have its origins in Mesopotamia (Leavitt, 1990). According to the humoral theory, Haly Abbas, who died in 994 AD, concluded that varicose veins are full of black bile as a result of hardworking and prolonged periods of standing. By pushing these humors with bandaging back into body Abbas suggested that it could result in madness.



Figure 1.1: *Components of humoral theory*

Avicenna (980-1037) thought that it was impossible to cure old people from venous ulcers (Belcaro G. , 1997). Ambrose Pare (1510-1590) stated that varicose veins came about as a result of melancholy temper in men and because of pregnancy in women. Others, (Vicray, 1636; Le Dran, 1731; Heister, 1768) also believed that ulcers were difficult to heal and that injury would release humors which were considered to be toxins. Consequently, if they returned back to the body, they might cause madness (Belcaro G. , 1997).

Barbette, in the Menstrual theory, (Barbette, 1675) mentioned that suppression of the menstrual cycle during pregnancy leads to the accumulation of menstrual blood in the leg veins. He thought that venous ulcer is a defence mechanism of the body by which it excretes humors. Pigeaux (1843) believed that the suppression of the menstrual cycle during pregnancy results in abortion and ulcer, Ettmuller (1688) and Astley Cooper (1824) both supported the menstrual theory; they believed that the menstrual cycle is necessary for the escape of evil. Astruc (1761) was the first who concluded that there is no relationship between the menstrual cycle and the accumulation of bad humors. The menstrual theory was

the dominant idea throughout the eighteenth century all the way up to the beginning of nineteenth century (Belcaro G. , 1997).

Wiseman (1676) concluded that varicose veins could come about due to circulatory failure of the venous system. In 1775, Hunter suggested the connection between thrombosis and phlebitis. Home wrote in (1801) that venous ulcer healing would be more difficult if it is associated with varicose veins. By concluding that ulcers on the gaiter area are associated with varicose veins, Hodgson in (1851) presented more understanding of the pathophysiology of venous ulcer. Hodgson, also mentioned that the dilatation of veins were due to obliteration of one of the associated veins (Belcaro G. , 1997).

There is a dramatic change in understanding of venous disease after Rudolf Virchow, a German physician (1821-1902), stated the three factors responsible for the formation of venous thrombosis namely, stasis, change in blood coagulability and injury to the endothelial surface (Virchow's triad) (Torrey & Deepi, 2004). In 1867 Gay stated that the presence of varicose veins is not necessarily why the venous ulcer will develop but could actually be due to other venous conditions like venous thrombosis (Gay J 1868) and he was the first to use the term venous ulcer (Belcaro G. , 1997).

Homans in 1916 introduced the term “post-phlebitic Syndrome”, he concluded that it came about as a consequence of incompetent perforating veins around the ankle as a result of deep venous thrombosis. Homans emphasized that there is strong association of valve incompetence in perforators as a result of thrombosis and developing of recanalization (Mc Mullin, 1990).

Piulachs and Vidal-Barraquer concluded that high venous oxygen saturation in patients with venous insufficiency might be due to arteriovenous fistula (Piulachs & Vidal-Barraquer, 1995). They thought that the blood shunting could produce venous ulcer through anaemic anoxia. The concerns about the venous shunting theory were addressed by Browse in 1989, reaching the conclusion that demonstration of fistula needs physiological techniques (Belcaro G. , 1997). In addition, developing of dynamic studies like plethysmography failed to demonstrate high venous shunting in venous insufficiency limbs (Abramson & Fierst, 1946).

In 1972, Bajordal found that the reflux of venous blood in leg veins is the main reason for the increase of venous pressure in people suffering from venous insufficiency. Franzeck (1984) showed that microcirculatory changes in venous ulcer are associated to low PO₂ readings in the ulcer area. As the study states, “areas of white atrophy tcPO₂ was extremely low, in the proximity of 0 mmHg” (Franzeck , Bollinger , Hutch, & Hutch, 1984).

1.5 Anatomy of lower limb Venous System

1.5.1 Veins

Veins are the channels that carry out the blood towards the heart (Keith, 2014). The function of the veins in the body, apart from pulmonary veins and portal vein, is to carry deoxygenated blood to the heart; low oxygen blood gives the veins the dark blue appearance. The veins have thin walls and have the ability to expand in comparison to arteries; therefore 80% of blood volume occupies the venous system.

Embryology:

The primitive venous system consists of three major components, all of which are bilaterally symmetrical:

1. The cardinal system, which drains, the head, neck, body wall and the limbs.
2. The vitelline veins, which initially drain the yolk sac
3. The umbilical veins, which develop in the connecting stalks and carry oxygenated blood from the placenta to the embryo.

All three systems undergo extensive modification during development. In the cardinal and vitelline systems, the longitudinal veins on the left side of the body tend to regress, whereas those on the right side persist and give rise to the great veins (William, 2001).

Histology:

There are three sizes of veins:

1. Venuels: the smallest veins, which drain blood from capillaries then converge to form small size veins. Venuels contain endothelial cells, although they are few in number they are capable of exchange of substances through their walls. Magnification is necessary to see venuels (Rhoades & Pflanzer, 2003).
2. Small and medium size veins: These contain a small amount of smooth muscles and tunica externa is the thickest layer. The medium size veins are provided with one-way valve, these valves are designed to prevent back flow of blood when the blood moves against gravity in the lower limbs. Examples of small and medium size blood vessels are superficial veins and deep veins are found in the upper and lower limbs.

3. Large veins: These are characterised by the presence of bundles of longitudinal smooth muscles and well-formed tunica adventitia. An example being the superior and inferior vena cava veins.

Venous drainage of lower limbs is composed of superficial and deep venous systems. Both of which contain valves and are connected by perforators.

1.5.2 The superficial venous system

Superficial veins are located in the subcutaneous connective tissue. They are two main veins, the Great saphenous vein and the Small saphenous vein both of which originate from the dorsal venous arch of the foot.

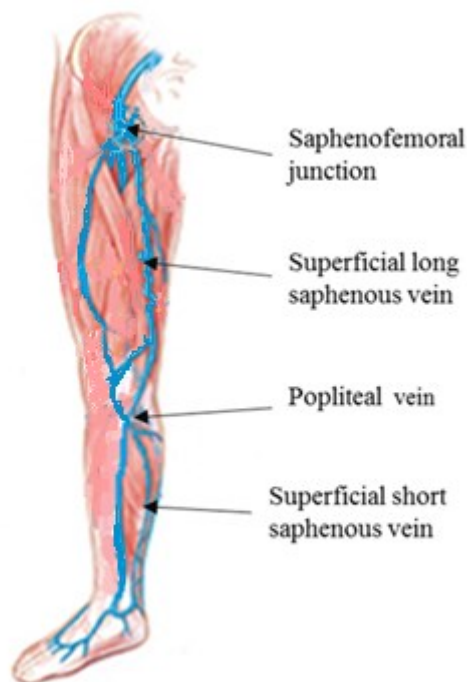


Figure 1.2: *Superficial venous system of lower limb*

Great saphenous vein (Long saphenous vein): This is formed by the medial side of the dorsal venous arch that then ascends anterior to medial malleolus, anastomose freely with the

small saphenous vein, eventually being emptied into the femoral vein (**Figure 1.2**). The Great saphenous vein contains 10-12 valves, they are greater in number below the knee than in the thigh. When it ascends through the lower limb it receives fewer tributaries. They communicate in different places with the small saphenous vein. Tributaries of the posterior and medial sides of the thigh frequently unite together to form the accessory saphenous vein. This vein, when present, becomes the main connection between the great and small saphenous vein. Close to its termination into the femoral vein the long saphenous vein receives the superficial circumflex iliac, superficial epigastric and external pudendal veins.

Small saphenous vein (lesser saphenous vein): This vein arises on the lateral border of the foot as a continuation of the lateral side of the dorsal venous arch; it ascends up on the posterior surface of the leg and then penetrates deep fascia on the back of the knee. The small saphenous vein empties directly into the popliteal vein in the popliteal fossa.

The saphenous veins receive tributaries. Their diameter does not change while they ascend; the explanation for this is that the blood drained into the saphenous veins is continuously shunted into the deep venous system by numerous perforating veins.

1.5.3 The perforating veins

These veins perforate the deep fascia near their origin from the superficial venous system and contain one way valves that only permit the flowing of blood into the deep veins (**Figure 1.3**). These veins penetrate through deep fascia at an oblique angle. Therefore, when the muscle contraction occurs there is an increase of pressure underneath deep fascia and the perforators are then compressed. Compression of perforating veins also assists valves in preventing back flow of venous blood into the superficial veins.

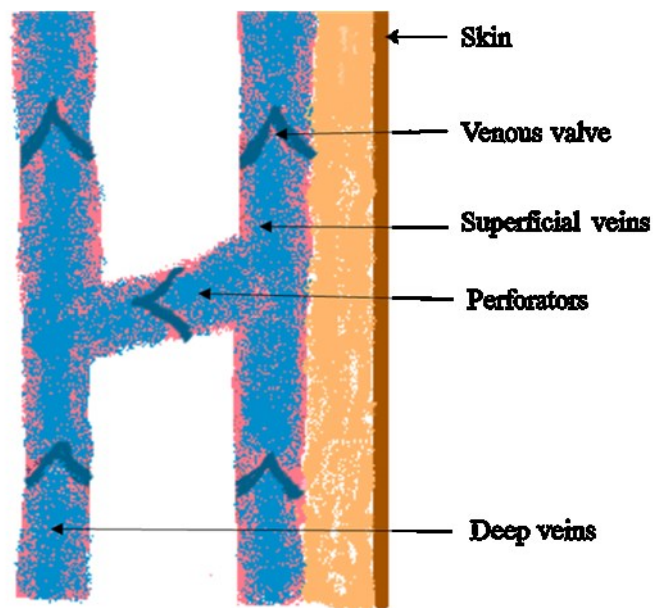


Figure 1.3: Perforating veins

The perforating veins play a major role in the development of CVI. In limbs with CVI, the ambulatory venous hypertension leads to recanalization of the deep venous system, this process result in destruction of the valves and increase in deep venous pressure. As a result of the high deep venous pressure, the perforators in the lower limb, especially on the gaiter area become dilated and the valves within these perforating veins become incompetent. Finally, the high deep venous pressure is transmitted back through the incompetent perforating veins to the superficial veins causing dilated and incompetent superficial venous system (O'Donnell TF, 2010).

The soleus muscle is enclosed in a strong sheath of elastic fascia, this investment within such strong fascia means that a high pressure develops when the muscle contracts. The rise in the pressure within the facial investment is called calf muscle pump, contraction of the calf muscles squeezes the intramuscular veins pushing the venous blood upwards to the deep

veins. Forcing the venous blood upwards only occur by the presence of competent perforating veins, which prevents blood being pushed outwards into superficial veins (Cockett, 1956).

1.5.4 The deep venous system

The deep venous system of the lower limbs is composed of few veins. These veins accompany all arteries and occur as paired rather than as a single vein as frequently illustrated. Deep veins are usually considered to be interconnecting veins that flank the accompanied artery and are contained in a fibrous sheath alongside the arteries whose pulsation would help in compressing and moving blood into veins.

Perforators continuously supply the deep veins, the anterior tibial vein on the anterior leg, the medial and lateral planter veins form the posterior tibial and the fibular veins on the posterior surface. All three deep leg veins empty into the popliteal vein on the back of the knee; the popliteal vein then becomes the femoral vein in the thigh.

Venous blood is drained from thigh muscles through veins by the perforating arteries associated to the profunda femoris artery. It moves into the deep vein of the thigh, the profunda femoris vein, which terminates into the distal end of the femoral vein.

During exercise, the blood flows from the superficial veins into the deep veins. The flow is accelerated by the contraction of the calf muscles. In contrast, the blood flow will be slower in the standing position due to the gravitational effect and the absence of calf muscle function. Blood will be propelled to the femoral vein and eventually to the external iliac vein under the effect of calf muscle contraction. There is more variation in the deep veins than arteries they accompany. We can ligate both superficial and deep veins if needed.

1.5.5 The venous valves

Venous valves are “flaps of endothelium with *cup-like valvular sinuses* that fill from above” (Keith, 2014). The vein wall is thicker at the base of the valve cusps due to an increase in the number of smooth muscle cells in the media (Caggiati, 2013).

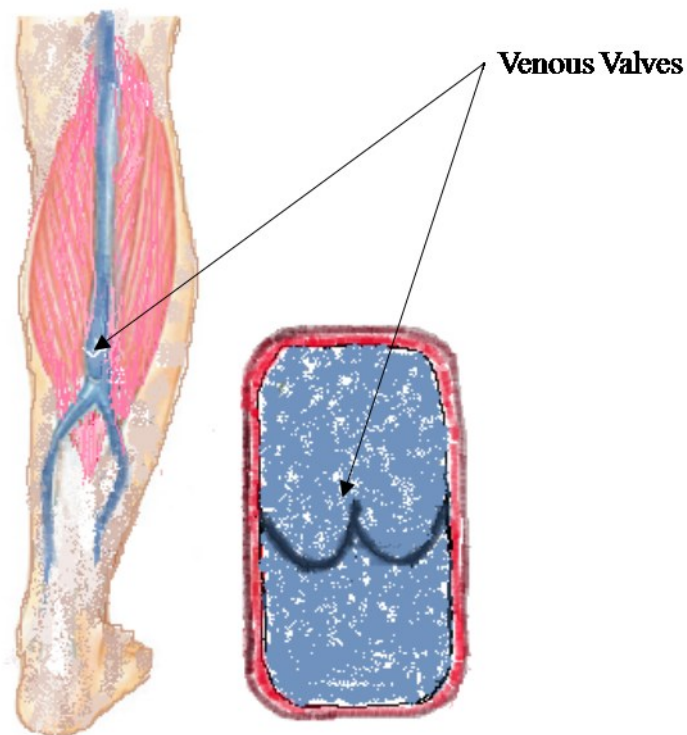


Figure 1.4: *Venous valves*

When the sinuses are full with blood the cusps will occlude the vein lumen thus preventing flow back of venous blood distally (**Figure 1.4**), breaking of venous blood into segments, in the saphenous vein, is another role of these valves; this phenomenon will lead to decreased venous hypertension. Both effects will help the muscovenous pump to overcome gravity.

Ludovicus Vassaeus was the first who mentioned the presence of valves in the veins in his *Anatomen Corporis Humani Tabulae Quatuor* book published in 1544 (Caggiati & Bertocchi, 2001). The function of the venous valves was indicated by Andrea Cesalpino in 1559, more interestingly Fabricius defined a test to assess venous valves location and function (Caggiati, 2013). Kampmeier and Birch succeeded in describing the five basic morphogenic events of valve development in 1927; these events have been confirmed recently by Bazigou and colleagues, (**Figure 1.:5**), (Bazigou, et al., 2011).

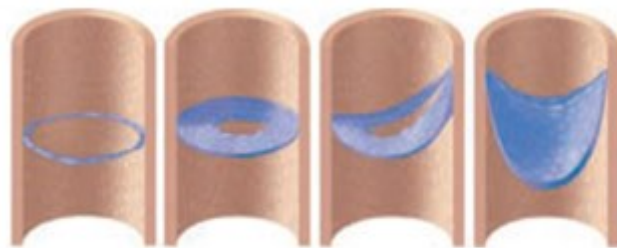


Figure 1.:5 Steps of venous valve development

(Modified from Bazigou et al, 2011)

It has been mentioned by Kampmeier and Birch, in 1927, that the appearance of lower limb valves is only seen “after the heart begins to beat and the primordial muscles begin to move.” (Kampmeier & Birch, 1927). Their findings have not been contravened by other authors.

Numerous studies have looked at the number and location of venous valves with some controversy. No valves in the inferior vena cava, apart from sporadic monocuspid valves have been recorded, as Franklin KJ mentioned (Franklin K. , 1987). Banjo AO, in his study, denied any valves existed within the inferior cava; one sporadic and frequently incomplete valve can be found in the common iliac vein of 1% to 7% of human lower limbs. Banjo

recorded normal valves present within the external iliac vein and the femoral vein. Interestingly, he found that Caucasians have lower incidence of valve numbers in comparison to Africans supporting his view on the high incidence of varicosity in Caucasians. Kampmeier and Birch, believe that the internal iliac vein has no valves, while its main branches are valvular. Recently, La Page et al mentioned that a well-developed valve is present in 7.6% of people (LePage , Villavicencio, Gomez, Sheridan, & Rich, 1991).

1.6 Physiology of the Venous System

1.6.1 Veins functions

For a considerable time, people have considered veins to have no function apart from being a passageway for blood transportation from lower limbs to the heart; however with the development of research, scientists discovered other vein functions. One of the main additional functions is to work as reservoirs for the blood when needed by the heart and helps maintain venous pressure. Veins have the capacity to conduit blood and regulate blood pressure because they constrict and dilate when required (Widmaier, 2014). Broadening of the venous system is crucial because it decelerates the blood flow. The propelling of blood towards the heart is another important function performed through the venous pump mechanism. Physiological stress induces an increase in sympathetic stimulation, which in turn leads to the displacement of blood from peripheral veins to the heart. (Levick J, 2010). There is an exchange of some materials between the venules and the interstitial tissue, the same is seen with the capillaries, although the permeability of venules to macromolecules is more than in the capillaries (Widmaier, 2014).

1.6.2 Vein wall structure

The wall of the vein is composed of three layers: The inner layer, tunica intima, the middle layer, tunica media and the third layer, tunica adventitia (**Figure 1.6**).

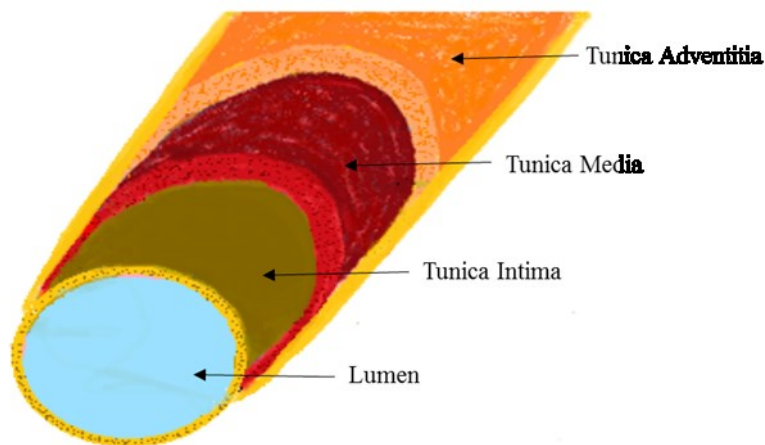


Figure 1.6: Vein wall structure

The intima is composed of a group of endothelial cells lying upon a connective tissue layer. It is the main guard of the plasma. It is also responsible for the secreting of many vasoactive materials, like vasodilators. In the veins of the lower limbs the intima has semilunar valves, which are one way valves preventing the blood from falling down when the calf muscles relax.

The media work as a mechanical support due to its contractile ability. It is composed of a group of smooth muscle cells; these cells are embedded in a network of matrix and collagen fibres. Two layers of elastin bound to the media.

The adventitia is composed of a connective tissue layer. Its function is to connect the veins to the surrounding tissue. The adventitia contains vasa vasorum; where blood vessels supply the media. There are sympathetic fibre terminals embedded in the adventitia, these terminals are

responsible for releasing noradrenaline which cause vasoconstriction of blood vessels. Despite Sympathetic neurons that are the main stimulator to veins, smooth muscles of veins will response to hormonal, vasoconstrictors and dilators.

1.6.3 Venous return

There are major factors that can affect the venous return: gravity of the body, tone of peripheral veins, blood volume and calf muscle pump. In a normal situation the strength that drives the return of venous blood to the heart is the difference in pressure between peripheral venous system and the right atrium. Generally, the pressure in the lower limb venous system is low, about 10- 15 mmHg while the pressure in the right heart is around 0 mmHg. The pressure required to move blood towards the heart is low, around 10 mmHg; this is because of low resistance offered by the peripheral veins due to their large diameter and thin walls. Disposability of the veins gives them the ability to work as a reservoir for blood which constitutes about two thirds of blood volume.

1.6.4 Gravity

The effect of gravity on the human body appears clearly when standing for long periods of time with an increasing feeling of heaviness in the legs. In a standing position, the gravity is responsible for redistribution of 500 ml of blood from the thorax to the lower limb venous system. Central venous pressure (CVP) will reduce as a consequence of the pooling of venous blood; this will lead to a decrease in stroke volume. On lying down, there will be a redistribution of blood from the lower limbs to the thorax that increases CVP and the stroke volume.

1.6.5 Blood volume

Two thirds of the blood volume is distributed in the venous system and one third occupies the arterial system, because of that, any change in blood volume will cause a shift in the venous blood volume. Hypovolaemia, resulting from haemorrhage or dehydration for instance can cause severe reduction in the venous volume which can cause serious consequences such as hypotension or shock. On the other hand, blood transfusion or fluid overload can cause an increase in venous pressure and stroke volume.

1.6.6 Sympathetic nerves

Constrictor muscles of the veins are under the influence of sympathetic nerves; these peripheral constrictors will shift the blood from peripheral veins into the central veins. As a result, the central nervous system can regulate venous return and stroke volume. Stress, deep respiration, exercise and shock can contribute, to a certain extent, to the raising of venous pressure. Conversely, with the hot weather or with any factor related to vasodilatation, like spinal anaesthesia, central venous pressure (CVP) will reduce significantly. This phenomenon explains the fainting or dizziness seen in hot weather.

1.6.7 Calf muscle pump

The calf muscle pump has important functions, the principle function being to maintain venous return to the heart, it also decreases blood volume in lower limb venous sinuses and enables more blood to be made available to be pumped to other organs of the body. The muscovenous pump is the contraction of the calf muscles, where it squeezes the venous blood accumulated inside venous sinuses and pushes it up to the deep veins of the body.

Emptying calf muscle veins by the muscloveous pump leads to a decrease in the venous pressure in peripheral venous system.

The calf muscle mechanism is based on the concept that, the deep venous system squeezes blood proximally into the popliteal vein under the effect of the calf muscles contraction that compresses the deep veins (**Figure 1.7**). Eventually, the deep veins pressure is reduced, resulting in venous blood being moved from the superficial venous system into the deep veins. The calf muscles pump occurs in a reciprocal mechanism; it is a regular and parallel action of calf muscle contraction followed by movement of blood from the superficial venous system into the deep venous system through perforating veins. Perforating veins contain one way valves preventing the return of venous blood from deep veins into superficial veins when the calf muscles contract. For a proper and regular calf muscle pump, venous valves must remain healthy otherwise blood will return back into the superficial venous system and cause venous hypertension, then to the subcutaneous tissue causing oedema, lipodermatosclerosis and eventually venous ulcer. When there is an escape of blood into the subcutaneous tissue around the ankle joint, the pressure inside the venous mesh will be around 100 mmHg whereas in a normal situation, the pressure around the ankle is 0 mmHg. Researchers believe this high pressure is the trigger for lower limb venous insufficiency and ulcer (Cockett, 1956).

The deep calf veins are either within the muscles, called intramuscular veins (the gastrocnemial veins and the soleal sinuses) or intermuscular (posterior tibial, anterior tibial and peronial veins). The intramuscular veins are considered pump chambers, these veins are thin walled and have no valves whereas the intermuscular veins have valves and their walls

are thicker (Cockett, 1956). Calf muscles are firmly enclosed by dense strong fascia, this tight fascia facilitates the generation of high intramuscular pressure during muscle contraction which helps in squeezing intramuscular veins. Venous return to the heart will depend significantly on the calf muscles function and their investing fascia (Cockett, 1956).

In a standing position the pressure in the lower limb superficial veins is equal to the blood column height between that point around the ankle joint up to the heart, which is about 120 cm or 80-100 mmHg. In a static position, this pressure is equal inside the muscle sheath (deep veins) and in the superficial veins, during exercise; the pressure in the superficial venous system will be zero (Cockett, 1956). Exercise may play role in decreasing venous hypertension within the superficial venous system in chronic venous insufficiency (CVI) patients.

Smirk, in 1936, first recorded the venous pressure measurements at the ankle whilst walking. Further researchers reported that venous pressure in the lower calf veins was reduced during exercise from about 80 mmHg to 40 mmHg and from approximately 90 mmHg to 25 mmHg in thigh veins (Pollack & Wood, 1949) (Walker & Loncland, 1950). Pollack and Wood concluded that, regular contraction of calf muscles on standing or sitting position will decrease the venous pressure in calf muscle veins. By reducing venous pressure the calf muscles maintain equilibrium between the venous pressure and hydrostatic pressure preventing development of venous hypertension in normal circumstances; in addition the calf muscle pump decreases the chance of ankle oedema formation resulting from high venous pressure when in the standing position.

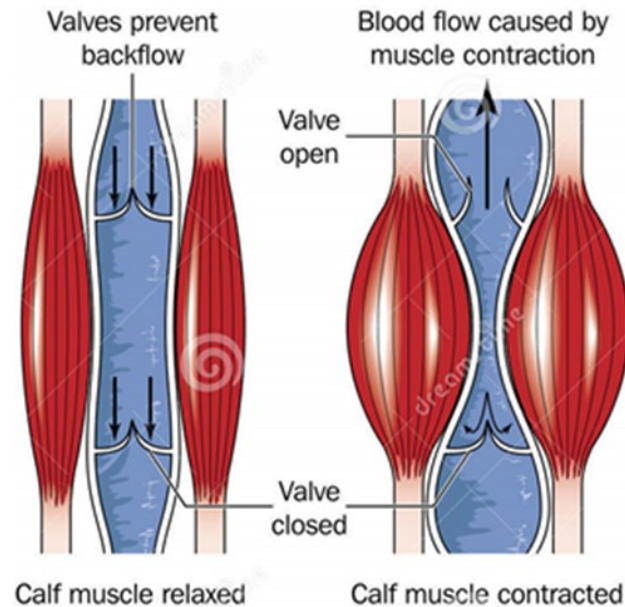


Figure 1.7: *Calf muscle pump*

(Modified from www.dreamstime.com)

1.6.8 Contraction and relaxation of calf muscles

Stegall found that the dorsiflexion exercise of feet causes a drop in superficial venous pressure from 90 mmHg to less than 10mmHg, he recorded that 30% of the power needed for blood circulation during exercise will be produced by the calf muscle pump (Stegall, 1966). Further researchers reported that venous pressure reduced significantly during exercise, approximately by 65-80%. Cockett reported that regular muscle contraction produced a pressure of 90 mmHg in side calf muscle veins while the pressure in the superficial venous system was around zero (Cockett, 1956). Ludbrook. J recorded that pressure inside the calf muscle veins could be increased up to 200 mmHg during contraction of calf muscles, he concluded that the pressure produced by muscle contraction depends on the following factors:

the thickness of the investing sheath of muscles, force of contraction and the direction of muscle fibres. Researchers reported that the pressure generated by calf muscle contraction is so powerful that it was able to push up blood above knee tourniquet when inflated at 90-100 mmHg. This high pressure is able to overcome the resistance of hydrostatic pressure and open venous valves. The pressure created by calf muscle contraction causes complete obliteration of intramuscular sinuses and exerts enough pressure to compress the intermuscular veins.

The amount of blood ejected proximally into popliteal vein is approximately 2ml/100 ml of tissue (Stegall, 1966). Ludbrook, reported that 60% of blood accumulated during standing will propel proximally in each single calf muscle contraction, whereas only 20% is ejected from the thigh veins during the same calf muscle contraction, the explanation for that being that the thigh does not contain intramuscular sinuses.

During relaxation of the calf muscles, blood will accumulate into venous sinuses, as these sinuses are relieved from the compression force applied by contracted muscles, at the same time popliteal vein valves closed preventing return-back of blood into the deep veins of the calf muscle. During the period of relaxation, the deep venous system is filled with venous blood coming from the superficial venous system, followed by the calf muscle contracting again to push accumulated venous blood up.

1.6.9 Foot venous pump

Researchers demonstrated a network of venous plexus in the foot. Although some variation in describing the plexus anatomy was seen, most of the scientists consider the plantar venous pump to be located in the deep intramuscular space. Foot venous plexus composed of dermal

and sub dermal veins pass alongside the foot plantar surface. These veins drain immediately to the medial and lateral marginal veins, also they drain into the medial and lateral plantar veins.

Investigators suggested that during each step when walking, the medial and lateral plantar veins push up the venous blood into the superficial and deep veins, this phenomenon occurs with a sequential movement of the foot and calf pumps. Foot venous pump mechanism occurs smoothly through three phases. Firstly, the weight-bearing phase: whilst walking the compression produced squeezes the venous reservoir; the second phase occurs when the foot is fixed, resulting in the compression of the venous pump into the musculo-tendinous plane and thirdly, the suspension phase, occurs when the foot is lifted from the ground leading to the pump being refilled.

During rest time, the planter venous pump is at rest. On standing and under the influence of gravity, the weight of the column of blood produces a pressure of about 90 mmHg. Walking up to 20 steps causes ankle venous pressure to fall down to 30mm Hg, this decrease in pressure is due to the effect of the planter venous pump. The falling in venous pressure enhances the refilling of the venous pump again which occurs cyclically (Uhl & Gilot, 2012).

1.7 Deep venous thrombosis

1.7.1 Introduction

Venous thromboembolism (VTE) is a very common clinical problem caused by the formation of blood clots in veins (thrombus) (*Figure 1.8*). The most common example of VTE is deep venous thrombosis (DVT) of the deep veins in the lower limbs and pelvis. Thrombus formed

in the lower limb veins can be logged into systemic veins particularly pulmonary arteries causing pulmonary embolism (PE) which is a fatal condition. Most importantly in relation to this study, deep venous thrombosis is the main cause for developing VLU in addition to the damage of venous valves.

Non-fatal venous thromboembolism can lead to other consequences, like post-thrombotic syndrome. Studies have estimated that 350,000 to 600,000 people in America suffer from DVT and PE each year, and that 100,000 deaths may occur yearly in relation to this illness.

Many studies have shown that at least one third of all patients having major surgery will develop or be affected by DVT at some point (Kakkar, Howe, Flanc, & Klarke, 1969). A report by the House of Commons Health Committee in 2005 found that 25000 people in the UK die from preventable hospital-acquired VTE annually.

The tragedy associated with deep venous thrombosis is that the clinical picture is easy to overlook because the signs and symptoms of VTE are much generalised and could be mixed up with other diseases. In fact, in many cases the clinical presentation is silent and there are no obvious signs at all, sometimes the only sign is the fatal PE. Moreover many of those patients who survive this problem will have serious consequences like chronic venous insufficiency and venous ulcer which will affect their quality of life.

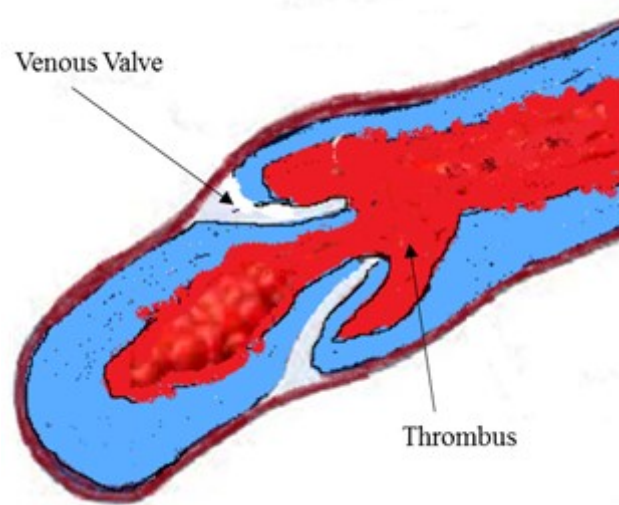


Figure 1.8: *Deep venous thrombosis*

1.7.2 Pathophysiology of DVT

All studies discussing the pathophysiology of DVT started with Virchow's triad. Rudolf Virchow, the 19th century pathologist (1856), was the first who was able to recognize that there are three factors responsible for the development of vein thrombus. These three elements are termed as Virchow's triad, namely, hypercoagulability, damage to the vein endothelium and stasis of venous blood (Torrey & Deepi, 2004). If we look at all risk factors associated with VTE we will find that nearly all of them will fall into one or more of these three categories. Venous stasis can be produced by anything that will slow the blood flow, such as dehydration and varicose veins. When the venous blood stagnates, micro thrombi will be formed which cannot be washed up by movement of fluid; these thrombi might grow and eventually will propagate. However, stasis in itself is not enough to trigger the development of VTE, Aronson et al recorded that ligation of rabbit jugular veins for up to 60 minutes was not enough to cause venous thrombosis (Aronson & Thomas, 1985). Injury to the vein lining endothelium (intimal damage) can be produced by intrinsic or external injury such as trauma.

Hypercoagulability can occur as a result of biochemical imbalance between blood components, one of these could be the increase in tissue activation factors associated with low antithrombin and fibrinolysis. In normal situations, there is a balance between factors that enhance and suppress coagulation. Any disturbance in this natural equilibrium may result in the development of hyper coagulation.

Studies have shown that the thrombus usually forms in the veins where there is disturbance or slow blood flow, such as soleal sinuses or behind valve pockets. Places, such as venous branch points and behind valve cusps usually form the best sites for developing thrombus, most of which start in the calf muscle veins in relation to prolonged bedrest and immobilized patients (Gibbs, 1957).

1.7.3 Risk factors

There are a number of factors contributing to Virchow's triad, such as endothelial damage and venous wall dilatation, in addition, individuals may have individual risk factors, for example previous thromboembolism, advanced age, malnutrition, dehydration and genetic trait related to hyper coagulopathy.

Coagulopathy, whether inherited or acquired later, is the most important factor. Studies have shown that hypercoagulability conditions are recognised in 25% of patients who develop venous thromboembolism (Thomas & Roerts, 1997). Gerhardt A et al found that the incidence of hypercoagulable conditions are even higher in individuals having recurrent venous thromboembolism and recurrent VTE.

It has been shown that the common risk factors acting as triggers for developing VTE in patients who already have hypercoagulable state (Bloemenkamp, Rosendaal, Helmerhorst,

Buller, & Vandenbroucke, 1995). In such cases there must be direct attention to those patients who have coagulable state when they are exposed to the common DVT risk factors.

Researchers reported in 1990 that general anaesthesia produce venous dilatation in 2% to 28% in patients and up to 75 % in patients who had intravenous infusion of one litre saline during surgery (Coleridge-smith, Hasty, & Scurr, 1990). Coleridge-Smith et al concluded that intraoperative venous distension precipitates DVT in individuals undergoing surgery, it was also suggested that loss of muscle tone as a result of muscle relaxants may produce venous stasis. Comerota et al suggested that muscle handling during surgery might lead to venodilation whereas, manipulation of bone causes venoconstriction; the same researches also suggested that suppression of venous dilation during surgery decreases postoperative DVT (Comerota, Stewart GJ, Alburger, Smalley, & White, 1989).

1.7.4 Clinical picture

Clots can lead to partial or complete blocking of the involved vein, this blockage can cause certain symptoms, including pain, discomfort, fever, tenderness, warm skin, swelling and redness or discoloration involving the affected area. However, nearly half of patients with DVT may have no symptoms at all (Piazza & Goldhaber, 2006). For some individuals who have their DVT for the first time, they will remain under possible risk of experiencing subsequent episodes during the rest of their lives (Prandoni , et al., 1996) (Heit, et al., 1999). Some patients, when developing deep venous thrombosis, symptoms disappear once the disease has disappeared, in general 30 percent of individuals affected by the disease will suffer from symptoms, such as pain, discolouration of skin, swelling and venous ulcers (Mohr, et al., 2000) (Prandoni , et al., 1996) (Heit, et al., 1999).

1.7.5 Prevention and treatment of DVT

It is thought that venous thromboembolism (VTE) is the most common preventable clinical problem in hospitals. Proper VTE prophylaxis reduced the hospital acquired VTE significantly. In spite of that, some patients will develop VTE; this failure has no clear justification yet and the risk factors for inpatient venous thromboembolism remain despite thromboprophylaxis (Tzu-fei, et al., 2014). Many preventive measures are available for prevention of VTE; however each one of them has its own risks and benefits.

Prevention of VTE has two approaches, which need to work in tandem where possible, pharmacological and mechanical approaches. Pharmacological preventive measures include, low dose unfractionated heparin (UFH), low molecular weight heparin (LMWH) and vitamin K antagonists, while the mechanical approach to VTE prophylaxis includes compression stockings and the use of intermittent pneumatic compression devices. Permanent inferior vena cava (IVC) filters can be used in prevention of pulmonary embolism in patients who develop new VTE and they will need urgent surgery, however they are not indicated for prophylaxis of primary VTE (Tzu-fei, et al., 2014).

1.7.6 Economic impact of DVT

The economic impact of DVT is not only related to the DVT itself but mainly to the consequences of venous thrombosis such as VLU. In spite of the fact that a lot of attention has been paid to the implementations of measures for the prevention and treatment of DVT, a gap still exist between these measures and the planned goals. DVT still cost the economy a vast amount and the cost to patients may be far greater.

Accurate and high quality data that assess the economic burden of DVT and PE are lacking. In the UK, 1 in 1000 people is affected by DVT (NHS Choices, 2014) and the figures from the House of Commons Health Committee, 2005 suggest that 25000 die from preventable hospital-acquired VTE a year. In the United States, Epidemiologic studies estimate 900,000 Americans could have VTE each year, and the death rate around 300,000 per year in relation to DVT and PE. The annual economic cost of VTE in the United States is estimated to be \$3.2 to \$15.5 billion (Hiet, 2008).

1.7.7 DVT and CVI

Development of CVI and venous ulcer is mainly related to the recurrence of DVT. Recurrence mostly occurs when the patient has a sudden event “spontaneous”, it is more likely to happen if it is not initiated by predisposing factors such as surgery, trauma, pregnancy or contraceptive pills (Heit, et al., 2000), (Prandoni , et al., 1996). For unknown reasons the risk of recurrence of DVT is higher in men than women (Kyrle, et al., 2004).

Chronic venous insufficiency (CVI) or post-thrombotic syndrome may complicate the initial episode along with the risk of recurrence, 30% of patients who suffer PE or DVT may develop CVI within the next 20 years or immediately after the episode (Mohr, et al., 2000) (Prandoni, et al., 2004). Kahn SR et al research concluded that CVI associated with DVT can cause similar impacts on the quality of life to that caused by other debilitating diseases such as arthritis, heart or lung disease (Kahn, et al., 2004) (Kahn, et al., 2005).

1.8 Venous leg ulcer

1.8.1 Introduction

Venous leg ulcer is a common clinical problem. Although venous ulcer is the cause of a great deal of morbidity, there is no standard definition of venous leg ulcer. The lack of standard definitions for venous leg ulcer may play a role in the failure of organising standard guidelines for management of the condition. There might be confusion if we limited the venous ulcer healing time, as venous ulcer may be healed before the expected 6 weeks. Venous ulcer may be defined as discontinuation of epithelium due to venous insufficiency.

Venous ulcer is the end result of chronic venous insufficiency (CVI) which occurs as a result of a defect in the venous system, either due to venous valve damage or obstruction of veins. VI can lead to venous hypertension, oedema, lipodermatosclerosis and inflammatory changes to the skin and eventually venous leg ulcer (Shami, Sarin, & Scurr, 1997) (Brem, Kirsner, & Falanga, 2004) (Nelson, et al., 2006).

Researchers reported that 40–50% of the adult population presented with chronic venous insufficiency and 1–2% of the adult population suffer with venous ulceration (Abbade & Lastoria, 2005). Venous ulcer is a debilitating clinical problem, that often takes months or years to heal and the recurrence rate is very high, up to 70%. Eventually it can lead to a vicious cycle of ulceration, healing, and recurrent ulceration. Achieving healing of venous leg ulcer is often a difficult and lengthy process that is further complicated by significant comorbidities. As a result, it can become a lifelong chronic condition causing a huge burden on individuals and states.

The venous leg ulcer has been well known for millennia, and yet there remains no proper effective treatment for this very common condition. Not surprisingly, a chronic problem as common as VLU has led to the development of different modalities of treatment, many of which are unproven. The compression therapy has been tried since 3000 BC; surprisingly, this technique is still the accepted “gold-standard” treatment of VLU worldwide. A variety of forms of treatment are becoming more available, however none of them has become accepted as the single effective treatment. Treatment of venous leg ulcer is not an easy task to achieve; it involves the management of chronic and complicated pathophysiological clinical condition, where there is a high rate of recurrence and infection. In addition, VLU patients suffer with a lot of comorbidities, they are most often a debilitated elderly group of patients and most of the time VLU patients are isolated and have little or no support. The treatment approach of such complicated condition needs changing, especially with the fact that there is no single effective treatment at present; there is a need to accept the idea of taking a multidisciplinary approach. These approaches could include compression therapy and suitable, easy, feasible, non-invasive and cost effective treatment such as exercise.

1.8.2 Epidemiology

The prevalence of venous leg ulcer varies from one study to another. Shami S., et al looked at a few Swedish studies, one of them reported prevalence of 6.4/1000 whilst the other reported a prevalence of 3.05/1000 of population (Shami, Sarin, & Scurr, 1997). A study conducted in Ireland revealed a prevalence of 15.2/1000 of population, whilst in North America, chronic venous leg ulcer affect between 500,000 and 2 million individuals per year and over 50% of leg ulcers are considered to be venous leg ulcer (Lazarus, Mahmud, Umair, & Nisa, 2014).

Collins & Seraj estimated the prevalence of venous leg ulcer in the US at about 1% of the population (Collins & Seraj, 2010), while Robertson L et al, estimates the percentage of the population affected with VLU at more than 2.5 million per year (Robertson, Eans, & Fowkes, 2008). A cross sectional study conducted in Scotland looked at a sample of 1566 subjects with venous ulcer aged between 18-64 years, in this study, the prevalence of venous leg ulcer was estimated to be 1% of the study group. (Schmid-Schonbein, et al., 2006). This study might not represent the reality of venous leg ulcer problems in Scotland; the study ignored a significant number of the population above the age of 64. Sarkar P K and Ballantyne concluded that there are 400 000 subjects with venous leg ulcer present in the UK at any one time, other researchers reported UK prevalence of venous leg ulcer disease between 1.5 and 3.0/1000 of population. Graham et al, conducted a systemic review for lower limb ulceration, he found variations between the different studies, where prevalence rates were found to be between 1.2 and 3.2 per 1,000 of population. Meaning, a lower figure of between 70,000–190,000 subjects, in the UK, would be complaining of venous ulcer at any time (Graham , Harrison, Nelson, Lorimer, & Fisher, 2003). There is no clear idea about geographical variation in the prevalence of venous insufficiency (VI) although it has been mentioned that rates of VI are higher in developed countries as opposed to those in undeveloped countries (Eberhardt & Raffetto, 2014). These findings may be misleading as they could be due to a lack of research having been conducted in the undeveloped countries or may be due to a lack of reporting venous problems by individuals until later stages of ulceration.

The occurrence of venous leg ulcer showed sharp increases with age (Graham , Harrison, Nelson, Lorimer, & Fisher, 2003). In a study examining the population of a London Borough,

Moffatt et al and colleagues reported high dependency of venous leg ulcer on age. Venous leg ulcer rates increased significantly in those aged more than 85 years; with an increase to 8.29 (men) and 8.06/1000 (women) from 0.34 and 0.54/1000 respectively. This study may not represent the real problem in the UK as it was localized to one London borough and only involved 113 patients (Moffatt, et al., 2004). Lian W reported an increase in venous leg ulcer with age to about 20/1000 in individuals aged above 80 years (Laing, 1992). Data showed that there is going to be an increase in the aging population in the near future; where it is expected that the number of those living with debilitated conditions will increase by 23% in the next 25 years. As a result, the venous leg ulcer incidence is most likely to increase as aging population and associated risk factors increase; putting further burden on health services (Yarwood-Ross & Haigh, 2012).

Sex distribution of venous leg ulcer is not clearly demonstrated, however most studies have shown increase in chronic venous insufficiency incidence among women (Graham , Harrison, Nelson, Lorimer, & Fisher, 2003) (Bergan, et al., 2006). Callam et al conducted a study in the areas of Lothian and Forth Valley, Scotland, an area that has a mixed population of about one million that is made up of both rural and urban dwellings. The purpose of the study was to identify subjects receiving treatment for chronic leg ulceration. It reported that the majority of patients were mostly women, out of the 1477 patients who were recognised by the study; 1089 were women and 388 were men with a ratio of (1:2.8) (Callam, Ruckley, Hrper, & Dale, 1985). In contrast, in the Edinburgh Vein Study, where 1566 patients, aged 18-64 years were examined from 12 general practices throughout Edinburgh, the prevalence of

venous disease was higher in men; prevalence of varicose veins was 39.7% in men versus 32.2% in women (Lee, Evans, Allan, Ruckley, & Fowkes, 2003).

The reporting of the prevalence of chronic venous ulcer was limited by many factors including the lack of standard definitions of CVLUs, inconsistent outcome measures, suboptimal comparison groups and the lack of a uniform reporting standard (Lazarus, Mahmud, Umair, & Nisa, 2014).

1.8.3 Impact of venous ulcer

The management of venous leg ulcer presents an increasing medical and socio-economic challenge worldwide. Venous ulcer is a long-term problem that often takes months or even years to heal completely and it frequently reoccurs, this problem consumes huge volumes of medical staff time and healthcare service resources. With lack of proper management and high recurrence rate, venous ulcer becomes a lifelong debilitating condition with cycles of ulceration, healing and recurrence.

The socio-economic impact of venous leg ulcer is dramatic, patients affected by VLU lack the ability to engage in occupational and social activities, resulting in impairment of the quality of life and creating financial constraints upon them. Venous leg ulcer impact on individuals and the state has been reported in literature, with consequences such as increased levels of pain, reduced quality of life, limitation of movement, lack of functional ability and social isolation (Persoon, et al., 2004). Disability associated with venous leg ulcer leads to loss of productivity, estimated to be around two million workdays annually and early retirement was found in >12% of workers suffering from VLU (Robertson, Eans, & Fowkes, 2008).

Moreover, there are significant financial burdens placed upon the patients and states, the estimated cost being about 2–3% of total healthcare expenditure in the western world (Finlayson, Edwards, & Courtney, 2011). The annual cost to the NHS in the UK has been estimated at £300 million. This does not include the loss of productivity due to venous ulcer illness (Laing, 1992). The cost of treating venous leg ulcer in Western European countries is estimated to be 1% of the total annual healthcare budget (Van Gent, Wilschut, & Wittens, 2010). In the United States, venous ulcer treatment costs \$1.0-1.6 billion for more than 6 million patients (Phillips, Stanton, Provan, & Lew, 1994) (Eberhardt & Raffetto, 2014). A prospective cross-sectional study conducted in Germany in 2010, involving 23 specialized wound centres, aimed to determine the cost of venous ulcer treatment; researchers found that the mean total cost of a venous ulcer treatment per patient per year to be €9569, of which 92% were direct costs and 8% were indirect cost (Purwins, et al., 2010).

Such huge consequences of venous ulcer on patients and states will require a new approach for the management of venous ulcer, especially where compression therapy, the classical treatment of venous leg ulcer is associated with high recurrence rate and leads to a significant number of patients being either unwilling or being unable to use it.

1.8.4 Description of Disease

Venous leg ulcer is an ulcer that occurs on the gaiter area of lower limb; between the ankle joint and the knee joint and mostly affects the full thickness of the skin. The underlying pathophysiology of venous leg ulcer is venous hypertension that is caused by reflux of venous blood into superficial venous system. This occurs as a result of obstruction of the lower limb veins such as deep venous thrombosis, damaged venous valves or due to

insufficiency of the calf muscle pump. Venous hypertension will lead to chronic venous insufficiency which may involve, superficial venous system, deep venous system and perforators joining those two systems (**Figure 1.9**), (Huisman, Baker, & Wittens, 2013).

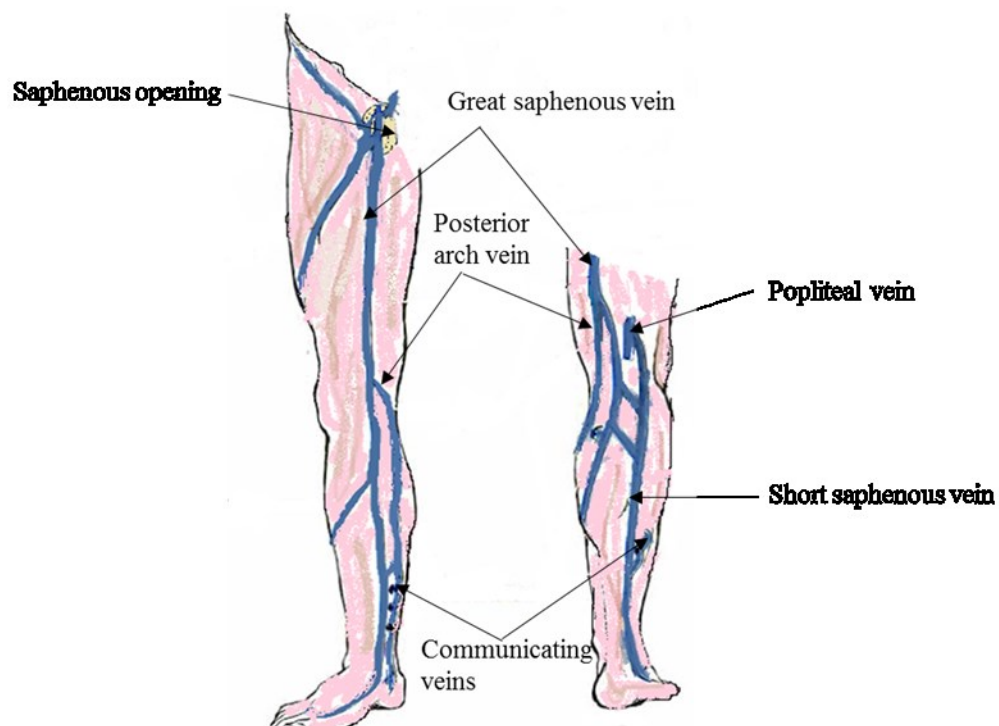


Figure 1.9: Lower limb venous system

Chronic venous insufficiency is defined as a condition which affects the venous system of the lower limbs and is associated with “persistent ambulatory venous hypertension causing various pathologies, including pain, oedema, lipodermatosclerosis and ulcerations” (Eberhardt & Raffetto, 2014). The identification of the specific cause of venous insufficiency predisposing to a venous ulcer may aid a proper and more effective management plan. The anatomy and function of the venous system needs to be known in order to understand the pathophysiology of the venous insufficiency and venous ulcer.

The peripheral venous system has a vital function that includes working as a reservoir for blood and for transporting blood to the heart from peripheral tissue. For normal function of the peripheral venous system, there are important factors that need to be available, namely the presence of healthy patent's superficial and deep venous systems, presence of functioning venous valves and normal calf muscle function. Normal calf muscle function is important for movement of venous blood towards heart against gravity and functioning one way bicuspid valves is crucial for this process.

The superficial venous system which is composed of the long saphenous vein, the short saphenous vein and the accessory veins lies between the skin and the muscular facial layer. The deep venous system is located beneath the muscular facia and composed of veins that follow the correspondent arteries and venous sinuses. The perforating veins connect superficial veins and deep veins and pass through the facia; these perforating veins contain valves that allow movement of blood from superficial veins to deep veins and not vice versa (Gloviczki P. , 2001).

The contraction of the calf muscle works in coordination with venous valves to permit the travelling of blood towards the heart against gravity. When the calf muscle contracts, it squeezes the venous plexi pushing venous blood up, at this moment the valves open leaving the blood to move into the deep veins and preventing flow into perforators and superficial veins. As muscle contraction ceases the pressure in the calf veins decreases, low venous pressure in the calf muscle veins will allow blood to flow again from superficial veins into the calf veins. Any disturbance of this cycle leads to venous hypertension and related consequences.

The pathophysiology changes start when there is damage in the peripheral venous system causing disturbance of the normal mechanism of circulation of blood through the venous system. The damage may occur through three mechanisms, venous valves damage, which is the most common problem, obstruction of veins, mainly by deep venous thrombosis and calf muscle dysfunction. These mechanisms may produce an increase of venous pressure that would become obvious whilst standing or in the sitting position, venous hypertension eventually leads to microcirculatory changes at skin level, which eventually develops into venous ulcer (Gschwandtner & Ehringer, 2001) (Shami, Sarin, & Scurr, 1997).

Valve damage can come about as a result of different mechanisms such as weakness of the vein wall or flapping of the valve leaflets, direct injury and phlebitis or as a result of hormonal disturbance during pregnancy. When the valve damage occurs at superficial veins, there will be a reflux of blood into these veins, causing dilated and tortuous veins. Dysfunction of valves occurs at two levels, namely saphenofemoral and saphenopopliteal junctions, as a result, venous blood accumulates in long saphenous, short saphenous and accessory veins which in turn leads to venous hypertension, oedema and venous ulcer. Researchers demonstrated that superficial venous system incompetence showed in 90% of cardiovascular disease (CVD) and 84% of venous ulcer patients (Meissner, et al., 2007).

Valve incompetence in the communicating perforators may lead to flow back of blood from deep veins to superficial veins, this flow back is characterised by high pressure volume (Delis, Husman, Kalodiki, Wolfe, & Nicolaides, 2001) (Labropoulos, et al., 1997). This high pressure may enhance dilatation of superficial veins and increase hydrostatic pressure, ultimately oedema and skin changes develop. Valve damage can occur at the deep venous

system, such as femoral or iliac veins, where deep venous system incompetence occurs mostly due to deep venous thrombosis (Neglen, Thrasher, & Raju, 2003). Valve incompetence causes rapid refilling of the veins because damaged valves cannot prevent venous blood from falling back. As a result, the deep venous pressure is elevated, resulting in high venous pressure in the presence of normal calf muscle function (Kahn & Ginsberg, 2004).

Obstruction of veins may be from inside such as with deep venous thrombosis, from within the vein itself in recurrent phlebitis or from outside, when pressure is caused by a pelvic tumour. Venous obstruction will cause backflow of venous blood resulting in an increase of venous pressure, damage to valves and secondary calf muscle dysfunction as it is working against the backflow pressure (Eberhardt & Raffetto, 2014). Post obstructive venous insufficiency, as in deep venous thrombosis, may lead to severe consequences such as chronic venous insufficiency and venous ulcer.

Calf muscle function is a vital factor in keeping normal venous circulation, without regular calf muscle contraction venous blood will accumulate in the peripheral venous system. Dysfunction of the calf muscle could be primarily due to neuromuscular problems and muscle wasting conditions, such as those seen in the elderly or debilitated patients or after trauma. Calf muscle dysfunction mostly occurs as a result of venous reflux or venous obstruction, as a result of inefficient emptying of the peripheral venous system, the venous pressure inside the calf muscle venous sinuses will increase (Araki, et al., 1994). Venous pressure increase will put a greater load on the calf muscle function, leading to fatigue and eventually calf muscle dysfunction.

Haemodynamic changes in the lower limb veins reflect within microcirculation and produce venous microangiopathy changes in the skin (Pappas, Duran, & Hobson, 2001). These microangiopathic changes present as elongation and tortuosity of capillaries with increased collagen and elastic fibres in the capillary bed and increased pericapillary oedema (Shami, Sarin, & Scurr, 1997).

Venous hypertension may result in severe venous insufficiency through different mechanisms, distension of veins may result in the impairment of the venous wall function leading to an increase in hydrostatic pressure and enable the escaping of blood contents into interstitial tissue. The abnormality of capillaries associated with the haemodynamic changes may lead to increased permeability across the venules. Disturbance of normal capillary permeability result in the escaping of fluid and extravasation of red blood cells and other blood components into the interstitial space. Circulating leukocytes, vascular endothelial factor and other inflammatory cells will express themselves as a complicated inflammatory process (Yong & Khwaja, 1990).

There have been several theories explaining the development of venous microangiopathy, including fibrin cuff formation, hypoxia, ischemia, growth factor trapping and white blood cell trapping; however there is no universal acceptance of any one of these explaining why venous microangiopathy develop. Therefore it is important to examine the most significant theories in greater detail.

1.8.5 Homan's theory

Homan, in 1917, suggested that stasis of the blood in the venous system was the cause of venous ulcer (Homans, 1917). He referred the development of ulcer to the hypoxia produced

by venous stasis, as venous blood accumulation results in reduced skin oxygenation and eventually anoxic cell damage. Homan's theory was widely accepted although in 1929, Blalock reported high level of oxygen obtained from the femoral vein of patients with varicose veins compared with that acquired from normal patients (Blalock , 1929). Although Blalock's findings have not been supported and there is no explanation offered for his findings. Moreover, the venous ulcer involves superficial veins in a specific area, namely the gaiter area while the femoral veins drain venous blood from the entire lower limb.

1.8.6 Fibrin cuff theory

This theory was first mentioned by Browse and Burnand in 1982 with the publication of their hypothesis explaining venous ulcer pathophysiology (Browse & Burnand, 1982). They build their hypothesis on the findings of enlargement of capillaries in the skin around the ulcerated area; they suggested that as venous pressure increases inside the veins, it causes dilatation of pores between endothelial cells forming the capillary walls. These enlarged pores allow molecules, in particular fibrinogen, to pass through them and to form fibrin cuffs in the perivascular area. These cuffs act as a barrier that prevents oxygen from reaching the skin and will result in ischemia and ulcer (Butler & Coleridge smith, 1994).

The fibrin cuff theory was supported by Leach RD, who reported that there was an increase in the fibrinogen levels in venous blood collected from the dog's hind limb after ligating the femoral vein (Leach & Browse, 1981). Presence of a high fibrinogen level in a ligated femoral vein could be accompanied by other blood components and inflammatory markers as a part of the injury induced inflammatory process. Presence of fibrin in venous ulcers was

also observed by some histological studies (Burnand, Whimster, Naidoo, & Browse, 1982), (Falanga, Moosa, Nemeth, Alstadt, & Eaglstein, 1987).

Several investigators provide evidence against this theory; Michel (1990) concluded that the fibrin cuff was unlikely to act as a significant barrier to molecules the size of oxygen (Michel, 1990). Further research has also suggested that if the fibrin cuff prevents oxygen diffusion, then reduction of the fibrin would result in ulcer healing, on investigating the effect of fibrinolytic agent on ulcer healing, researchers failed to find any significant difference from the placebo (Layer, Stacey, & Burnand, 1986).

1.8.7 White cell trapping theory

Moyses reported in 1987 that in normal subjects, 15-20% of white cells that entered the foot, after 40 minutes whilst in the sitting position, failed to leave venous blood, (Moyses, Cederholm-Williams, & Michel, 1987). In this study the white cell count was measured before and after 40 minutes of sitting. It concluded that these lost white cells remained in the legs venous blood, accumulating either in the microcirculation or in the tissues. These findings were supported by Thomas and colleagues, (Thomas, Nash, & Dormandy, 1988).

These findings led Coleridge Smith and colleagues to propose their 'white cell trapping theory' (Coleridge Smith, Thomas, Scurr, & Dormandy, 1988). They suggested that white blood cells stick inside the capillaries of the skin, while an increase in venous pressure when in a standing position will lead to a decrease in the capillary blood flow rate, resulting in the trapping of white blood cells. Occlusion of capillaries will lead to localised ischemia and eventually venous ulcer. There was no clear evidence to support the idea that states, tissue hypoxia resulted from capillary plugging. Subsequently the authors themselves refuted part

of their theory, and they suggested that although the white blood cells may be trapped in capillaries, it does not cause capillary occlusion to the level that will impair skin perfusion.

1.8.8 The growth factor trap theory

In the growth factor trap theory (*Figure 1.10*), Falanga and Eaglstein, suggested that fibrinogen, α_2 -macroglobulin and other macromolecules that leak from blood to the dermis “bind to or trap” growth factors and other haemostatic molecules and make them unavailable for the repair process in venous ulcer (Falanga V & Eaglstein WH, 1993). They conclude that the venous ulcer microenvironment becomes hostile for the healing process because of the ineffectiveness of growth factors. This theory was supported by studies which revealed high growth factor levels in venous ulcer, however the tissue exudate from venous ulcer did not show proliferation of fibroblasts, endothelial cells and keratinocytes in vitro, (Bucalo, Eaglstein, & Falanga, 1989). Other researchers suggested that it is difficult to explain that growth factor trapping within skin capillaries may result in inflammation, skin changes and venous ulcer, (Browse N. , Burnand, Irvine, & Wilson, 1999). Furthermore, the growth factor therapy has failed to solve the problem of venous ulcer. Growth factors are a common element for almost all inflammatory conditions for instance as seen in arterial ulcers, does this mean that arterial ulcer result from trapping of growth factors by fibrinogen and other macromolecules.

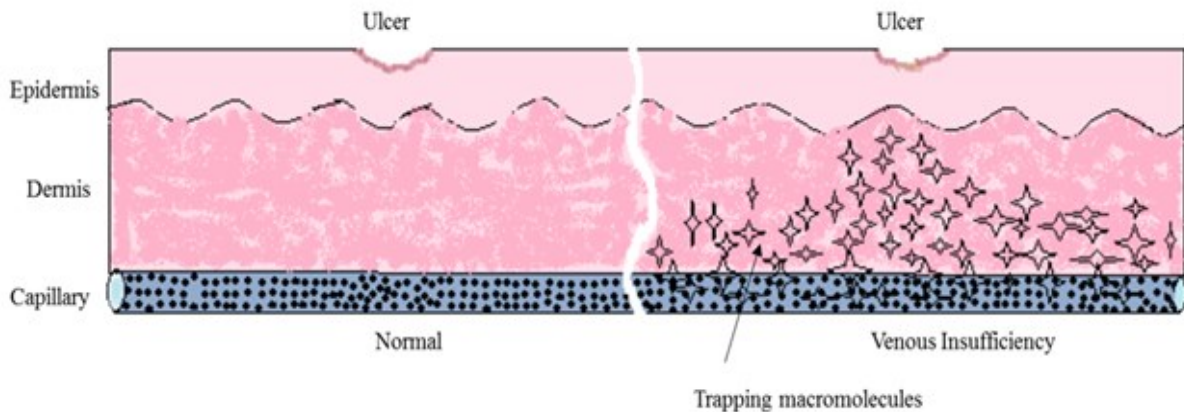


Figure 1.10: *Trapping theory in venous leg ulcer*

1.8.9 Summary of pathophysiology

It is obvious that there is a full understanding of haemodynamics of venous insufficiency but it is not clear yet how these haemodynamics lead to venous ulceration. Additionally, there is a clear agreement that venous leg ulcer is caused by localized microvascular ischemia (Franzeck, Haselbach, Speiser, & Bollinger, 1993). However, there is no clear understanding of the mechanism of this microvascular ischemia.

As we previously mentioned there are reservations that have been reported as to whether the fibrin cuff theory provides a logical explanation for skin changes and ulceration in CVI. If the fibrin is solely responsible for developing skin hypoxia, one would expect an improvement in the CVI picture with fibrinolytic agent (Cheatle, Sarin, Coleridge Smith, & Scurr, 1991). For the white cell trapping theory, it is believed that the supporting evidence is generally circumstantial. Although there is proof of white cell activation in venous ulcer, there is no clear evidence supporting the causative role of the leucocyte; therefore, any inflammatory response, caused by any trigger, may lead to the same findings.

Homan's theory implies that stasis is present in the venous blood; his theory has led to the assumption that venous stasis will lead to a decrease in skin oxygenation, which in turn induces anoxic cell death (Cheatle, Sarin, Coleridge Smith, & Scurr, 1991).

All of the theories outlined consider venous hypertension as the main factor that is responsible for the development of venous insufficiency and suggest that microcirculation changes are the trigger of cell damage. However, there is still controversy about the mechanism of microvascular ischemia.

Homan's was the first to suggest that venous stasis in cutaneous blood, may induce skin hypoxia in CVI patients (Abramson & Fierst, 1946). Franzeck UK reported that microangiopathy in the initial stages of CVI are characterized by the dilatation of capillaries as a result of continuous retrograde venous pressure, eventually becoming very dilated and tortuous under the effect of retrograde venous pressure (Franzeck, Haselbach, Speiser, & Bollinger, 1993). The findings of Franzeck UK et al, were supported by Anke Steins et al, who mentioned that in patients with CVI there is a "constant state of venous hypervolaemia", through the propagation of venous blood into cutaneous capillaries (Steins, Hafher, Hahn, & Junger, 2002). Franzeck UK et al also reported microthrombosis of capillaries and referred to "interactions between leucocytes and other blood cells with the vessel wall of post-capillary venules" (Franzeck, Haselbach, Speiser, & Bollinger, 1993). There is no clear evidence that such interaction can lead to thrombosis, on the other hand it is well known that Virchow's triad (hypercoagulability, stasis, endothelial injury) is the pathophysiology behind thrombosis. Devolvement of venous thrombosis if present may be better explained by venous stasis.

Venous stasis is defined as being, the accumulation of deoxygenated venous blood in the cutaneous capillaries. The back pressure and the accumulation of the deoxygenated venous blood in the cutaneous capillaries will diminish the space for the arterial oxygenated blood and this may be the explanation for low tcPO₂ in the venous ulcer area. Low tcPO₂ in venous ulcer was reported by Franzeck UK et al, and they considered it as a “localized, spot-like ischemia” (Franzeck, Haselbach, Speiser, & Bollinger, 1993). Other researchers have shown the same findings including (Clyne, Ramsden, Chant, Webster, & JH, 1985), (Stacey , Burnand, Pattison, Thomas, & Layer, 1987), (Kolari, Pekanmaki, & Pohjola, 1988), (Steins, Hafher, Hahn, & Junger, 2002), (Rosaria Cesarone, et al., 2008). The question that is raised by this is that while we agree that the majority of cutaneous capillaries are filled with deoxygenated venous blood rather than oxygenated blood, will deoxygenated hypervolemia be the cause of ischemia and eventually VLU. It could therefore be called ‘venous ischaemia’ or perhaps be recognised as a factor that is contributing to the “multifactorial microvascular ischemia” as Franzeck UK et al reported (Franzeck, Haselbach, Speiser, & Bollinger, 1993).

Treatment

Although venous leg ulcer is a very common surgical problem, causing significant burden to individuals and states, there is no unique treatment for it. Bandaging and compression is the classic method of treatment which has been used for thousands of years and has remained the same, however, over the last 100 years, other modalities have been used; mainly surgical and drug treatments.

1.8.10 Compression therapy

Compression treatment of leg ulcer is an ancient method of treatment that has been in practice since 3000 BC, compression therapy could be through the use of compression stockings or compression bandages (*Figure 1.12*). Over the last century researchers have been able to confirm that compression with stockings and bandaging helps in the healing of venous leg ulcer. Partsch et al published a detailed review showing the efficacy of compression treatments in venous leg ulcer. It was found that with high levels of compression, with the use of bandaging or stockings (20 to 30 mm Hg) became a more effective way of healing VLU. Healing may produce an improvement in the function of the calf muscle pump, an oedema reduction and a reduction of venous reflex (Partsch, Flour, & Smith, 2008). There is strong evidence (level 1A) that bandaging of the involved limb is effective in the healing of venous leg ulcers. In addition, there is level 1B evidence that strong compression stockings (30-40 mm Hg) leads to ulcer healing. Level 1A evidence also showed that compression hosiery (30-40 mm Hg) prevents recurrence of the venous ulcer.

Researchers suggest that compression treatment with bandaging or strong hosiery is the first line of management for venous leg ulcer treatment unless, it is contraindicated; such as in subjects with peripheral artery disease. Compression may be sufficient to cure 50 to 60 % of venous leg ulcers (Bergan, et al., 2006) . Importantly, compression treatment should be maintained and continued after ulcer healing, perhaps through the use of a light form of compression stockings to prevent recurrence.



Figure 1.11: Compression stockings

Intermittent pneumatic compression (IPC) has been used in the treatment of venous leg ulcers, IPC is a mechanical method that produces compression to swollen legs that can help to treat VLU. However, the evidence in support of such modality is weak (Coleridge-Smith, 2009). In a Cochrane review, a series of randomised controlled trials were reviewed for IPC treatment regimens in venous ulcer management. The authors identified nine randomised controlled trials of 489 individuals, where they concluded that there is some limited evidence to suggest that IPC may improve VLU healing when used with compression bandages (Nelson , Hillman, & Thomas, 2014).

There is little evidence that IPC could be used with compression treatment to improve ulcer healing, however most VLU patients are elderly and complain of many comorbidities. IPC might be difficult to use by VLU patients and therefore hospital wards may be the most suitable place for their use, particularly postoperatively.

Bandage classification

Type 1 compression bandages (14-17mmHg):

Products which have a simple dressing retention function. This should conform to a limb or joint without limiting patient movement

Type 2 compression bandages (18-24 mmHg):

Sometimes called short stretch bandages, these types of compression bandages are used to control oedema and swelling in the treatment of venous disorder of the lower limb. Type 2 bandages are also used for the leg ulcer treatment because of the limited elasticity.

Type 3 compression bandages (25-40mmHg):

Type 3a: Light compression bandages are able to provide and maintain a low level of pressure up to 20 mmHg on an ankle of average dimension

Type 3b: Moderate compression bandages are used to apply compression up to 30 mmHg on an ankle of average dimension.

Type 3c: High compression bandages may be used to apply high levels of compression up to 40 mmHg on an ankle of average dimension. This type of bandage is also suitable for the treatment of varicosity, post-thrombotic venous insufficiency and the management of leg ulcers and gross oedema in limbs of average circumference.

Type 3d: Extra-high performance compression bandages are capable of applying pressure up to 50 mmHg. These bandages can be expected to sustain and apply these pressures on the largest limbs for extended periods of time.

To apply compression bandage, wound needs to be cleaned regularly. Then a 3-4 layer bandage should be wrapped over the wound dressing by a skilled nurse, so that the highest

pressure is applied at the ankle gradually reducing in pressure towards the knee and thigh. In order to have a precise pressure applied to the wound area, the bandage may need to be re-applied every week or when the ulcer dressing is changed.



Figure 1.12: *Compression bandaging of venous ulcer*

1.8.11 Surgical treatment

Conventional Surgery:

A surgical approach is another option for venous leg ulcer treatment and is a treatment method that has been used for many years. Surgical stripping of the long and short saphenous veins was reported by Keller and by Babcock around 100 years ago (Coleridge-Smith, 2009) (**Figure 1.13**). There are different methods to remove the saphenous vein; however there are minor technical differences between them, in addition there is controversy about stripping the long saphenous vein below the knee as it might cause damage to the saphenous nerve.

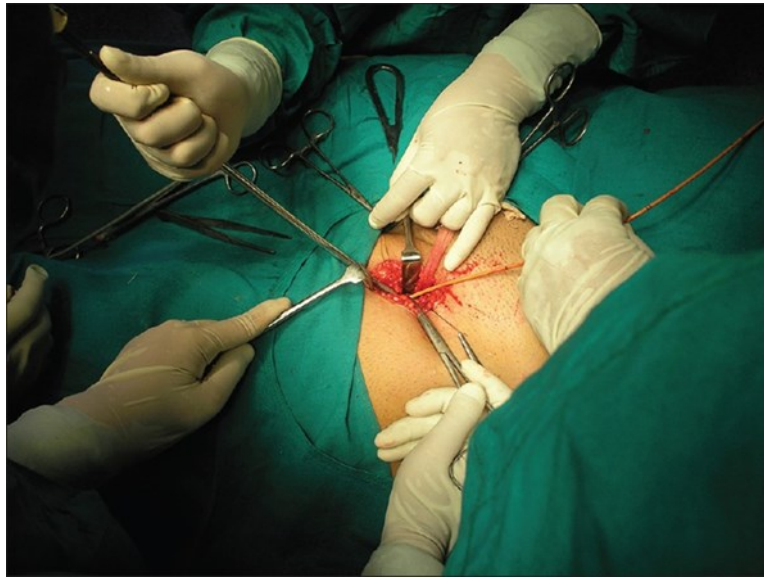


Figure 1.13: *Surgical stripping of the long saphenous vein*
(Modified from www.google.com)

Effect of Surgery and Compression on Healing and Recurrence (ESCHAR), a clinical randomized trial study investigated the role of saphenous vein surgery and compression in treating VLU (Barwell, et al., 2004). Researchers looked at 500 patients, over 24 weeks, who underwent treatment of venous leg ulcer either by compression alone or combined with superficial saphenous vein stripping. Barwell JR et al, found that 65% of patients within both groups had healed ulcers, but a recurrence of ulceration was seen in 12% of the compression and surgery group compared with 28% recurrence in the compression alone group. Investigators in the ESCHAR study concluded that surgical treatment of superficial venous reflux combined with compression bandaging does not improve ulcer healing compared with compression alone but reduces the ulcer recurrence levels at four years. These findings have been further supported by recent studies such as De Carvalho, (De Carvalho, 2015).

A prospective randomized multicenter trial took place that compared surgical treatment (combined superficial and perforating vein surgery) with compression therapy alone for VLU patients (Van Gent, et al., 2015). This study reported the results of a 10-year follow-up period, the researchers concluded that, the addition of surgical treatment in venous ulceration patients leads to a significant healing rate. The study reported a high rate of ulcer healing in the surgical group (58.9%), compared to the conservative group (39.6%), they also reported a very high recurrence rate in the conservative group only, about 94.3%. While the recurrence rate was 48.9% for the combined superficial and perforating vein surgery, there was no clear explanation for such high recurrence in the conservative group. Although they constituted 4%, there were diabetic patients included in the study, furthermore the study included subfascial endoscopic surgery. All these factors may explain why the findings differ from those found by Barwell et al.

Perforators Surgery:

Open surgery for treating perforating veins has been practised over the last 50 years, but most often only the serious wound healing problems are reported in literature. With the development of minimal invasive surgery, subfascial endoscopic surgery (SEPS) has become popular, researchers recorded less wound healing problems, as opposed to those seen in conventional open surgery where long incisions are made in order to divide the incompetent perforators (Valluru & Babji, 2015) (Bergan, Murray, & Greason, 1996). Open perforating vein surgery for venous ulcer treatment has not been commonly used and has not been investigated in detail, possibly because of the high levels of complication or maybe due to

replacement by SEPS. There is no known detailed clinical trial that have investigated the role of open perforating vein surgery.

The North American SEPS Registry collected information on 148 SEPS procedures, these procedures were performed in different centres throughout the US and Canada over a 3 year period. Researchers focused on mid-term outcome of 146 subjects, these patients were followed up, on average, for 24 months, 69% of them had active ulcers and 14% had healed ulcers. One hundred and three patients (71%) required surgery, (stripping, 70; high ligation, 17; varicosity avulsion alone, 16). The study reported that cumulative ulcer healing, at 1 year, was 88% and cumulative ulcer recurrence, at 1 year, was 16% and 28% at 2 years (Gloviczki, et al., 2002). We can conclude from these findings that the division of perforators is effective in healing ulcers on the other hand there is still a high recurrence of venous leg ulcer. The role of SEPS is not quite recognised through a detailed study and needs more investigations.

Deep vein surgery:

There have been a number of researchers who published papers about deep vein surgery (DVS) to treat deep venous incompetence. Masuda EM and Kistner RL, followed-up 48 patients (51 extremities) for a period ranging from 4 to 21 years with a mean of 10.6 years. These individuals did not have venous ulcer, even though the recurrence in this study was high (Masuda & Kistner, 1994).

A Cochrane review of randomised controlled trials of surgical treatment for patients with DVS included three trials involving 233 patients, but it is not clear whether these patients were complaining of VLU or not (Hardy, Riding, & Abidia, 2004). The authors' concluded

that valvuloplasty surgery produced a moderate improvement of venous symptoms over seven to ten years, however they have not recommend this modality for treating VLU. Unfortunately these trials were small, they have not used the same methods of valvuloplasty and there were different methods for assessment. Furthermore, it is not clear whether VLU patients were included or not in these trials.

Reconstructive deep venous surgery (RDVS) has also been examined. Over the last two decades researchers have been trying to develop stent-mounted bioprosthetic valves to replace diseased venous valves. The leaflets of these valves are made of synthetic or biological material (porcine small intestinal and pericardial tissue), these synthetic valves are related with autoimmune reaction, thrombosis, migration and incompetence (De Borst & Moll, 2012). Researchers believe that tissue engineering might represent a chance for improvement of synthetic valves in the near future (Rosales, 2015). The role of RDVS in the treatment of CVI is still not clear and not commonly used, it may need further work at the laboratory level before it is commonly seen in practise.

Other surgical methods for treating varicose veins have gained wide spread popularity including endovenous laser and radiofrequency ablation as well as ultrasound-guided foam sclerotherapy. Radiofrequency or laser produce thermal energy that is used to ablate incompetent veins. This can however, lead to thermal injury of the vein wall caused by the heat generated that will lead to thrombosis, fibrosis and obliteration of the vein, (Eberhardt & Raffetto, 2014). There are still not enough studies supporting treatment of saphenous vein incompetence with these modalities in patients with venous leg ulcer (Coleridge-Smith, 2009).

There is no detail randomised clinical trial yet published comparing these modalities with conventional surgical treatment of VLU patients. There has been a meta-analysis reported review of 119 studies, 12.320 limbs and the average follow up is 32.2 months. Subjects have included patients who have venous incompetency and there is no indication about the percentage of venous ulcer patients. Researchers concluded that the minimally invasive surgery appears to be as effective as conventional surgery in VLU treatment (Van Den Bos, Arends, Kockaert, Neumann, & Nijsten, 2009).

We can conclude that endogenous surgery may be as effective as venous stripping; therefore endogenous surgery could be more suitable for VLU patients as it is less invasive than conventional surgery. The other positive criterion of endogenous surgery is that it does not require general anaesthesia, which suits VLU patients more as the majority are elderly subjects with many comorbidities.

1.8.12 Skin grafting

Different types of skin grafts have been used for the treatment of VLU, including full-thickness grafts (autografts and xenografts); split-thickness grafts (autografts) and artificial skin (allografts). Skin grafting looks to be a suitable option for large and poorly responding ulcers since it covers the entire ulcerated area and there is the additional benefit of potential healing in an ideal environment. However, in the presence of an edema, common with VLU and the presence of other comorbidities, such as diabetes mellitus or cellulitis, skin grafting may not be effective (Seraj & Collins, 2010). A Cochrane review of 11 trials, in which various grafts were compared with standard VLU treatments including compression therapy. Authors found few high-quality studies to support the use of human skin grafting for the

treatment of a venous ulcer, they concluded that the evidence was not enough to support the effectiveness of skin grafting (Richmond & Maderal, 2013).

1.8.13 Drug treatment

Different medications have been used to heal venous VLU, however only a small number have showed evidence of efficacy (Coleridge-Smith, 2009).

Pentoxifylline, a methylxanthine used in the management of intermittent claudication, have been tried with VLU, Pentoxifylline is an inhibitor of platelet aggregation, and therefore it reduces blood viscosity and helps improve blood flow.

In a review of 11 studies, by Cochrane, the Pentoxifylline role in management of venous ulceration was examined. The study concluded that Pentoxifylline is effective in promoting VLU healing when used in combination with compression therapy (Jull, Waters, & Arroll, 2002). Pentoxifylline, may be used as monotherapy in venous ulcer patients who are unable to use compression therapy. Although, there is a number of studies that may support Pentoxifylline effectiveness in combination with compression therapy and possibly as monotherapy, the cost-effectiveness of pentoxifylline has not been identified (Collins & Seraj, 2010).

1.8.14 Antibiotics and antiseptics

Moore and his colleagues recommended antibiotics and antiseptics for treatment of VLU patients who are exhibiting signs or symptoms of infection, therefore all venous ulcer patients should be swabbed for culture and sensitivity and treated accordingly with the proper antibiotics when there is infection. Prophylactic use of antibiotics however is discouraged as

it has yielded little supportive evidence, with the exception of cadexomer iodine. Cadexomer iodine is a topical agent with antimicrobial properties (Moore, et al., 2010).

Three trials, compared cadexomer iodine to standard care where there was improved healing within 4–6 weeks, there was statistical significance in only one of the studies, in which patients were hospitalized for daily dressing of the affected area (Richmond & Maderal, 2013). However, not enough attention was paid to the possible hospitalisation drawbacks such as cost effectiveness.

1.8.15 Wound and skin care

Chronic venous insufficiency, associated with oedema and lipodermatosclerosis may lead to skin damage, therefore it is necessary to maintain good skin health and prevent infection. A moist wound environment will facilitate in the development of healthy granulation tissue and epithelialization, furthermore moisturizing skin with topical agents will help to reduce skin breakdown. Sometimes topical steroid agents may also be a necessary form of treatment. (Richmond & Maderal, 2013).

Bacterial overgrowth may complicate venous ulcers; thus, aggressive wound care is important to minimize complications. A variety of dressings and hydrocolloids can be used to control wound fluid drainage, where the most commonly used is a saline-moistened dressing. Foam dressing have the ability to absorb exudative wounds and excess fluid from an ulcer, meta-analysis examined six studies that showed no clear evidence supporting the view that foam dressings are superiority to other types of dressings (Dumville, Desphande, O'Meara, & Speak, 2013). Silver impregnated dressings have been used to control infection and restore tissue integrity for infected ulcers, but the benefit is controversial (Jones, Bowler, Walker, &

Parsons, 2004). In a Cochrane study, authors concluded that “there is some evidence to suggest that ibuprofen dressings may offer pain relief to people with painful venous leg ulcers”. The use of local anaesthesia EMLA (5%) appears to be useful in relieving pain during the debridement of venous leg ulcers. Briggs M et al, suggested that further research may be necessary in order to consider standardised pain treatment (Briggs, Nelson, & Martyn-St James, 2012). Research about venous ulcer wound dressings is still limited; although there are a variety of dressings, the use of the most suitable dressing should depend upon the wound characteristics. Generally, sufficient evidence of the influence of dressings and topical agents when managing venous leg ulcers has not been shown in relation to VLU healing (Palfreyman, Nelson, Lochiel, & Michaels, 2006).

1.8.16 Nutrition

Nutritional deficit is a common presentation in venous ulcer patients as they are generally elderly and affected by several comorbidities. Nutritional requirements should be supplemented to those patients according to their needs. Glucose levels should be kept under control in diabetic patients as carbohydrates are the main source of energy in the body, ulcers need energy for the healing process particularly for collagen synthesis (Arnold & Barbu, 2006). The minimum daily protein requirement of 0.9 g/kg/day is necessary for collagen synthesis and epidermal proliferation. Cell membrane synthesis requires fats and fatty acids; phospholipids are important for the epidermal barrier layer synthesis; synthesis of intercellular matrix and inflammatory reaction (Lansdown, 2004) . Vitamin C is vital for collagen synthesis and the integrity of the capillary wall; iron supplements are recommended when iron deficiency anaemia is present, as iron is needed for the transportation of oxygen

and synthesis of collagen; Zinc is also important in collagen formation and protein synthesis (Rojas & Phillips, 1999).

In a prospective study that reviewed 296 VLU patients over 5 years, researchers found that VLU patients suffered with nutrient deficiency such as vitamins C, E, and B complex, protein, and minerals. They also reported that supplementation of dietary requirements was necessary in the ulcer healing process. Investigators concluded that careful monitoring and control of blood glucose, to be <120 mg/dL, was vital for venous ulcer healing especially in diabetic patients (Cheryl, Michael, Hughes, & Robert, 2006). Nutrition deficiency is a common problem in VLU patients and affects wound healing significantly, therefore careful monitoring of nutritional requirements is essential and supplementation of a deficit is mandatory.

1.8.17 Oxygen therapy

The presence of oxygen is vital for wound healing, as it is the main precursor for energy metabolism in the cell. Oxygen is involved in all phases of the wound healing process including oxidative eradication of bacteria, angiogenesis, reepithelialisation process, extracellular matrix formation, cell motility, and collagen synthesis and in the remodelling of the wound. Recent studies have been addressing the role of O₂ in wound healing via reactive oxygen species (ROS) production. ROS are thought to serve as cellular messengers in the wound healing process (Gordillo & Sen, 2003). Appreciating the importance of oxygen has led researchers to explore the therapeutic modality to aid wound healing. However, whilst there is some supportive evidence to this idea there are contradictory views against the use of hyperbaric and topical oxygen therapy to improve wound healing (Fries, et al., 2005).

Hyperbaric oxygen therapy (HBOT):

Many studies have suggested that increasing oxygen tension at the wound site may promote and speed up wound healing. Therefore, the use of oxygen therapy has been used to aid wound healing for both acute and chronic wounds. Hyperbaric oxygen therapy is one of the methods used to deliver oxygen where it's mainly used in the treatment of chronic wounds. HBOT is the administration of 100% oxygen at a pressure of greater than 1 ATM (Broussard, 2004).

Studies supporting HBOT use have been criticized; these studies have small sample sizes, poor treatment protocols and poor evaluation of the patients' comorbidities (Fries , et al., 2005). HBOT therapy disadvantages include middle ear barotrauma; hypoglycaemia and the most serious reported adverse effect is generalized seizures (Hampson & Atik, 2003). Considering the serious complications of HBOT and lack of large detailed clinical trials, it is difficult to determine whether the benefits outweigh the risks.

Topical Oxygen Therapy:

Some researchers have suggested topical oxygen therapy as an alternative to HBOT. Studies have considered topical oxygen therapy as it offers the convenience of being less toxic and it can be used at home.

The mechanisms of topical oxygen therapy are less understood apart from its effects on wound infection. It is still not clear whether supplemental oxygen affects wound healing through its local or systemic effect. Studies of topical oxygen, have been criticized due to the lack of appropriate sample sizes, not involving different types of wounds and for not recognising the aging population. Topical oxygen therapy is contraindicated for smokers due

to safety reasons. The most serious concern is that there are few studies on this treatment, and none of these studies have been case-controlled in humans (Rodriguez, Felix, Woodley, & Shim, 2008).

We can conclude that there is wide agreement that oxygen has a key role in the wound healing process and will accelerate healing; however, hyperbaric oxygen and topical oxygen therapy are associated with complications that make both modalities a non-popular choice. Moreover, there is a lack of large randomized controlled trials. Oxygen is mandatory for wound healing; there might be an urgent need for a safer method of delivering oxygen to the wound that excludes the pitfalls of both hyperbaric oxygen and topical oxygen therapy.

1.8.18 Exercise therapy

Several studies have reported the role of exercise in venous ulcer healing. The majority of these studies focused on the effect of exercise on improving the calf muscle function, which is relevant to the majority of VLU subjects who are elderly with limitations of movement that may cause calf muscle dysfunction (Heinen, et al., 2012) (Nicholas, Andrea, & Alejandra, 2013) (O'brien, Finlayson, Kerr, & Edwards, 2014). Venous leg ulcer patients are less mobile and active in comparison with similar aged-matched people. As a result, the calf muscle function is often impaired which in turn leads to delayed healing (Richmond & Maderal, 2013).

Calf muscle pump dysfunction is considered to be the main predisposing factor in developing VLU. Failure of the calf muscle pump, in some cases, is related to the lack, or restriction of mobility, at the ankle joint, especially in elderly patients. Muscle dystrophy or disuse due to physical injury such as direct trauma or fracture may also lead to calf muscle dysfunction.

Moreover some other illnesses such neuromuscular illnesses, rheumatoid arthritis and osteoarthritis may play a role in the development of the calf muscle pump failure.

Patients involved with venous insufficiency may develop calf muscle failure. This is due to the obstruction of calf veins, with venous thromboembolism the obstruction caused will increase the workload on the calf muscle. In addition, the walking pattern in long standing VLU patients, changes from light standing on the toes to a difficult slow “duck-like” pattern standing on heels, this change results in deformation of the knee and ankle joints (Tierney, Burke, & Fitzgerald, 1993).

Improvement of calf muscle dysfunction depends on the cause of failure, however the key principles in treating muscle dysfunction is exercise and physiotherapy, regular gradual continuous exercise could play a role in improvement of the ankle joint movement and strengthen the calf muscle function. Compression therapy may improve the calf muscle pump by decreasing the edema and venous pressure within the calf veins (Struckmann, Christensen, & Lendorf, 1986).

A prospective trial recruited 31 patients (CEAP 4, 5, 6) to rehabilitate the muscle pump action through an exercise program (Padberg, Johnston, & Sisto, 2004). The outcomes were measured after 6 months after completing exercise to strengthen the legs, researchers found that the calf muscle indicators (Mean ejection fraction and Mean residual volume fraction) returned to a normal range within the therapy group and there was no change in reflux amount or severity scores. Investigators concluded that exercise could re-establish calf muscle pump function in CVI patients and may prove beneficial as a supplemental therapy. Although the sample size is small and there were not enough details of the exercise

programme, the study is encouraging and provides evidence for the effect of exercise on calf muscle function.

Another prospective study prescribed walking for 40 newly diagnosed VLU patients, participants were provided with compression treatment and monitored over a 12 week period or until their ulcer had fully healed (Meagher, Ryan, Clarke-Moloney, O'laighin, & Crace, 2012). Subjects were either allocated to control or exercise groups, Sixty-seven per cent of the participants (exercised group) were healed by week 8, compared with 35% of those who did not do any exercise. All 40 patients were newly diagnosed, in addition the sample size may have been small and therefore the study may not reflect the real picture of VLU patients. The percentage rate for the healing of venous ulcer could offer important evidence and may support the idea that exercise may be helpful in VLU management.

In conclusion there is enough evidence to suggest that exercise does improve wound healing, however there is no agreement about how exercise improve wound healing. The most acceptable explanation for this phenomenon is that it is a multifactorial process. There may be other factors that play a role in the process of wound healing during exercise, one of them could be the increase in tissue oxygenation on a microvascular level due to the improvement of skin circulation.

1.9 Summary

VLU is a complicated clinical problem. VLU involves predominantly elderly patients suffering from several comorbidities and limitation of movement, they lack family support, and finally the management of VLU has not changed that dramatically in years. As a result, venous ulcer continue to put huge burden on individuals and states.

The classic approach to treat VLU is through compression therapy, this modality of treatment has not changed for thousands of years. Unfortunately, compression therapy is associated with a high recurrence rate, where it may be up to 80%, in addition many patients are not willing to use it, either because it is uncomfortable or difficult to wear, especially among the elderly. The next most popular approach is conventional surgery (stripping of saphenous veins), which is mostly used as an adjuvant therapy to compression therapy. We know that only one third of venous insufficiency patients present with superficial venous incompetence, moreover, most of VLU patients may not be fit for surgery mainly because of comorbidities. Researchers have tried different types of modalities to treat VLU, however the majority lack the acceptance, either because they are not effective or because there is no available well organised clinical trials available.

Generally, people believe that the aim of VLU treatment is to maximize perfusion; therefore sufficient oxygen must be available for the healing process. The available strategies to enhance ulcer healing include compression therapy, surgery and other alternatives. However, recurrence is very common and healing is impaired in many patients, some researchers have mentioned a multidisciplinary approach to the treatment of leg venous ulcer. There might be a need for feasible, safe, effective, easily available and suitable treatment for the venous ulcer population. Exercise could be the most suitable alternative therapy for VLU patients as an adjuvant or preventive therapy, exercise has the ability to be both a preventive and effective treatment therapy. Moreover, exercise is easy, can be adjusted according to patient requirements, can be performed at home or within groups, is cost effective, avoids the side effects of medications and offers positive health and social benefits. Exercise has shown a

positive role in the treatment of peripheral vascular disease, in addition there are a few encouraging studies about the effect of exercise on wound healing. For all these reasons, the present research study is investigating the influence of exercise on VLU healing.

2 CHAPTER TWO: INTEGUMENTARY SYSTEM (SKIN AND APPENDAGES)

2.1 Introduction

The skin is the largest organ in the body that covers about 2 m². The skin consists of two components: the epidermis or outer layer and the dermis or inner layer. The epidermis is composed of five layers. The five layers, listed from the surface to the deepest layer are: the stratum corneum, the stratum lucidum, the stratum granulosum, stratum spinosum, and stratum basale (*Figure 2.1*).

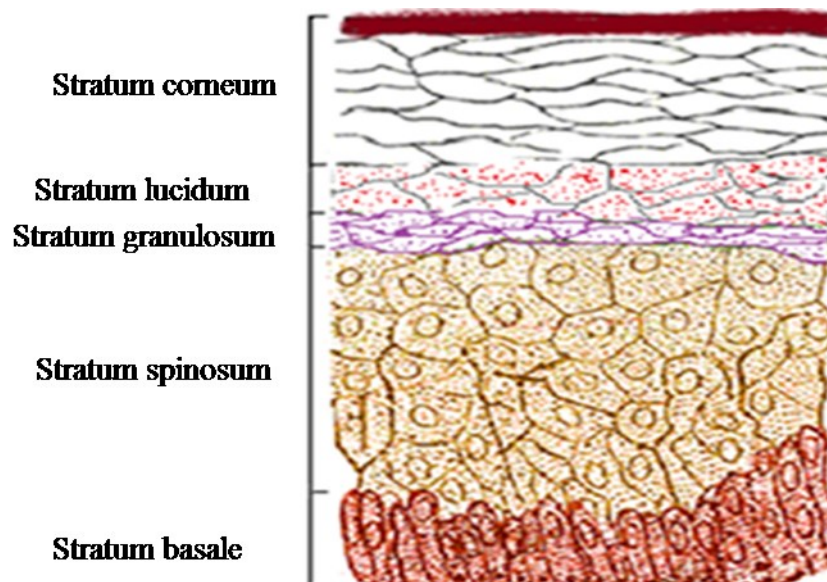


Figure 2.1: Epidermal layers

The stratum basale, spinosum and granulosum layers are metabolically active and are sometimes called the germinative zone or stratum Malphigii. The stratum lucidum, which presents normally in the thick skin of the soles and the palms and the stratum defined as cornified zone.

The epidermis is a stratified epithelium composed primarily of stratified squamous epithelium, which is a multi-layered tissue continuously undergoing the processes of cell

formation, maturation and loss. Basal layers cells are generally cuboid or columnar in character, when these cells move upwards into the epidermis they go through transformation; the structure is altered and their shape becomes flattened. Ultimately, they will be shed at the surface of the skin. The dermis is formed by dense connective tissue that contains blood vessels, lymphatic vessels and nerves.

There is great deal of variation in thickness of skin in different parts of the body, it tends to be thinner on the anterior than posterior part of the body and the skin in women is thinner than that found in men. The dermis is connected to the epidermis and to the underlying deep fascia or bones, between dermis and underlying tissue is the subcutaneous or superficial fascia (**Figure 2.2**) (Williams, Warwick, Dyson, & Bannister, 1989).

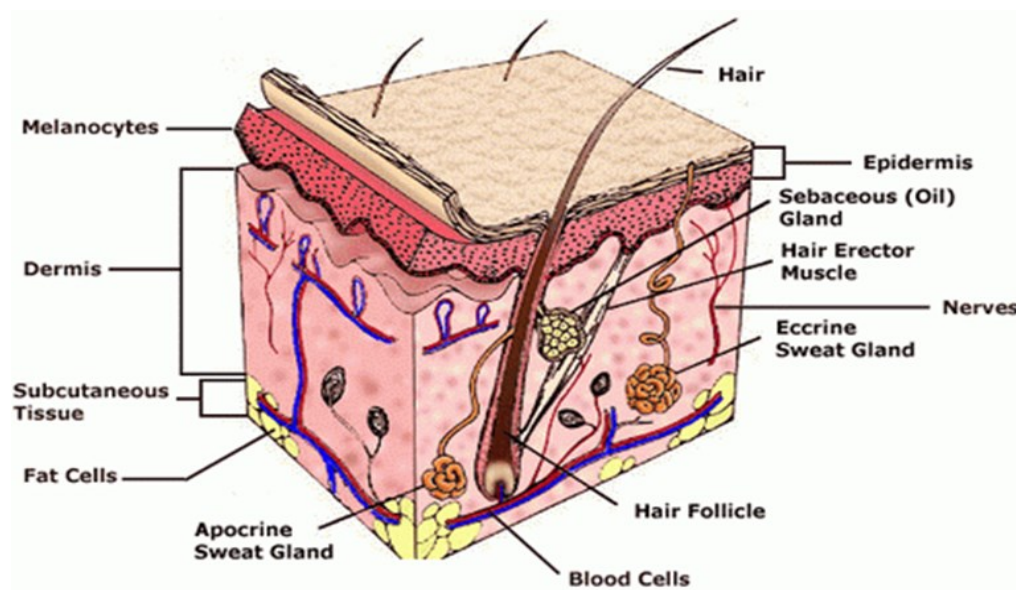


Figure 2.2: *Anatomy of the skin*

(Modified from www.google.com)

The integument function depends mainly on an extensive network of blood vessels and sensory receptors embedded in the dermis. The general function of skin and its appendages includes:

1. Protection of all underlying tissue and other organs against trauma, chemical attack, fluid loss and regulating of heat or cold
2. Maintenance of normal body temperature either by insulation or evaporation
3. Excretion of water, electrolytes and glands waste
4. Vitamin D synthesis, which is an important steroid in calcium metabolism
5. Storage of lipids in the epidermis and subcutaneous layer
6. Detection of sensation including, pain, touch and temperature

The Epidermis

The epidermis is the topmost living layer of the skin, which consists of four layers of keratinocytes. These four layers go through gradual differentiation as they grow from the basal layer up to the top layer, the stratum corneum. The turnover time of epidermal cells is about one month. There are other cells which are embedded in the epidermis including melanocytes, langerhans cells, and merkel cells.

The Keratinocytes

The keratinocyte is the most important cell of the four major types of cells that form the epidermis; the keratinocytes is formed by mitosis of the stem cells in the stratum basale. Keratinocytes move upwards gradually to the surface of the skin. As they move from the stratum basale, the cell surfaces become folded and tightly connected with adjacent cells. They appear spiny and are often called "prickle cells" in the stratum spinosum. They begin to

flatten when they move upward from stratum spinosum. In stratum the cells start to produce and secrete a glycolipid which coats the cell surface resulting in the creation of a thick water resistant cement that is imbedded between the cells. Eventually, they lose their nuclei and become dead cells to form the stratum corneum.

The Melanocytes

The melanocyte cells are pigmented cells, located at the base of the skin and store melanin. Melanin is the brown pigment that gives the skin its colour. Melanin is manufactured from the amino acid tyrosine and is reserved in the intracellular vesicles called melanosomes. Larger melanosomes are found in darker-skinned people explaining why the skin colour is darker. Melanin offers protection to the epidermis and dermis from the damaging effect of sunlight which contains damaging ultraviolet rays. Melanocytes are present mainly in the epidermis, but are also abundant in connective tissues of the dermis and eye but to a far lesser extent. Their numbers differ according to the region, hormonal and genetic factors.

The main hormones stimulate the melanocyte are oestrogens, progesterone and hormones from the adenohypophysis as well. Ultraviolet exposure is the main environmental factor that affects the location and number of melanocytes. In general the ultraviolet effect is photo-oxidative in nature and reverses if the exposure is stopped. Long-term exposure of the skin to ultraviolet light can damage the fibroblasts, resulting in impaired maintenance of the dermis which could lead to premature wrinkling. More harmful damage from cumulative exposure to ultraviolet light could develop from chromosomal damage to melanocytes; this damage can result in skin cancer. Skin cancer rates have increased dramatically in recent years because of continuous depletion of the ozone layer in the earth's upper atmosphere. An increase in skin

cancer rate has been reported in America, Canada, Europe and Australia where there has been a significantly greater loss of ozone layer coverage (Maartini, 2006).

Nonpigmented Dendrocytes

The Langerhan's and Granstein cells are derived from the bone marrow and are constantly being renewed; they also play a role in the protection of the body through the immune system. Langerhan's cells interact with suppressor T cells whilst the Granstein cells help T cells by acting as markers of antigens. They have filaments that spread between the surrounding cells. These cells are sensitive to ultraviolet light in addition to their immune response.

The Dermis

The dermis is the deeper layer of the skin lying underneath the epidermis, the dermis consists mainly of dense soft connective tissue that contain blood vessels, collagenous and elastic fibres, hair follicles, nerves, sensory receptors and glands.

The dermis layer provides considerable mechanical strength and elasticity to the skin as it contains bundles of collagen fibres that are mostly arranged in a parallel line. It is important that surgical incisions are made along these parallel fibres in order to cause minimal damage to the skin.

The dermis is composed of two major components: the papillary and the reticular layers. The papillary layer consists of areolar tissue, containing lymphatics, capillaries and sensory fibres that innervate the skin surface. This layer derived its name from the dermal papillae which projects between the epidermal ridges. The reticular layer, located deep to the papillary layer

is composed of a tight dense meshwork of connective tissue that contains collagen and elastic fibres. These collagen fibres expand superficially behind the reticular layer to interconnect with those of the papillary layer, meaning there is no clear boundary between the two layers. Collagen bundles of the reticular layer extend distally into the subcutaneous layer. The dermis contains all connective tissue cells such as sweat glands, hair follicles and sebaceous glands, which are scattered throughout the dermis (**Figure 2.3**).

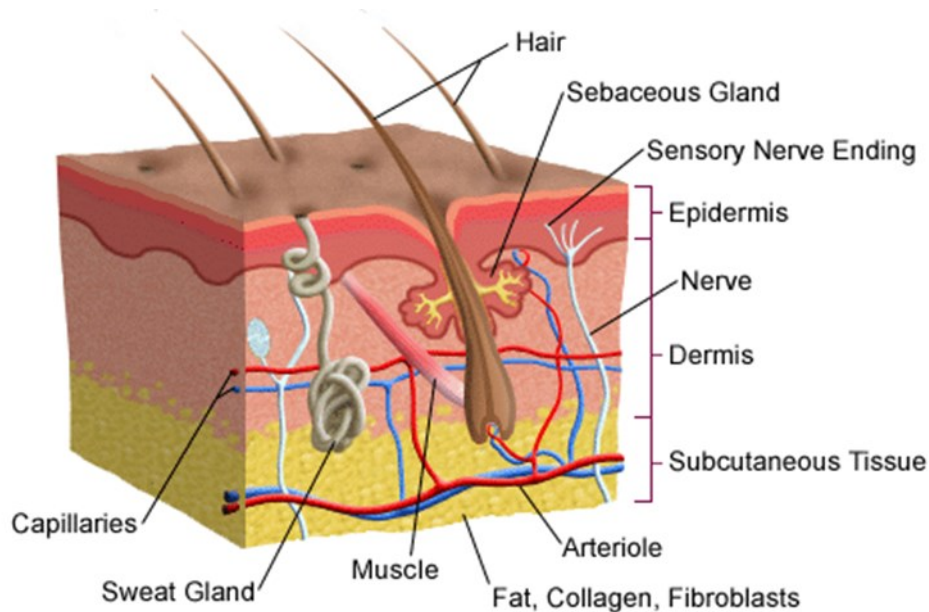


Figure 2.3: Dermis of the skin

(Modified from www.stanfordchildrens.org)

2.1.1 Dermal elasticity and Strength

The skin contains two types of fibres, collagen fibres that are very strong and have the ability to resist stretching. Although they are able to be twisted and bent the elastic fibres which can be stretched will return to their original length and form. The beautiful function of these two fibres is that elastic fibres permit flexibility whilst the collagen fibres restrict that flexibility in order to prevent damage to the skin. There is another factor which plays an important role in skin flexibility and resilience; these are known as skin turgor, which may be lost during dehydration. Dehydrated skin remains peaked when pinched while hydrated skin will flatten out when touched. Sometimes the damage to the abdominal skin during pregnancy or after weight gain may exceed the elastic capability of the dermis.

2.1.2 Innervation of the Skin

The skin is filled with extensive networks of nerves and sensory receptors, so any touch or contact with the epidermis will initiate nerve impulses. Skin nerve fibres control the function of blood vessels, glands secretion, but also modify the sensory receptors which are located in superficial and deeper layers of the epidermis. The sensory neurons that provide the sensation of pain and temperature are extended through the epidermis; there are specialised receptors which are sensitive to deep pressure and vibration only.

The innervation of epidermis is by somatic axons coming from nerve bundles that run in the subpapillary dermis. Intraepidermal nerve fibers (IENFs) lose their schwann cells sheath as they pass through the dermal–epidermal junction and go through the skin surface, sometimes with the extension of horizontal branches.

Function of Skin Nerves:

Generally, skin nerves were considered to be transducers of certain types of sensory stimuli (e.g. pain, temperature and touch) and also to play an important role in maintaining tissue structure.

Researchers have demonstrated a very complex network involving skin nerves and epidermal cells. Studies have suggested that keratinocytes and other epidermal cells are able to communicate with neighbouring nerve fibres and could well participate effectively in the transduction of specific physical and chemical stimuli. Studies in vitro have shown that the activation of sensory neurons by mechanical stimulation of the keratinocytes can be achieved through a mechanism involving ATP and other receptors. Furthermore, researchers found that keratinocytes secrete chemical substances (e.g. β -endorphin, neurotrophins, ATP, and interleukins) that can affect sensory neurons (Lumpkin & Caterina, 2007). Skin nerves produce certain neuropeptides which exert various effects on skin cells and blood vessels, resulting in hyperthermia, erythema, itch and oedema (Gibbins, Wattchow, & Coventry, 1987).

2.2 Skin blood supply

Skin covers all of the body's surface area, underneath the epidermis there is an extensive network of blood vessels, these blood vessels reflect changes occurring through the vasculature of the body. As the skin is easy to observe, changes in skin colour can be very useful in diagnosis of illnesses affecting other organs, such as jaundice, melanocyte stimulating hormone secreting tumours, vitiligo and Addison's disease.

Cutaneous blood vessels arise from underlying source vessels (**Figure 2.4**). Each source blood vessel is divided into 3-dimensional vascular territories from the bone to the skin called

the angiosome. These cutaneous vessels emerge either directly from the source arteries (septocutaneous or fasciocutaneous perforators) or as terminal branches that come off muscular vessels (musculocutaneous perforators). While these blood vessels extend to the skin, the cutaneous vessels pass through the connective tissue framework and give branches to adjacent appendages such as muscles, fat, bones, fascia and nerves.

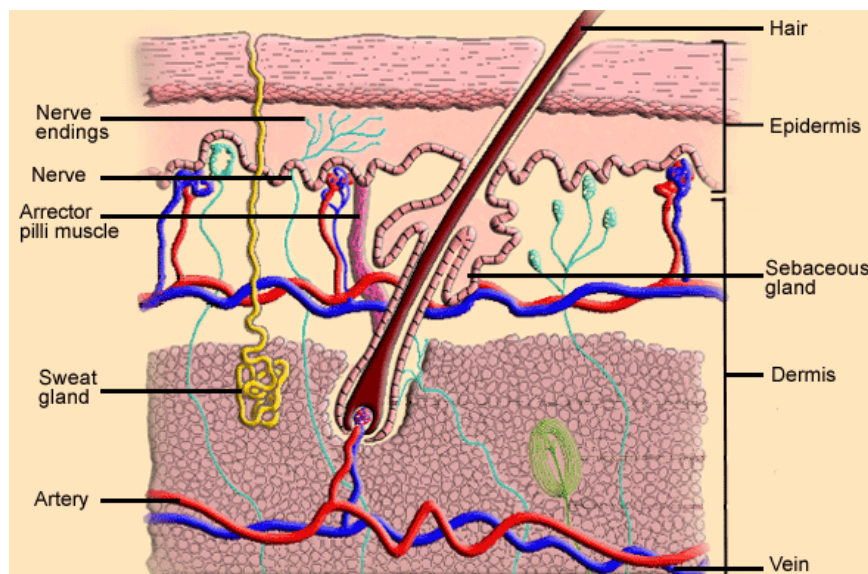


Figure 2.4: *Cutaneous blood vessels*

(Modified from www.google.com)

The Cutaneous blood vessels commence from the deep fascia near the intermuscular or tendons and travel to the skin. In the skin, they form extensive subdermal and dermal plexuses.

In the dermis, blood vessels are arranged horizontally in the form of superficial and deep plexuses, these blood vessels are interconnected by other communicating vessels organized in a perpendicular direction to the skin surface. Cutaneous vessels eventually connect to other

cutaneous vessels to form an extensive cutaneous vascular network. Clinically, the horizontal network of blood vessels can help with skin flap survival (Taylor & Pan, 1998).

Arteriovenous anastomoses are commonly seen in the deeper layers of the dermis especially in areas subject to change in temperature such as the ears, hands and feet. Glomera, a number of arteriovenous anastomoses, exist in large numbers in the dermis. Each glomus is composed of afferent arteries arising from branches of cutaneous arteries that extend to the skin surface. These vessels relax under the effect of autonomic vasomotor control, during relaxation; blood is diverted away from the superficial plexus resulting in a reduction of heat loss (Mc Gregor & Morgan, 1963).

2.3 Lymphatics

Lymphatic vessels in the skin run parallel to blood vessels. Skin is confronted by different types of pathogenic organisms; therefore it is supplied with an extensive lymphatic network; however bacteria could invade these lymphatic vessels causing lymphangitis (*Figure 2.5*). Lymphatic capillaries emerge within the interstitial spaces of the dermis; these capillaries are unvalved and embedded in the dermis and drain into valved deep dermal and subdermal lymphatic plexuses. These plexuses unite to form larger lymphatic channels, which run through filtering lymph nodes before joining the venous circulation near the subclavian vein on the left side of the body and to the internal jugular vein on the right side.

Recognising and responding to foreign cells, cancer cells and microorganisms is the main function of the lymph in the body. These targeted cells by lymph are either destroyed by direct action of T lymphocytes or by indirect action of B lymphocytes that differentiate into plasma cells and produce antibodies



Figure 2.5: *Inflammation of subcutaneous lymphatic vessels*
(Modified from www.google.com)

2.4 Wound

Skin is the first line of defence to the body; therefore it is very common that this barrier get wounded. There are different definitions for the wound, all essentially deal with one idea which is associated with a break through the skin or a cause of damage to the skin in one way or another. The definition of an ulcer is not that different from that of a wound, both share the same characteristics, ultimately an ulcer creates a loss of the continuity of the skin. Therefore an understanding of the wound, in its pathophysiology sense, enables one to recognise the factors that affect the healing of the wounds and understanding venous leg ulcer.

There are different ways of classifying wounds that are dependent upon the various factors involved. Acute or chronic wounds that depend on the length of healing time, the nature of

the wound, whether it was accidental, where it has been caused without reason, or intentional where a wound has been caused on purpose such as through a surgical procedure. Another classification is that of an open wound or a closed wound, open wounds can be caused for example by a bullet injury where there is communication between the inside of the body and the external environment, whereas with a closed wound there is no such communication an example of which would be that of a closed fracture. Depending on the depth of tissue damage, we can classify the wounds to partial thickness wounds; that involve damage to the two superficial layers only (epidermis and dermis) or as full thickness wounds which are deeper and involve damage to the subcutaneous layers. Wounds also divided according to degree of contamination with pathologic organisms, clean wound, contaminated wounds and dirty wounds. Depending on the origin of the cause, wounds can be external due to external factor like a stab wound or internal due to internal triggers such as immune deficiency, infection, or deep venous thrombosis (Percival, 2002).

The skin is the largest organ in the body and the first protective barrier of the body, any injury or damage to the skin induce disturbance of the protective mechanism which makes the body prone to infection and susceptible to the depression of immunity. Understanding the factors related to the wound process helps to evaluate and manage the wound as these factor determine the wound healing process.

2.5 Wound healing

There are two types of wound healing dependent upon the opposition of the wound edges, either primary intention healing, where the two edges comes close together usually facilitated by a mechanical aid such as a suture. Secondary intention healing, where the wound is healed

by epithelisation, where it usually takes time for the skin to heal completely, a venous ulcer could be seen as a good example of secondary intention healing.

Healing of the wound is a natural defence mechanism that occurs through a series of cellular events that come into action once the body is injured, including resurfacing, reconstitution, and restoration. The processes involved in healing are a complex phenomenon and the phases of wound healing usually overlap one another. Generally the process of wound healing is expressed in terms of four phases with each overlapping the other; they are haemostasis, inflammation, proliferation, and maturation (**Figure 2.6**) (Gosain & DiPietro, 2004).

Healing of chronic wounds is different from the healing of acute wounds and might take longer. Although there is no clear difference between acute and chronic wounds, healing of the acute wound progresses through the four normal stages of wound healing, without overlapping between each stage. The healing process in acute wound will show clear signs of healing within one month, while chronic wounds do not progress smoothly through the healing phases and quite often halt in one of the stages. Moreover, the chronic wound healing process does not settle within four weeks and often takes a longer period of time.

Hemostasis is the first phase where we see the clot formation, this is followed by the inflammatory phase which is controlled by the inflammatory cells, after this angiogenesis and epithelialization occur during proliferative phase, and eventually the collagen network begins to extend through the wound leading to it beginning to contract.

Within these four phases, there is a complex set of events which include chemotaxis, phagocytosis, formation of new collagen, collagen degradation and collagen remodelling.

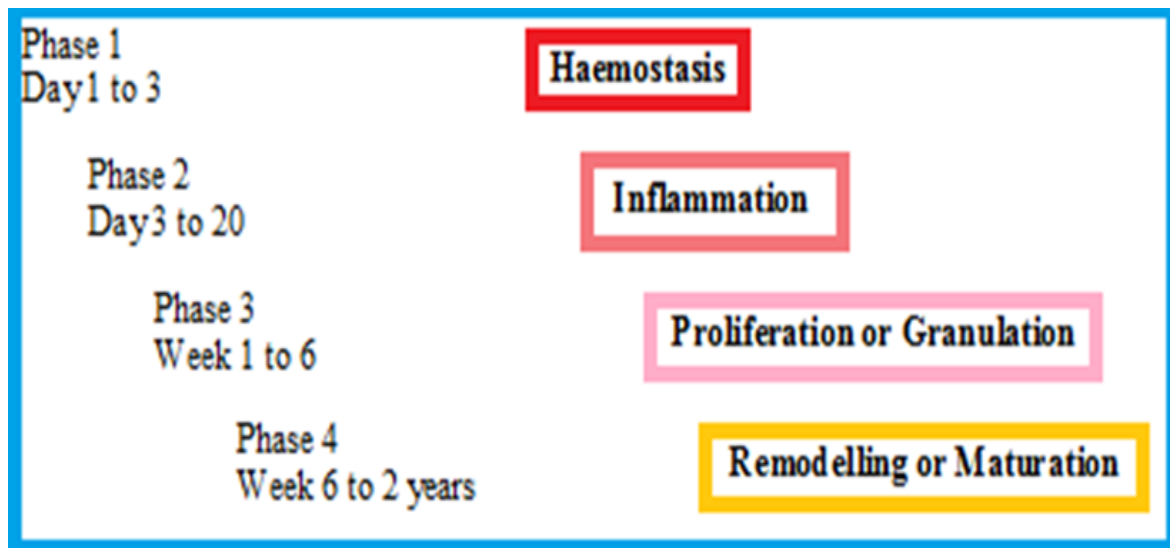


Figure 2.6: Wound healing phases

Furthermore, there are other events that combine with the inflammatory phases such as angiogenesis, epithelization, and the production of new glycosaminoglycans, these materials are crucial for the wound healing process (Mathieu, 2006).

2.5.1 Haemostasis

As soon as the wound occurs, platelets flow into the wound, and these platelets play an important role in the formation of the hemostatic plug by aggregating with the other components of blood components. In the interim, there is a release of chemotactic factors such as cytokines, chemokines, and hormones into the wound. Next there is a release of vasoactive mediators (epinephrine, prostaglandins, norepinephrine, thromboxane and serotonin) that cause the temporary blanching of the wound. These vasoactive substances induce vasoconstriction of blood vessels which decreases the blood loss. Damaged subendothelium including collagen and certain tissue factors also play a key role in the activation of platelet aggregation that forms the primary plug (clot formation). The activation

of platelets induces the release of chemotactic factors such as platelet derived growth factor (PDGF), serotonin, histamine and proteases. Mediators released by platelet activation invite inflammatory cells to the wound, leading to the next step in the wound healing process.

2.5.2 The inflammatory phase

The inflammatory phase happens within hours of injury. With the increase of permeability initiated by platelet activation the cells and factors that are important for healing diffuse from the intravascular to extravascular space. In the meantime the clot formation disappears as its stimuli is depleted and plasminogen is converted to plasmin which is an enzyme that assists in lysis of cell.

The vasoconstriction period occurs at the beginning of wound injury and lasts for only 10-15 minutes; vasodilation is followed by vasoconstriction a process that lasts much longer. Vasodilation is mediated by interleukins including histamine, kinins, leukotrienes and prostaglandins. The vasodilatation period is important in the process of healing as it increases the blood flow into the wound, flooding the wound with inflammatory cells and the necessary factors which are capable of fighting infection and debride the devitalized tissue.

The cellular aspect of inflammatory phase includes the migration of neutrophils, macrophages and lymphocytes to the wound. Neutrophils have two functions, firstly to clear the wound of bacteria and necrotic tissue and secondly to release inflammatory mediators accompanied by oxygen free radicals. The neutrophils are the dominant cells for the first 48 hours although their absence does not prevent healing.

Macrophages are the most important cells in the inflammatory phase. Macrophages also have other functions such as secreting enzymes (collagenases and elastases), releasing PDGF and

cytokine that stimulate the chemotaxis and induces proliferation of smooth muscle cells and fibroblasts. In addition, macrophages secrete certain factors that initiate endothelial cells migration and angiogenesis formation.

Migration of T lymphocytes into the wound starts 72 hours following injury. The release of interleukin 1 attracts T lymphocytes to the wound. The major role of Lymphocytes is antibody production and cellular immunity, additionally lymphocytes secrete lymphokines such as basic fibroblast growth factors and heparin-binding epidermal growth factors.

2.5.3 Proliferative Phase:

The main event in the proliferative phase is the formation of granulation tissue which occurs 3-5 days following injury. Granulation tissue includes fibroblasts, inflammatory cells, and neovascular matrix which is composed of proteoglycans, fibronectin, glycosaminoglycans and collagen.

2.5.4 Epithelialization

The epithelialization is the formation of epithelium over a wound surface. An epithelial layer in an incisional wound for instance, is developed within 24-48 hours. Structural changes that have occurred at the wound edges allow epidermal cells to separate from other epidermal cells and their basement membrane. Adnexal structures contribute in repithelialization particularly in superficial wounds such as burns.

Researchers found that wounds in a moist environment have a faster course of epithelialization therefore applying occlusive and semioclusive dressings during the first two

days after injury may optimize tissue humidity and epithelium formation (Mustoe & Gurjala, 2011).

Fibroblast cells begin to deposit in the wound 3-5 days after injury however it may last for 14 days. The growing of fibroblasts in the wound starts when the number of inflammatory cells declines. Skin fibroblasts migrate and differentiate in response to a group of factors that include, the platelet-derived growth factor (PDGF), fibronectin, the fibroblast growth factor, the transforming growth factor and C5a.

Deposition and synthesis of collagen is a crucial event in the wound healing process. Amino acids like hydroxylysine and hydroxyproline strengthen the collagen framework and enable it to form tight cross-links. Proline and lysine hydroxylation residues based on the availability of oxygen, ferrous iron, α -ketoglutarate and vitamin C. The deficiency of oxygen and vitamin C, result in underhydroxylated collagen therefore, is more liable to breakdown. The rate of collagen synthesis is affected by age, tension, pressure, and stress affects. Collagen synthesis starts 3 days after injury but may last for 2-4 weeks. Type I collagen constitute about 80% of the collagen in normal skin, the remaining is primarily type III collagen.

2.5.5 Angiogenesis

Good blood supply is vital for newly formed tissue. Angiogenesis depends mainly on macrophage which is crucial for the initiation of and production of macrophage-derived angiogenic factor. Two factors are important to angiogenesis, the fibroblast growth factor, which is secreted by macrophage and the vascular endothelial growth factor which is produced by the endothelial cell. As the need for new blood vessels decreases angiogenesis

ceases, in addition newly formed blood vessels which become unnecessary dissolve by apoptosis.

2.5.6 Contraction

Contraction of the wound begins almost simultaneously with collagen synthesis mostly 5-15 days after injury. Contraction, is defined as the movement of wound edges closer together to promote the closure of the wound. The rate of contraction is dependent on tissue laxity and wound shape. Loose tissues contract faster and more efficiently than tissue of poor laxity and square wounds contract more efficiently than circular wounds. Wound contraction depends mainly on the myofibroblast and its connection within the extracellular matrix and myofibroblast proliferation. Contraction of the wound may be affected by radiation and drugs causing delay of the wound healing. Contracture of the wound is different from contraction, contracture is a pathologic process characterized by excessive contraction that restricts the underlying tissues motion and is mostly caused by applying exaggerated stress to the wound.

2.5.7 Factors affecting wound healing

Wound healing is a complicated process that involves the participation of multiple organs and it is composed of four phases (Gosain & DiPietro, 2004). There are many factors affecting wound healing through interference with one or more of these four phases, some of them are local factors and some are general factors. These factors include oxygenation, age, sex, infection, hormones, stress, diabetes, medications, smoking, alcoholism, nutrition and obesity (Guo & Dipietro, 2010).

2.6 Skin changes in venous leg ulcer

Ulcer is a local defect in a surface of an organ or a tissue associated with inflammation and can be acute or chronic. In an acute ulcer, the predominant picture is intense polymorphnuclear infiltration and dilation of vasculature in the margins of the ulcer; with features of chronicity. Fibroblastic proliferation is the main picture of chronic ulcer that leads to fibrosis and scarring to the base and margins of the ulcer. With chronicity there will be accumulation of inflammatory cells such as lymphocytes, plasma cells and macrophages.

The pathophysiology of venous disease is believed to be as a result of venous incompetency that leads to venous hypertension. Alteration of venous hemodynamic, could be due to occlusion or partial recanalization as a result of deep vein thrombosis or due to longstanding varicose veins, even though most people with venous ulcers have no history of deep vein thrombosis (Collins & Seraj, 2010). Prolonged venous hypertension leads to oedema within the involved leg, oedema causes a disturbance to the normal capillary hydrostatic pressure (Simon, Dix, & Mc Collum, 2004). An oedema and a disturbance of pressure at the capillary level plays a major role the development of skin changes which includes lipodermatosclerosis and skin ulceration (**Figure 2.7**). These changes may interfere with the normal transfer of nutrients which will eventually escape into interstitial tissue and cause tissue ischemia under the effect of high interstitial pressure that will induce the collapse of the capillaries (Fagrell, 1982). Oedema is a predominant feature of chronic venous insufficiency which may result from one or more of four factors (Shami, Sarin, & Scurr, 1997).

1. Increase in capillary filtration due to high intracapillary pressure as a result of venous hypertension.

2. Loss of the veno-arteriolar reflex
3. Increased capillaries permeability to proteins
4. Inability to clear interstitial fluid and proteins because of lymphangiopathy

Under the effect of oedema and inflammatory changes induced by the escaping of blood cells and inflammatory markers into surrounding tissue, the tissues on the gaiter area becomes ischemic, especially during dependency of the lower limbs, as the gravity augments the effect of oedema. These consequences are superimposed by the effects of reperfusion injury that comes about after walking or elevation (Simon, Dix, & Mc Collum, 2004).



Figure 2.7: *Skin changes in venous insufficiency*

These series of changes on microcellular level lead to more changes on the macrocellular level resulting in the common symptoms seen with chronic venous insufficiency, including itching, skin changes, pigmentation, swelling, eczema, lipodermatosclerosis and eventually resulting in ulceration.

3 CHAPTER THREE: DIAGNOSIS AND INVESTIGATION OF CHRONIC VENOUS INSUFFICIENCY CAUSING VENOUS ULCER

3.1 Introduction

Identifying the aetiology of leg ulceration is the most important step in the diagnosis of venous leg ulcers. Having a clinical picture and physical examination can help the physician to create a proper differentiated diagnosis (*Table 3.1*).

Differential Diagnosis of leg ulcer includes
1. Venous insufficiency
2. Arterial disease
3. Neuropathic ulcers
4. Lymphedema
5. Neoplastic ulcer
6. Vasculitis
7. Polycythaemia Rubra Vera
8. Sickle cell disease
9. Traumatic (burns, cold injury, pressure, radiation)
10. Pyoderma Gangrenosum

Table 3.1: Differential diagnosis of leg ulcer

Venous ulcer diagnosis is generally clinical and should start with a full history and clinical examination. History should include any risk factors associated with venous ulcer such as age, sex, body mass index, cardiovascular disease, arthritis, mobility, diabetes, immune disease, trauma history and deep vein thrombosis (Simon, Dix, & Mc Collum, 2004).

The most common location for venous ulcers is over bony prominences; particularly on the gaiter area (ankle to mid-calf) (**Figure 3.1**), recurrence of the ulcer on the same area is highly suggestive of venous leg ulcer (Collins & Seraj, 2010).



Figure 3.1: *Venous leg ulcer, gaiter area*

Other findings include lower extremity varicosities, oedema, venous dermatitis that may be associated with hyperpigmentation, hemosiderosis or hemosiderin deposition in the skin and lipodermatosclerosis associated with thickening and fibrosis of normal adipose tissue underneath the skin (Collins & Seraj, 2010).

3.2 History

There are different types of leg ulcer and treatment modalities may differ depending on the aetiology of the ulcer, therefore appropriate diagnosis of the wound is crucial. Taking a proper history would be the initial step in the assessment of a leg ulcer, knowing the time of the onset and the related clinical picture would be needed as well as the chronicity and any diagnostic or treatment modalities that have already been taken. CVI clinical picture concluded mainly in CEAP classification

Table 3.2).

CEAP classification of chronic venous disease	Clinical classification
C0	No visible or palpable signs of venous disease
C1	Telangiectasies or reticular veins
C2	Varicose veins
C3	Oedema
C4a	Pigmentation or eczema
C4b	Lipodermatosclerosis or atrophie blanche
C5	Healed venous ulcer
C6	Active venous ulcer

Table 3.2: CEAP classification

It is important to know the social history, including smoking, occupation, social support and social isolation of the patient. The availability of clinical care throughout the day and knowing what the patient accessibility to this care is crucial, for instance, is the patient able to perform dressing changes or do they need help. All of these factors need to be taken into consideration as they would determine the most appropriate management plan. For elderly or obese patients it might be difficult for them to reach their lower legs; meaning self-care and bandaging will become an extremely difficult task.

Past medical or surgical history is also important, including attention given to other clinical problems that might give a similar picture to that of a venous leg ulcer or may interfere with the diagnosis or prognosis. Existence of cardiovascular disease, diabetes Mellitus, peripheral artery disease, chronic kidney disease, venous thromboembolism, autoimmune disorders, inflammatory bowel disease, connective tissue disease and sickle cell disease could be present when diagnosing a leg ulcer (Halabi-Twail, et al., 2008).

Sometimes a venous ulcer can present in the existence of a mixed arterial-venous ulcer. In such a case treatment of venous hypertension alone will not cure the patient when combined with significant arterial disease. Therefore, peripheral arterial disease needs to be ruled out by performing proper examination of the patient. This should be through a clinical examination where it can be established if there is presence of any associated features.

3.3 Physical examination

It is important to perform physical examination of VLU patient in order to differentiate it from other types of ulcer. A physical examination would include assessing the general condition of the patient to identify any associated signs such as varicose veins, limb size and

shape of the leg, examining the skin around the ulcer for any skin changes, lipodermatosclerosis, pigmentation or hemosiderin deposition in the skin (Collins & Seraj, 2010). Examining the ulcer itself is very important, assessing the ulcer edge, identifying the presence of granulation tissue, epithelial edge and vascularisation of the area. A venous ulcer is mostly irregular and shallow associated with fibrin, granulation tissue and slough in the base of the ulcer. The examination should include the contralateral limb even if it is normal, careful examination of the ulcer permits the exclusion of similar problems and will allow the implementation and arrangement of the correct management plan.

Examining the involved limb should involve examining joints above and below to exclude joint problems like rheumatoid arthritis and to identify any limitation of movement. To assess for peripheral arterial disease by examining peripheral pulses, checking posterior tibial artery and dorsalis pedis artery pulses. Checking skin temperature and capillary refill is important to exclude peripheral arterial disease. Assessment of a peripheral pulse and measuring the ankle-brachial index (ABI) can exclude peripheral arterial disease most of the time. If $ABI > 0.8$ this will confirm enough arterial blood flow to the lower limbs. Compression therapy cannot be indicated before excluding peripheral arterial disease, compression therapy is contraindicated if $ABI < 0.7$.

A diabetic ulcer is a very common differential diagnosis of a venous ulcer, therefore careful assessment of the involved limb for diabetic neuropathy, pain, touch, position, sensation and site of the ulcer is essential. Gathering relevant information would help in making a definitive plan for management of the ulcer.

3.4 Investigations

Non-invasive procedures

In addition to clinical presentation and history, there are other diagnostic modalities utilized to diagnose venous insufficiency and identify ulceration aetiology. Detailed anatomical and functional information about the venous system of the lower limbs can be obtained by modern non-invasive procedures. Generally these procedures can be repeated frequently with little or no problems with no harmful effects to the patient in comparison to invasive methods. Most of the equipment used in non-invasive procedures are affordable and easy to use.

Reliable quantitative information can be obtained when using non-invasive macro- and microcirculatory equipment. Duplex ultrasound scanning is a useful tool used to assess the macrocirculation, and venous microangiopathy. The Laser Doppler flowmetry is a non-invasive method used to assess perfusion. Transcutaneous PO₂ measurements can also be used to study the perfusion of the skin. Capillary filtration can be assessed by plethysmography.

3.4.1 Ankle brachial index (ABPI)

ABPI is obtained through looking at the ratio between the blood pressure in the lower limbs and the blood pressure in the upper arms. In comparison to the arm, lower blood pressure in the leg is an indication of peripheral vascular disease. Ankle brachial pressure index is calculated by dividing the ankle systolic blood pressure by the systolic blood pressures in the

arm (**Figure 3.2**). Researchers have found ankle brachial index (ABI) to have high specificity in the peripheral arterial system (Lazareth, Taieb, Michon-pasturel, & Priollet, 2009).



Figure 3.2: *Measurement of ABPI*
(Modified from www.google.com)

3.4.2 Duplex US

Duplex US is an investigation method that involves the combination of two elements:

1. Traditional ultrasound which uses the sound waves to create a picture of the anatomy of the vessel without assessing the flow through it.
2. Doppler ultrasound, colour Doppler ultrasound uses the sound waves to identify blood flow through the blood vessel and measure the velocity of blood.

The Doppler Effect was described by Johann Doppler who showed that the frequency of sound reflected from a moving object varies according to the velocity of the object (Nayman, 1974). The first person to suggest using the Doppler ultrasound in the study of blood flow measurements was Franklin DL (Franklin, Scheegel, & Rushmer, 1961). His suggestion was based on his observation that high-frequency sound waves become scattered when they fall on a moving target.

The Duplex ultrasound scan has become the test of choice for the assessment of leg veins because it is a simple non-invasive method and gives quantitative and qualitative information about blood vessels including veins. It evaluates the anatomy of the veins, venous reflux and venous hypertension. With colour duplex scanning we can localise the site of reflux by calf compression and release while the patient is standing because reflux is the effect of gravity. Moreover it allows quick identification of the venous system and its anatomical variations.

In the popliteal vein, although it can detect reflux, it sometimes cannot detect the level of the termination of the short saphenous vein (Strandness & Sumner, 1976). Reflux in the Gastrocnemius or the Giacomini vein can give false positive results indicating deep venous reflux despite the presence of competent popliteal valves. These deficiencies have been overcome by duplex scanning (Belcaro, Rulo, Vasdeski, Williams, & Nicolaides, 1989).

Duplex ultrasonography, is used routinely nowadays in the assessment of the venous system. It has the ability to confirm the presence of venous obstruction or valvular incompetence in veins and is used in planning different venous procedures (Obermayer & Garzon, 2010).



Figure 3.3: *Show position of the probe during lower limb examination*

On examination, the leg should be relaxed with the knee slightly bent and supported on the opposite leg

Figure 3.3). Venous reflux can be identified by a reverse blood flow of >0.5 second following the release of firm calf compression (**Figure 3.4**).

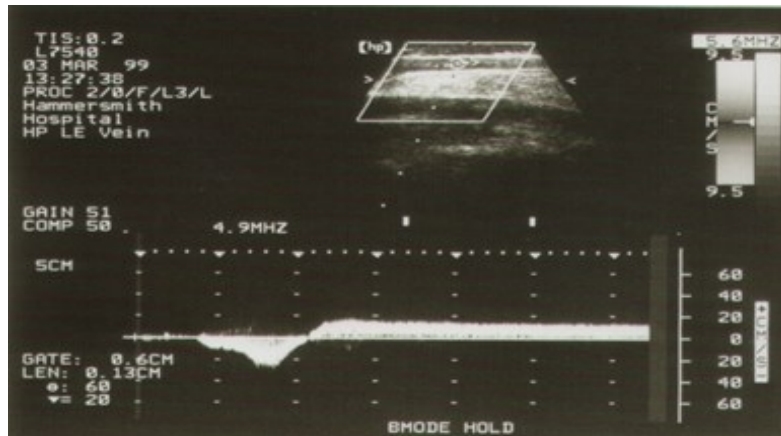


Figure 3.4: Venous reflux during distal augmentation manoeuvres

The sapheno-femoral junction, long saphenous vein, sapheno-popliteal junction, short saphenous vein, the deep veins and all perforating veins should be examined for patency and competence. Duplex ultrasonography is not always necessary in all cases of suspected venous insufficiency unless an intervention is being considered (Obermayer & Garzon, 2010).

3.4.3 Air Plethysmography.

An air-filled bag applied around the leg can detect volume changes in the venous volume of the limb, by using pressure changes during the application of the bag. A plastic sleeve is tightened around the leg and filled with air to a pressure of 6-8 mmHg. This pressure is sufficient for the leg to be in a comfortable condition without interference with venous flow. The device is calibrated in order to monitor the venous volume change. Venous refilling can also be measured by this method following exercise.

3.4.4 Photoplethysmography (PPG)

This is a non-invasive method used to detect changes in blood components. It was first used for the assessment of the arterial system. Later on, people started to rely on its benefit in assessing the venous system. The principles of photoplethysmography in the venous system depend on the variation of the absorption of the light by haemoglobin circulating in the veins. Light reflected from the red blood cells is collected by a photoelectric detector. The basics of the test show, when there is high venous pressure, the skin venous plexus is full, and therefore haemoglobin absorbs the light. Whereas, when venous pressure falls, the venous volume decrease in dermal venous plexus and light absorption become less.

On the practical side, in photoplethysmography a typical measurement obtained after



Figure 3.5: PPG test

emptying of lower limb veins is usually by 10 dorsiflexions of the ankle joint whilst in the sitting position, (**Figure 3.5**). To assess venous reflux, a probe containing a light source and a light sensitive diode is applied on the skin; this probe would be able to detect changes caused by alteration in the venous pressure resulting from venous obstruction or position change (Belcaro, Rulo, Vasdeski, Williams, & Nicolaides, 1989). Ninety-five percent venous refilling times are normally greater than 20 seconds; shorter than 20 seconds refilling means presence of venous reflux (**Figure 3.6**). Refilling Time (RT) of veins and Calf muscle Power (MP) was measured during VPPG test.

Photoplethysmography can be used as a screening test for venous disease however, it is difficult to differentiate between superficial and deep vein incompetence compared with duplex scanning, especially with the new generations of duplex scanners.

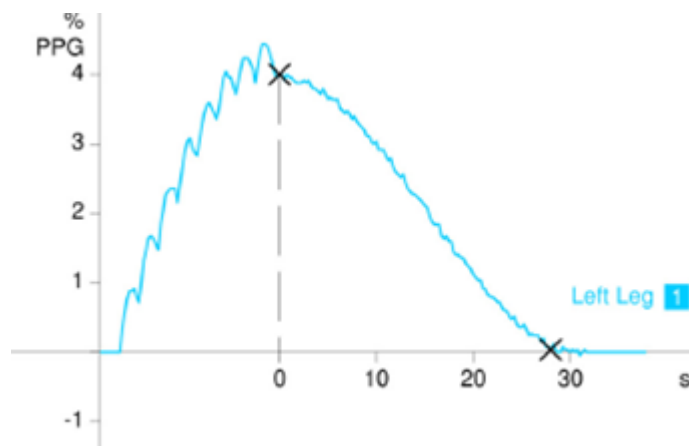


Figure 3.6: Shows a typical tracing obtained from a PPG examination

3.4.5 Light reflex rheography

Light reflex rheography is composed of many light emitters, these emitters shine on the skin and the light reflected from the skin is collected by a detector. Its principles are the same as photoplethysmography, and the way in which assessment of venous system is done is similar. Light reflex rheography has advantages over PPG, the main one being the ability to cover a larger area of skin, also a greater number of emitters are used. For the achievement of a known amount of skin transmission, the system is designed to be calibrated.

3.5 Investigations of the skin microcirculation

There are different types of investigations for assessing skin microcirculation. The main two reliable methods especially associated with the research of venous ulcers are Transcutaneous

Oximetry (TRC) and Laser Doppler Flowmetry (LDF); both of these investigations are feasible, accurate, easy to use and available.

3.5.1 Laser Doppler

Skin perfusion is dynamically changing; therefore the method used to measure blood flow has to be safe, sensitive and reproducible. Moreover, it is not measuring the basal blood flow only but measuring vascular reactivity, researchers found that LDF is suitable tool for that especially when coupled with provocation tests.

Laser Doppler Flowmetry is a non-invasive method which was developed in the last two decades of the 20th century and was not used as a clinical tool until the last few years of the 20th century (Belcaro, Rulo, Vasdeski, Williams, & Nicolaides, 1989). Since the 1970s several instruments have been used with development and improvement. In the 1980s fibre-optic based laser Doppler perfusion monitoring (LDPM) techniques were introduced. Laser Doppler flowmetry is used in research of hemodynamics as a technique to quantify blood flow in tissues such as skin and on these same principles it can be used within the clinical environment.

LDF use in clinical practise is limited because it does not give absolute readings of blood perfusion, however LDF is used widely in other fields such as research, pharmacological industry and wound healing. LDF is also used to measure blood perfusion in other organs like the kidneys, the intestine, the liver muscles, the brain and the heart but to lesser extent in comparison with the skin.

Mechanism

The principles of LDF theory can be explained by illuminating the skin or any other tissue with single-frequency light and then measuring the frequency distribution of the reflected light from the moving particles within the blood such as red blood cells. The Doppler effect is physical phenomenon which occurs between two objects and it includes a wave source and a wave receiver. The relative motion between the wave source and the wave detector leads to a change in the wave frequency. The difference between the frequencies is called the Doppler frequency shift.

The light used is monochromatic consists of a single frequency because the frequency shift will cause frequency broadening of the original beam. The moving object will scatter the light and the shift of the frequency depends on this object movement, the direction of the incoming light and the scattered light direction.

Most LDF instruments utilise helium-neon gas or gallium aluminium arsenide elements to produce a weak laser beam with low tissue penetration. Low-power laser (usually a laser diode) can produce a beam which penetrates the skin at certain depth (

Figure 3.7). This type of laser beam does not damage the tissues under evaluation or induce an increase in tissue temperature. Only moving parts in the sample volume (blood cells) will cause a Doppler shift in the light frequency. The power in the signal depends on the number of the moving particles. These measurements are useful to monitor the effect of exercise, heat, medications, etc. on targeted micro-sized vascular areas.

There are problems with probe attachment that could limit some applications while movement of the fibre optics during measurement can result in artefacts that may make the

interpretation of results difficult. Another disadvantage is its inability to detect absolute blood flow. Finally variability in measurements is a problem; this must be taken into consideration when taking measurements and interpreting results.

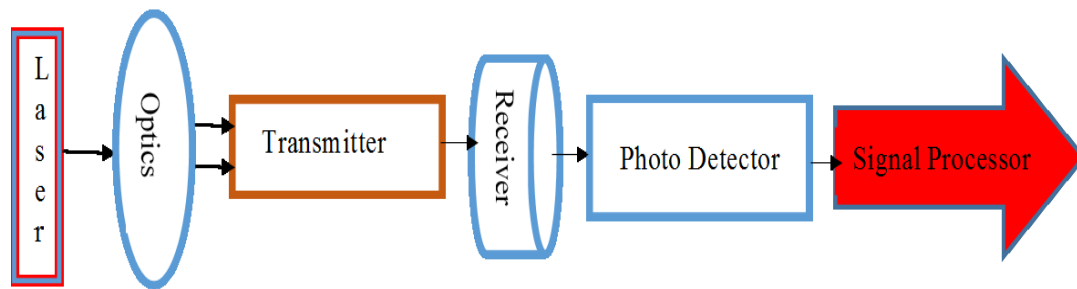


Figure 3.7: Laser Doppler mechanism

On the other hand, LDF has several advantages over other methods: it is non-invasive and it can measure dynamic changes in microcirculation without being affected by the underlying muscle blood flow. Moreover it is easy to apply, reproducible and available. In conclusion LDF remains the gold standard for the assessment of skin microcirculation and its reactivity in the research field as well as in a clinical environment.

3.5.2 Transcutaneous Oximetry (TCPO₂)

Transcutaneous measurement of PO₂ and PCO₂ provide instant, continuous information of the body's ability to deliver O₂ to tissue and remove CO₂ via the CVS. It is, however, important to realize that the electrode is measuring the gas tension of the underlying tissue and not the arterial gas tension. When hemodynamic conditions are stable, transcutaneous correlate well with arterial values, but this does not necessarily mean that the measured values are identical.

Huch et al demonstrated the possibility of measuring oxygen partial pressure on skin neonate in 1969 by applying the electrode to the skin; he heated the skin to 44° to enhance skin permeability by causing maximum capillary vasodilatation (Huch & Lubbers, 1969). Researchers have shown that transcutaneous oxygen pressure (tcPO₂) has significant correlation with cutaneous oxygen delivery.

This fact was very useful for detecting skin perfusion and viability of peripheral tissue (Eickhoff & Jacobsen, 1980), (White , et al., 1982), (Cina, et al., 1984). Transcutaneous O₂ provides information on the O₂ supply to the tissue and is dependent on:

1. O₂ uptake of the respiratory system.
2. The O₂ transport capacity of the blood.
3. The condition of the circulatory system.



Figure 3.8: *Transcutaneous PO₂ measurement in venous ulcer*

TRC measurement of PO₂ and PCO₂ based on heating element in the electrode elevates the temperature in the underlying tissue (**Figure 3.8**). This increases the capillary blood flow and the partial pressure of O₂ and PCO₂ and makes the skin permeable to gas diffusion. Transcutaneous oximetry may be an alternative to ABI in screening for arterial disease in patients with venous leg ulcers (Bianchi, Zamiri, Mc Intosh, Dawe, & Douglas, 2008).

3.5.3 Transcutaneous PO₂ in venous disease

Most investigators have found tcPO₂ to be reduced in extreme cases of venous disease (e.g. in venous ulcer), while the results are normal in the unaffected skin (**Figure 3.8**). This is distinctly different in arterial disease, where reduced tcPO₂ can be detected in wider areas. An exception represents the liposclerotic skin where the overall tcPO₂ is reduced, but to a lesser degree than in the immediate proximity of the ulcer. It has to be pointed out that there is pronounced overlap with normal values, except at the edge of ulcers where the tcPO₂ values are homogeneously lower.

3.5.4 Factors affecting tcPO₂ in venous disease

Oedema:

Oedema reduction, achieved for instance by external intermittent compression, did not influence the tcPO₂ reading which could be interpreted to mean that oedema per se has no effect on tcPO₂. This question does not seem to be settled because an increase in tcPO₂ after oedema reduction was recorded close to the ulcer after compression bandaging. tcPO₂ measurements might be increased when the oedema is decreased as the normal blood circulation may improve following the oedema subsiding.

3.6 Investigations, invasive procedures

Invasive procedures are rarely used in the diagnosis of venous insufficiency problems. These types of investigations can be associated with side effects which could sometimes be serious, for instance, bleeding, infection, hypovolemia, and renal problems. The availability and accuracy of different types of non-invasive procedures decreases the need for invasive procedures.

3.6.1 Capillary microscopy

This is a simple non-invasive method that is used to investigate the nutritional capillaries in the skin directly. Using capillary microscopy enables researchers to identify any morphology abnormalities. Some research have used some tracers like fluorescein to measure blood flow in the retina (Kohner, Hamilton, Saunders, Sutcliffe, & Bulpitt, 1975), Goodman et al reported the measurement of capillary blood velocity (Goodman, Guyton, Drake, & Loflin, 1974).

Capillary microscopy is the only technique in blood flow measuring in which nutritive flow alone is being measured. The main problem with capillary microscopy is that it is only applicable for certain areas of the body such as the nailfold and only a small sample of capillaries can be studied at a certain time. Movement of subjects during the examination is another problem; however capillary microscopy is still the gold standard for measuring nutritive capillary blood flow. Skin capillaroscopy involves using a fiberoptic video microscope where an optical surface sensor is attached to a TV monitor. It also eases the lower limb examination while the patient changes position (Thelesius, 1992).

3.6.2 Venography

Venography involves the injection of a contrast medium into one of the veins of the lower limb, either through a foot or leg vein, followed by imaging of the superficial and or deep veins by x-ray (**Figure 3.9**). Deep venous thrombosis is one of the main risks of venography, contrast causes damage to the venous intima and predispose to thrombosis (Albrechtsson & Olsson, 1976). However, with the introduction of non-ionic contrast media, the risk of developing deep venous thrombosis has been significantly reduced. Venography of the lower limb can be divided into: ascending venography and descending venography and varicography.

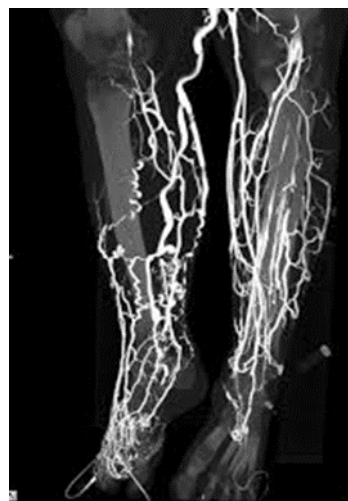


Figure 3.9: *Venography of lower limbs veins*

(Modified from www.google.com)

Ascending venography

Ascending venography is used for identifying venous anatomy, to distinguish between primary, and secondary disease, incompetent perforators and to define the presence or

absence of obstruction. With the development of duplex scans, the importance of venography has declined, however venography remains the gold standard method for determining the accuracy of new techniques.

3.6.3 Ascending functional venography

This procedure is designed to identify the deep to superficial reflux, during this procedure a table is tilted up to 60°, the legs are specifically positioned thus enabling the patient to perform voluntary plantar flexion. An injection of contrast is made into the deep veins, after that the patient is asked to do planter flexion. On planter flexion, the contrast moves upwards into the deep veins, during relaxation the contrast moves backwards from deep to superficial veins.

3.6.4 Descending venography

This technique is able to detect the extent of venous reflex and at the same time identify the valvular damage to the deep veins. It is also used to define the reflux from the superficial or deep veins, this criteria has little importance today due to the availability of duplex scans. Descending venography can be useful during surgical reconstruction or with recurrent varicose veins if the duplex scan is not conclusive.

3.6.5 Varicography

Varicography is a simple technique useful for defining the anatomy of the veins; it can also be used for delineating venous anatomy prior to recurrent varicose vein surgery. It is a simple technique that involves the direct injection of contrast into superficial a varicose vein (Stonebridge, Chalmers, Beggs, Bradbury, & Ruckley, 1995). The patient lies on their back

for the X-ray with the table tilted 10-20 ° up. When the contrast media injected into the varicose veins, the contrast drained into the deep system is defined.

3.6.6 Foot vein pressure measurements

Foot vein pressure measurement is useful in the assessment of venous insufficiency because venous hypertension is the main predisposing factor to VI. The procedure includes cannulation of a dorsal foot vein and measurement of venous pressure using pressure transducer. After cannulation of the veins, the patient is asked to exercise the calf muscle pump, exercise can be through dorsiflexion or walking on a treadmill (Hojensgard & Stump, 1949).

Foot vein pressure measures the foot pressure at rest, foot pressure on exercise, and the time needed for the foot pressure to return back to the resting level after exercise has ceased. In normal condition, the resting pressure is equal to a column of blood from the right atrium through the veins to the foot, while during exercise the pressure in the foot falls to less than 25 mmHg. The time needed for the pressure to return to the basal level after exercise has ceased is around 25-30 seconds (*Figure 3.10*).

If the foot pressure fails to drop down during exercise or if there is a reduction in the time taken for the pressure to reach the basal level this would be an indicator of venous incompetence. Repeating the test with the use of tourniquets can give a clue if the reflux is due to superficial or deep venous incompetence (Shami, Sarin, & Scurr, 1997).

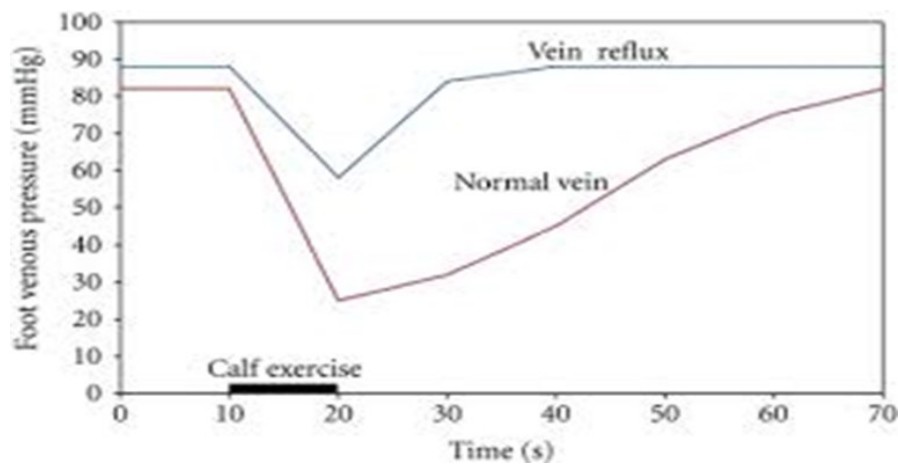


Figure 3.10: Foot venous pressure

(Modified from www.google.com)

3.7 Summary

For the diagnosis of venous ulcer there are many modalities available, some of them are invasive procedures and others are non-invasive. Non-invasive procedures are more popular for the diagnosis of venous leg ulcer as they have less side effects. Haemodynamic changes associated with venous ulcers are significant and are associated with microangiopathy changes of the skin and microvasculature changes at subcutaneous tissue level. These microangiopathic changes and associated physiologic changes in venous ulcers may be difficult to pick up by routine investigative measures like a duplex scan or PPG. Researchers have found that transcutaneous oxygen measurements and laser Doppler measurements can give a detailed idea about the venous ulcer changes on a microvascular level including $tcPO_2$ and blood flow through the skin. These diagnostic tools are non-invasive, easy to use, have no recognisable harmful effect and are easily available, moreover the new generation of these

instruments are accurate and delicate enough to minimise technical error. We used non-invasive measures throughout our study, we used duplex scans, PPG, laser Doppler and tcPO₂ measurements to identify all changes associated with venous ulcers and to measure the response after three months of regular exercise.

4 CHAPTER FOUR: EXERCISE AND WOUND HEALING

4.1 Physiology of exercise

Exercise exerts physiological demands on the body; the physiological response of the body to exercise depends on the strength, duration and frequency of the exercise as well as the environmental factors such as temperature, humidity and light. During physical exercise, the work load on body cells increases, in turn the requirements for oxygen and other substances necessary to provide energy to the skeletal muscles are increased as is the need for the removal of metabolites and carbon dioxide. Several changes occur throughout the body to meet exercise requirements; alterations of cardiovascular, metabolic, and respiratory functions occur in order to meet the increased demands of the cells. (Burton, Stokes, & Hall, 2004)

4.1.1 Adenosine triphosphate (ATP)

ATP is the common chemical coenzyme that provides energy for all forms of biological interactions in the cell and is vital for skeletal muscle work. ATP molecules are capable of releasing 7.3 kcal of energy and there is always a small amount of ATP stored in the muscle that is necessary to be able to perform exercise. For the human body to be able to perform normal resting body metabolism, the body needs more than half of body mass of ATP. ATP can be resynthesized very quickly from energy-dense molecules stored in the body.

4.1.2 Cardiovascular system changes during exercise

During exercise, the demand on oxygen supply increases dramatically, as a result, blood flow increases parallel to this in order to supply enough oxygen. Blood flow to the muscles during resting is usually 2–4 ml per 100g of muscle per minute; however, the blood flow might well

increase to nearly 100 ml per 100g of muscle per minute during maximal exercise. This sharp increase in blood flow occurs as a result of vasodilatory effect of metabolites such as adenosine, and PO_4 , which override the vasoconstrictor effects of noradrenaline. Low pH and an increase in temperature shift the oxygen dissociation curve for haemoglobin to the right during exercise, this shift permit the unloading of more oxygen from haemoglobin into the muscle. During exercise, muscle blood flow and coronary blood flow increase, whereas cerebral blood flow is controlled at a constant level and splanchnic flow will decrease. Other organs, such as the bowel and kidneys are ensured protection with enough blood flow. In contrast, Skin demands additional blood flow during exercise in order to enable skin function. (Gielen, Schuler, & Adams, 2010)

4.1.3 Circulatory changes during exercise

To supply the extra amount of blood needed for exercised muscles the cardiac output is increased, this increase in cardiac output is in direct proportion to the oxygen consumption increase. Cardiac output increase is achieved by the increase in both, heart rate and stroke volume. The chronotropic and inotropic effect on the heart is produced by stimulation of the noradrenergic sympathetic nervous system. There are other factors that influence the heart rate such as vagal inhibition through autonomic sympathetic response and carbon dioxide influence on the medulla.

In athletics, the contraction of the heart plays the vital part in the increased cardiac output during exercise because of the heart muscle hypertrophy. During strenuous exercise, stroke volume and heart rate both increase to about 90% of their maximum level. The change in blood pressure is moderate and is mainly due to the increase in cardiac output. The changes

seen in the venous system include significant increase in venous return as a result of calf muscle contraction, also include diversion of blood from the viscera and vasoconstriction. (Gielen, Schuler, & Adams, 2010)

4.1.4 Respiratory system changes during exercise

Exercise is an activity that carried out for a purpose of conditioning part of the body. For any biological work the cell needs oxygen to perform. During exercise, the cells of the body require more oxygen to meet the extra demand therefore ventilation might increase from 5–6 litres per minute during the resting time to more than 100 litres each minute during exercise. The relationship between ventilation and an increase in the workload is linear. The oxygen consumption during exercise also increases linearly with a physical activity increase. Oxygen consumption during resting time in an average young male is about 250 ml per minute, whereas in an endurance athlete, oxygen consumption is about 5000 ml each minute during very high intensity exercise.

Pulmonary ventilation is a combination of an increases in respiratory rate and tidal volume and reflects the increase in oxygen consumption and carbon dioxide production. Lung capacity, does not reach its maximum ability even during strenuous exercise and it has no role in the limitation of oxygen delivery to muscles during peak physical activity. In people with normal respiratory function, haemoglobin is fully saturated with oxygen throughout exercise (Burton, Stokes, & Hall, 2004).

4.1.5 Oxygen carrying capacity of the blood during exercise

Conditions associated with the reduction in the oxygen carrying capacity, such as anaemia, can affect the performance during exercise, they can produce shortness of breath and fatigue

even on mild exertion. An increase in the level of red blood cells in the body is a method that has been tried by some athletes. They remove red blood cells, storing and re infusing them, this method is called 'blood doping'. This method has been shown to improve maximal oxygen uptake by up to 10% (Burton, Stokes, & Hall, 2004).

4.1.6 Maximum oxygen consumption during exercise

Oxygen consumption of the body increase in a linear relationship with the increase in workload, however there is limit for oxygen uptake by cells, above a certain work rate the oxygen consumption reaches a plateau. Therefore, it is important for exercise to be gradual, regular and moderate in intensity in order to be effective in delivering regular amount of oxygen (Tiep, et al., 1987).

4.1.7 Oxygenation

Oxygen is a vital factor for cell life and metabolism, as it is the source of energy production through ATP which is the common chemical precursor that supplies energy for all forms of biological work (Burton, Stokes, & Hall, 2004). It is critical for each single step of the wound healing process. (Guo & Dipietro, 2010). Tandara AA et al, reported that Hypoxia is the main factor that affects wound healing (Tandara & Mustoe, 2004). During the process of wound healing the availability of oxygen takes on extra importance because of the increase of cell metabolism to fulfil different and complicated functions of cells such as synthesis of collagen and cell proliferation (Hunt, Zederfeldt, & Goldstick, 1969).

Oxygen has many crucial functions throughout the wound healing process; it protects the wound from infection, initiates angiogenesis as it is necessary for producing vascular endothelial growth factor. Oxygen increases keratinocyte differentiation, augment migration

of epithelial cells, induces fibroblast proliferation and synthesis of collagen, and builds up wound contraction. (Bishop, 2008).

In wounds, there are numerous factors would increase tissue oxygen demand, such as vascular disruption and increase in oxygen consumption by cells as they become more metabolically active. As a result of that the skin microcirculation at the early stages of the wound is predominantly hypoxic. In addition, clinical problems such as, a venous ulcer, peripheral vascular disease, diabetes mellitus exert an additional burden on the affected area which may all impair the blood flow, creating a condition of low oxygen supply. These circumstances create a hypoxic microvascular environment around the wound that will delay healing; this picture is more obvious in chronic wounds such as a venous ulcer. There is considerable experimental evidence showing the difference in cellular response between acute hypoxia and chronic hypoxia where, in chronic hypoxia the response is much slower than acute hypoxia. Additionally, there is a decrease in proliferation rate of dermal fibroblasts under chronic hypoxia (Siddiqui, et al., 1996). The conclusion that can be drawn is that the exercise effect on chronic wound is seen with prolonged regular exercise. Researchers recorded transcutaneous oxygen tension from 5 to 20 mm Hg in chronic wounds compared with 30 to 50 mm Hg in normal controlled tissue (Tandara & Mustoe, 2004). Tandara et al, demonstrated that an aged rabbits and young rabbits showed delayed wound healing under hypoxia.

There is strong evidence in many studies suggesting that oxygen stimulate reepithelialisation, (O'toole, et al., 1997). O'Toole and colleagues have reported that in vitro conditions, keratinocyte motility increased by hypoxia, these keratinocytes had increased expression of

certain proteins which are cytoskeletal proteins that have a role in cell motility (Mogford, et al., 2002).

4.1.8 Impact of aging on hypoxic stimulus

The majority of chronic wounds like a venous ulcer occur primarily in the elderly. In the elderly, their response to hypoxia is altered; as a result, wound healing is considerably delayed. Experiments on rabbit ears of aged animals showed delay in wound healing compared to middle aged-rabbits (Wu, Xia, Roth, Gruskin, & Mustoe, 1999). Generally, we can conclude that in patients with chronic wounds such as a venous ulcer, the alteration of response to hypoxia will cause a delay in the wound healing.

To summarise, for proper wound healing the body needs normal oxygen levels. Although hypoxia stimulates wound healing by initiating certain functions such as angiogenesis and allows the release of growth factors, the availability of oxygen is vital to maintain the healing process. Researchers tried to improve the wound oxygen level in order to improve wound healing, through among other things they used hyperbaric oxygen therapy (HBOT) and topical oxygen, however, each one of these therapeutic measure was related to a number of limitations. Hyperbaric oxygen therapy can cause both physiological and psychological side effects like middle ear barotrauma and hypoglycaemia, the most serious reported hazards of HBOT is seizures. Researchers also reported impairment of red blood cell function by HBOT in Vitro (Conconi MT, 2003). In addition they demonstrated an association between HBOT and mutations in human blood DNA, impair fibroblast growth and apoptosis (Spier, Denong, & Radermacher, 2002). Topical oxygen therapy has limitations for use in humans, for instance with relation to safety reasons in patients who smoke. (Kalliainen, Gordillo,

Schalanger, & Sen, 2003), most importantly, there have been no control studies in humans. Other researchers mentioned that exercise could be a suitable method to increase oxygen level in wound healing (Rodriguez, Felix, Woodley, & Shim, 2008).

4.2 Effect of exercise on wound healing

A poor healing wound causes a lot of concerns. Chronic wounds can cause social isolation, severe pain, limitation of movement, lack of productivity and a reduced quality of life. These troubles do not only affect individuals but can put a large financial burden on a country's economy, for example in the United States, chronic wounds are costing an estimated \$25 billion per year (Sen, et al., 2009).

Acute wounds can heal quickly, while a chronic wound takes longer to heal and is often difficult to manage. Chronic wounds most commonly affect the elderly who are suffering from debilitated conditions, chronic illnesses and have a poor physiologic response to illness; in addition older patients may have limitation of movement and lack of social support. Furthermore with the number of elderly people growing rapidly year on year worldwide, the number of chronic wounds such as venous ulcer has also increased.

Many methods of treatment have been tried to tackle chronic wounds, however there is no single effective treatment. In such circumstances where no such effective treatment is readily available, there is need for a simple economically friendly solution, this solution needs not only to be economic but suitable for use in district hospitals, nursing homes, outpatient clinics and the home. Exercise has been recommended by studies where improving exercise endurance can assist in the suppression of psychological distress (Dustman, et al., 1984).

It is well recognised that the lack of physical activity and a sedentary life style have a negative impact on wellbeing. A lack of exercise may cause obesity, joint problems, limitation of movements, cardiovascular problems, immunity depression and psychological problems. There is growing evidence that the lack of physical activity impairs wound healing. Radek KA and co-workers suspended rats' hind limbs to simulate a bed rest condition resulting in a condition of diminished physical activity then they examined the wound closure, angiogenesis and collagen deposition after 21 days, researchers concluded that, bed rest, not only causes muscle wasting and thinning bones but also wound vascularity is reduced significantly, wound closure is delayed and the function of the vital cells involved in wound healing are impaired. (Radek, Baer, Eckhardt, DiPietro, & Wade, 2008). The conclusion that can be drawn that exercise not only has a positive impact on wound healing but more importantly when there is a total lack of exercise the wound healing process is drastically impaired. The first study evaluating the effect of exercise on wound healing in living individuals was published in 2005, Emery et al and his colleagues investigated twenty eight healthy subjects (mean age 61.0 ± 5.5 years). Those individuals had aerobic exercise three days per week, one hour a day with moderate intensity exercise for a period of three months, in contrast, the other set of control patients were only carrying out normal daily activity. The wound closure in the exercised group (decreased less than 10% of original size) was faster than those in the control group (29.2 vs. 38.9 days) (Emery, Kiecolt-Glaser, Glaser, Malarkey, & Frid, 2005)

Emery et al, findings were supported three years later by a further study that was published in 2008. This study was conducted by Keylock KT et al, they investigated the effect of

moderate aerobic exercise on wounded older mice. Exercise started three days before wounding and lasted five days after wounding, the wounds of exercised mice contracted at a faster rate than that of the control mice group. In exercised mice, their wounds decreased to 80% of their original size, which occurred 51% faster than sedentary controls (Keylock, et al., 2008).

Both Emry et al and Keylock KT et al, explained the improvement of wound healing in exercised groups by the effect of exercise on the inflammatory phase of wound healing. Their conclusion was supported by suppressed inflammatory markers in exercised groups. Several studies reported positive relationship between faster healing and low inflammatory factors many of these studies rely on animal experiments (Martin, et al., 2003) (Szpaderska, Zuckerman, & DiPietro, 2003). Szpaderska AM et al, reported decreased inflammatory markers in mice wounds that healed faster compared with the control group. In most of these studies researchers found a correlation between low inflammation level and faster healing compared with higher levels of inflammation and an association with delayed healing.

Several studies showed evidence that regular physical activity and/or exercise could inhibit inflammatory marker levels in the blood, (Geffken, et al., 2001) (Colbert, et al., 2004) (Fairey, et al., 2005). Fairry et al, reported that; in a group of breast cancer survivors, Fifty-three postmenopausal breast cancer patients were randomly allocated to an aerobic exercise programme of 15 weeks. The exercise group exercised on cycle ergometers 3 times per week, they found that CRP levels decreased significantly in the exercised group compared with the control group, findings that were later supported by Jeongseon et al (Kim, Choi, & Jeong, 2013).

Others researchers have not been in favour of the idea that physical activity can decrease levels of inflammatory markers. Beavers Km, et al and colleagues investigated the effect of moderate-intensity physical activity on inflammatory factors in a group of 424 elderly patients (aged 70–89 years). The study did not provide evidence that regular exercise has an effect on inflammatory biomarkers (Beavers, et al., 2010). A further study conducted by another group of investigators in 2004, demonstrated no significant relationship between physical activity and levels of inflammatory markers (Verdaet, et al., 2004). They studied 892 male individuals, with no clinical CHD picture and no major ECG changes, within the same work environment, all participants were aged between 35–59 years old. In comparison to the previous study conducted by Beavers Km et al group, this study dealt with a younger age group and a larger number of subjects. However, both studies concluded the same result.

Brandt P et al, concluded in a study conducted in 2012 that the effect of exercise on wound healing is independent of alterations in inflammation (Pence, DiPietro, & Woods, 2012). Obese mice were exercised on a treadmill three days before wounding and 5 days after, researchers were unable to detect any differences in inflammatory markers. The interesting thing to note about this study is that the same group of researchers published their first paper in 2008 evaluating the effects of exercise on wound healing in aged mice that showed the suppression of inflammatory markers in addition to a decrease in wound size (Keylock, et al., 2008). The exercise pattern, the wounding procedure and time scale for the analyses described in both studies are similar and intended to be similar in their outcome. In both studies the wound healing improved significantly with exercise, however the Brandt P et al study outcome shows no relationship between inflammatory markers and wound healing in

obese mice. This conclusion leads one to raise questions about the hypothesis that exercise improves wound healing because it impairs the inflammation process in the wound.

Other researchers have suggested that exercise reduces wound size and improves wound healing due to its ability to decrease damage caused by free radicals thus accelerating healing (Shin, Lee, Song, & Jun, 2008) (Gomez-cabrera, Domenech, & Vina, 2008). In conclusion there is enough evidence to suggest that exercise does improve wound healing, however there is no agreement about how exercise could improve wound healing. The most acceptable explanation for this phenomenon is that it is a multifactorial process. There may be other factors that play a role in the process of wound healing during exercise, one of them could be the increase in tissue oxygenation on microvascular level due to the enhancement of skin circulation. Venous ulcer is a typical form of a chronic wound that requires a long and difficult course of healing time, exercise might be useful in the treatment of such clinical condition.

4.3 Summary

Millions of people around the world suffer from wound healing problems; these problems are associated with a variety of illnesses such as venous leg ulcer, diabetic ulcer, arterial ulcer and other such illnesses. There are so many factors that can impair wound healing, including, obesity, cardiovascular disease, sedentary lifestyle, old age, depressed immunity, medications, cancer and many more that can all affect the wound healing process making it a very complicated problem.

Chronic wounds such as VLU can create real problems for individuals such as loss of mobility, increased pain, lack of productivity and reduced quality of life. This is not only an

issue for patients, but it also affects the health budget of the states. Moreover the number of patients with delayed wound healing conditions is growing significantly as the aging population increase. For all these circumstances, finding a simple, effective, easy, low cost ways to tackle wound healing problems is mandatory and must be of high priority.

There is growing evidence that exercise may be the suitable solution, exercise is easy, cost effective, can be performed at home, and has many positive impacts on human body wellbeing without undesirable side effects if used in a moderate level and according to the needs of the individual patient.

5 CHAPTER FIVE: METHODS

5.1 Introduction

Participants recruited from the vascular clinic at Hammersmith hospital, Imperial College Healthcare NHS Trust. Local ethics committee approval was obtained from South West - Cornwall & Plymouth, National Research Ethics Service (11/SW/0307).

All selected patients approached by the investigator, explaining to them the clinical study, methods, duration and aim and objectives, patients who agreed to participate in the study signed an informed consent statement. 122 patients approached by the investigator and 42 were excluded from the study. Participants had thorough history and clinical examination, primary investigations as discussed below in this chapter to confirm the diagnosis and to check the competency of superficial and deep venous systems.

Inclusion criteria: Every venous leg ulcer patient willing to participate in the trial and able to do the exercise.

Exclusion criteria were:

- Peripheral arterial disease (ABPI <0.8)
- Venous leg ulcer with clinical signs of cellulitis or infection
- Venous leg ulcer with necrotic tissue or slough
- Other types of ulcer: rheumatoid vasculitis, diabetic foot and malignant ulceration
- Corticosteroids use
- Participation in a leg ulcer trial within one year
- Patients complain of dementia or who were disorientated
- Patients who are unable to perform the exercise

<i>Variable</i>	<i>No. or mean $\pm$ SD</i>
Patient, N0	78
BMI (kg/m²; mean $\pm$ SD)	31.79 $\pm$ 3.86*
Age (years)	65 $\pm$ 13*
Systolic blood pressure (mmHg)	132 $\pm$ 21*
Diastolic blood pressure (mmHg)	92 $\pm$ 14*
Sex	
Male	38
Female	40
Venous reflux	
Superficial	60
Deep & superficial	6
Deep only	12

Table 5.1: Basic data of participants

**Values presented as mean $\pm$ SD*

Eighty participants were recruited to two groups, each group composed of forty patients (**Table 5.1**). The first forty patients randomised to two groups, control group (20) and compression therapy only group (20). The next forty patients, randomly assigned to two groups, exercise only group (20) and stocking and exercise group (20). Group 3 and 4 performed exercise and group 4 was wearing bandaging therapy in addition to exercise.

Method of randomisation by a computer-generated random numbers sealed in envelopes by a member not allied to the research team. Two of recruited participants from the control group left the study because of other illnesses.

Group	Number of patients	Age (years)	M:F ratio	Ulcer size before exercise (cm)	BMI
Group 1 Control group	18	64.55±13.70*	10:08	2.70 (5.25-1.29)*	31.27±3.75*
Group 2 Compression therapy	20	69.15±12.05*	10:10	2.52 (4.18-0.63)*	32.40±3.84*
Group 3 Exercise only	20	64.95±12.97*	10:10	2.63 (4.02-1.35)*	32.62±3.76*
Group 4 Exercise and compression therapy	20	62.15±14.05*	10:10	2.39 (3.51-1.16)*	30.85±4.28*

Table 5.2: Patient characteristics, *Values in mean ±SD, ulcer size values in median and the interquartile range

5.2 Study design

Sample size:

80 patients recruited from the clinics and divided into four groups (**Figure 5.1**);

Group 1 control group, n = 20

Group 2 will receive compression therapy only, n =20

Group 3 will receive regular exercise only, n =20

Group 4 will receive compression therapy and regular exercise, n=20

Participants assessed at the start of the trial and after three months period of regular exercise, by the following parameters:

Ulcersize

Transcutaneous oxymetry

Laser Doppler flowmetry

All patients were subjected to ABPI and venous Duplex scan to assess their arterial venous status.

ABPI

Duplex Ultrasound scan

Range of ankle joint movement (group 3 &4 only)

At the end of the trial by the following parameters:

Ulcer size

Transcutaneous Oxymetry

Laser Doppler flowmetry

Range of ankle joint movement (group 3&4 only)

Data collected analysed using appropriate statistical method.

5.3 Materials and methods

80 patients with venous leg ulcer were included in this study. Participants divided into four groups, each group composed of 20 patients. Two patients from control group were not able to continue the study due to other illnesses.

1. Group 1 (Control group), Venous ulcer subjects, wearing no stockings and performing no exercise.
2. Group 2 (Stocking group), Venous ulcer subjects, performing no exercise and wearing stockings only.
3. Group 3 (Exercise group), Venous ulcer subjects, wearing no stockings, performing exercise only.
4. Group 4 (Exercise and stockings group), Venous ulcer subjects wearing stockings and performing exercise.

All four groups (80) participants were checked for peripheral arterial occlusive disease (ankle brachial pressure index >0.8). The four groups were comparable at baseline in terms of age, sex, body mass index (

Table 5.2).

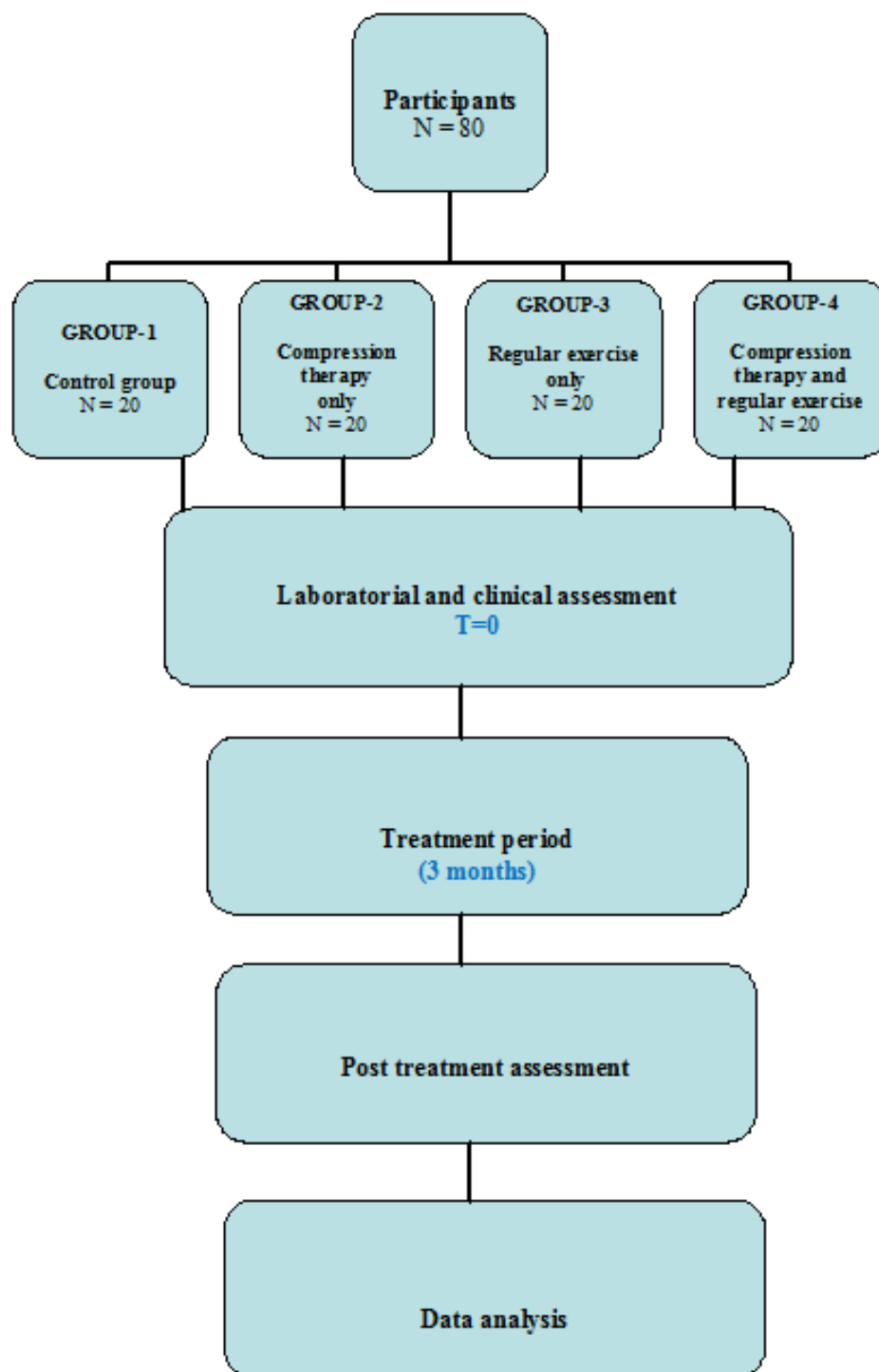


Figure 5.1: Study design

All measurements been taken at the beginning and the end of a period of three months of regular exercise. Colour duplex scan was performed for all examined subjects to demonstrate the presence of superficial, deep venous incompetence and absence of proximal obstruction (*Table 5.1*).

5.4 Exercise

Participants had full information about how to perform the exercise, they provided with log book to make sure that they perform the exercise at the proper time. Exercise composed of 10 dorsiflexions every hour while awake (*Figure 5.2*). Researchers found that regular foot movement increase the velocity of venous jet, decrease the pressure on the luminal side of the valve leaflets and initiate valve closure (Bergan, et al., 2006). This exercise has been shown to empty the venous blood from lower limbs completely, this stagnated deoxygenated venous blood will be replaced by fully oxygenated blood.



Figure 5.2: Showing dorsiflexion exercise

Dorsiflexion exercise is simple activity (Figure 5.3), easy to perform in sitting or sleeping position and fulfil the purpose which is emptying of accumulated venous blood. Moreover, the majority of venous insufficiency patients are elderly with other comorbidities that can limit their mobility; with the time participants might feel more comfortable to perform treadmill exercise for instance or participating in supervised regular exercise.



Figure 5.3: *Four layered compression with exercise procedure*

Participants who performed the exercise (group 3, 4), had the same exercise and had a thorough demonstration, and explanation of the exercise by the investigator also they have been asked to perform the exercise many times and instructions been given accordingly. All subjects received a diary and a leaflet to help them in performing the exercise on time, each participant will receive a phone call every 15 days by the investigator to make sure that the participant performing the exercise properly.

Only one VLU per patient was assessed in the study. If patients had more than one ulcer, then the largest was included. Dressing change frequency was at the clinician's decision, but on

average it took place twice per week. The Four groups were comparable at baseline in terms of age, sex and body mass index.

5.5 Instruments

There are invasive and non-invasive methods to investigate venous insufficiency in clinical practice and research. Choosing non-invasive methods in venous research could be more appropriate and might be more convenient for the participants. Invasive procedures might cause serious side effects to patients or complications like pain, scar, bleeding and infection, additionally non-invasive methods causing minimal discomfort. Researchers should try to use non-invasive measures as much as they can, however this should not affect the efficacy of the study. Therefore, we tried to use non-invasive measures in this clinical study (*Figure 5.4*).

Methods used in this thesis described and grouped in this section to avoid repetition although some of these methods might be described partially throughout the thesis.



Figure 5.4: Instruments set (laser Doppler & transcutaneous oximetry)

5.6 Doppler (CWD) examination

It is the preliminary test to evaluate all participants in the clinical studies. It is a pocket Doppler detector of blood flow and velocity, provided with 8 MHz probe applied on the relevant blood vessel.

Doppler procedure is the first evaluation that be able to exclude any peripheral arterial disease subjects. Lower limb arteries, namely posterior tibial artery and anterior tibial artery were checked to make sure that there is no involvement with peripheral arterial disease. All participants with ankle brachial Doppler pressure index below 0.8 were excluded from the study as they considered to have peripheral vascular disease.

The procedure performed while the subject lying supine on the bed and the probe applied on the skin at an angle of 40-60 degrees at the level of anterior tibial artery, posterior tibial artery and dorsalis pedis artery. In order to get the optimal signal from the artery water soluble gel applied on the skin this will facilitate transmission of waves into the probe.

5.7 Duplex scanning

Colour duplex (Toshiba Aplio) scanning was used to detect any abnormal flow through high resolution technique. Variations in venous flow and velocity appeared on the screen as a colour-coded scale of velocity, the proximal flow coded with different degrees of blue colour and the distal flow coded with red colour. On releasing calf compression while the patient was standing the retrograde flow indicated reflux blood in red colour through the superficial or deep veins under examination (Vasdekis, Clarke, & Nicolaides, 1989). Disappearance of

the colour flow through the vein under compression by a probe means that the lumen of that vessel was patent.

For evaluating the popliteal vein, the probe applied on the popliteal fossa skin pressing slightly and adjusted to identify the three structures in the popliteal fossa:, popliteal artery which is the deepest structure, second superficial is the popliteal vein, running parallel to the popliteal artery, the most superficial structure is the short saphenous vein, small diameter in comparison to the other two. It runs slightly oblique and join the popliteal vein at the saphenopoplital junction. All participants subjected to the colour duplex scan for the detection of incompetence or obstruction through the superficial and deep veins of lower limbs.

5.7.1 Venous reflux detection and localisation

First step was asking the patient to stand up on the healthy leg so the leg to be examined was relaxed, most of the time participants are elderly so they were supported when they moved their legs up.

Firstly, the probe applied on the groin area then on the popliteal fossa and the calf muscle compressed manually on these two occasions in order to eject blood up. If the valves of femoral, long saphenous and popliteal veins were competent then will be no abnormal signal or reverse flow on releasing of calf compression. On the other hand if there is retrograde flow detected, this considered to be an indication of reflux.

If there was no retrograde flow or obstruction detected, the participant was considered free of venous incompetence and colour duplex scan and other investigations were not necessary (Nicolaidis, et al., 1993).

5.8 Laser Doppler flowmetry (LDF)

LDF is a non-invasive method that has been developed over the last 30 years. It could be able to collect a sensitive, non-invasive, continuous, and real-time assessment of skin blood flow (Saumet, Kellogg, Taylor, & Johnson, 1988). Laser Doppler flowmetry has been used in research to study reflex control of cutaneous blood flow, in conjunction with other modalities like (Iontophoresis and local warming and heating). Furthermore, LDF used to evaluate the local effect of drugs on skin blood flow.

In this study we used the laser Doppler flowmeter (Moors Instruments Ltd., Axminster, Devon, UK), illuminates a small area of tissue with monochromatic light at a wavelength of 780 nm. Reflected, back scattered, light falls onto a photo multiplier. The laser power output at the tip of the probe around 1mW. This power has no hazard to the health of the subjects. The light receive on the probe consists of shifted light from moving objects inside the blood vessel mainly erythrocytes. The Doppler Effect occurs between two objects, namely a wave source and a wave receiver (erythrocyte), the relative motion of the wave detector and wave source causes a change in the wave frequency and wavelength. This change is recorded into a computer.

The LDF device needs to be calibrated primarily before any measurement to be taken, after that instrumental zero obtained then the biological zero has to be measured. Instrumental zero can be obtained by placing the probe against a white surface. The biological zero seem to be erythrocytes that remained in the blood vessels during arterial occlusion, still moving and producing some LDF components which recorded by the instrument. The LDF used in this study was used according to the manufacturer instructions.

It is well known that Laser Doppler recordings can be affected by interference and artefact. Research have shown that nearby structures can interfere with Laser Doppler readings (Soo-ampon, Vongsavan, Soo-ampon, Chuckpaiwong, & Mathews, 2003), therefore patient movement kept to the minimum, to avoid postural changes, avoid vibration and light changes.

The LDF recording taken 30-33 minutes (*Figure 6.1*). Baseline stage lasted 15 min. The occlusion taken 3 min at maximum; when there is strong leg pain, 1 min was the minimum time. After the occlusion period finished, the recording was continued for another 15 min to record the post occlusion reactive hyperaemia response (PORH). The place were to apply occlusion was the thigh immediately above the knee, 18 cm wide pneumatic cuff attached around the measured limb, a cuff applied above the knee was inflated to 150 mmHg to provide a vascular challenge a rapid cuff inflator system was used for quick inflation and deflation (Fernando Moralesa, et al., 2005).

All measurements were taken before 10 am to avoid the effect of long standing or working and particularly to avoid the effect of oedema. Laser Doppler recordings were taken in a room temperature of ($22\pm 1^{\circ}\text{C}$).

Before the measurement started, the participant had rest for at least 20 minutes to acclimatize and relax to avoid the problem of movement artefacts. The laser Doppler instrument was calibrated in a standard manner according to manufacturer's instruction with the probe against a stable white surface. The probe must be in close contact with the measured skin; in order to do so, using double adhesive rings fixed the probe holder against the skin, small quantity of gel applied to the skin surface to improve transmission of laser light through the skin.

Subjects were lying supine and the lower limb positioned stable with the aid of support to minimise movement. The site of the laser Doppler probe was 2 cm proximally or distally from the ulcer edge. All ulcerations, at the time of measurements are clean, not inflamed, no slough or necrosis and located at the internal perimalleolar area.

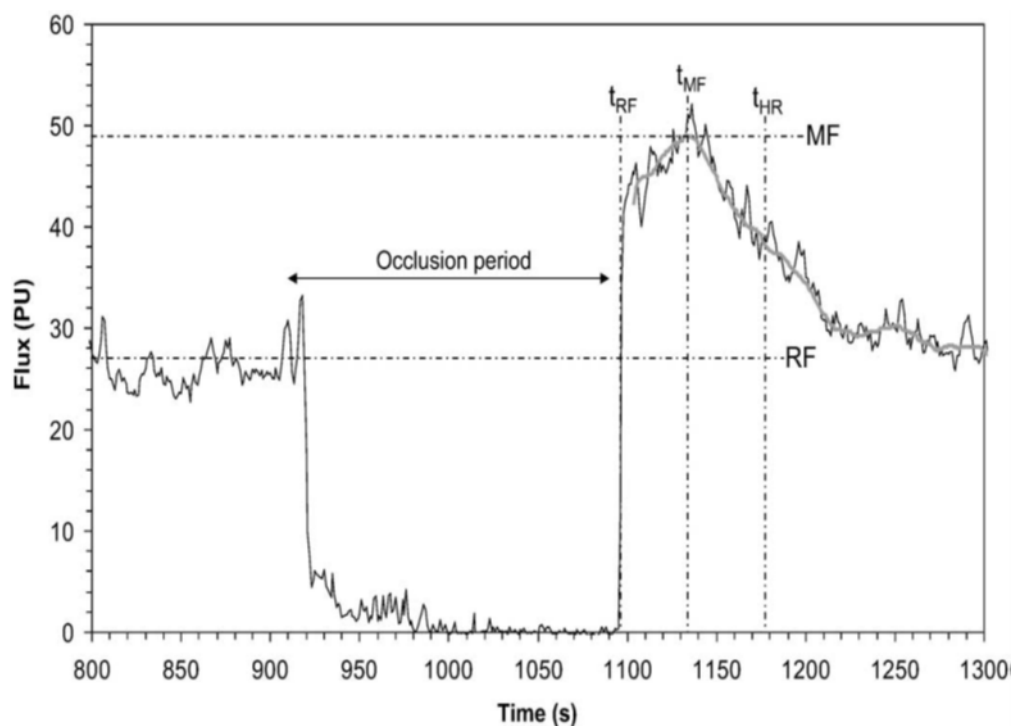


Figure 5.5: LDF recordings

(Modified from Fernando Moralesa et al)

Resting skin flux measured by applying the probe on the gaiter area within 2 cm away from the edge of the ulcer. Resting skin flux measured after 30 minutes of lying supine and acclimatisation in a room temperature ($22 \pm 1^{\circ} \text{C}$).

5.8.1 Resting skin flux (RF)

Resting skin flux measured by applying the probe on the gaiter area within 2 cm away from the edge of the ulcer. Resting skin flux measured after 30 minutes of lying supine and acclimatisation in a room temperature ($22 \pm 1^\circ \text{C}$). Intra-observer agreement was (74 %, K = 0.54).

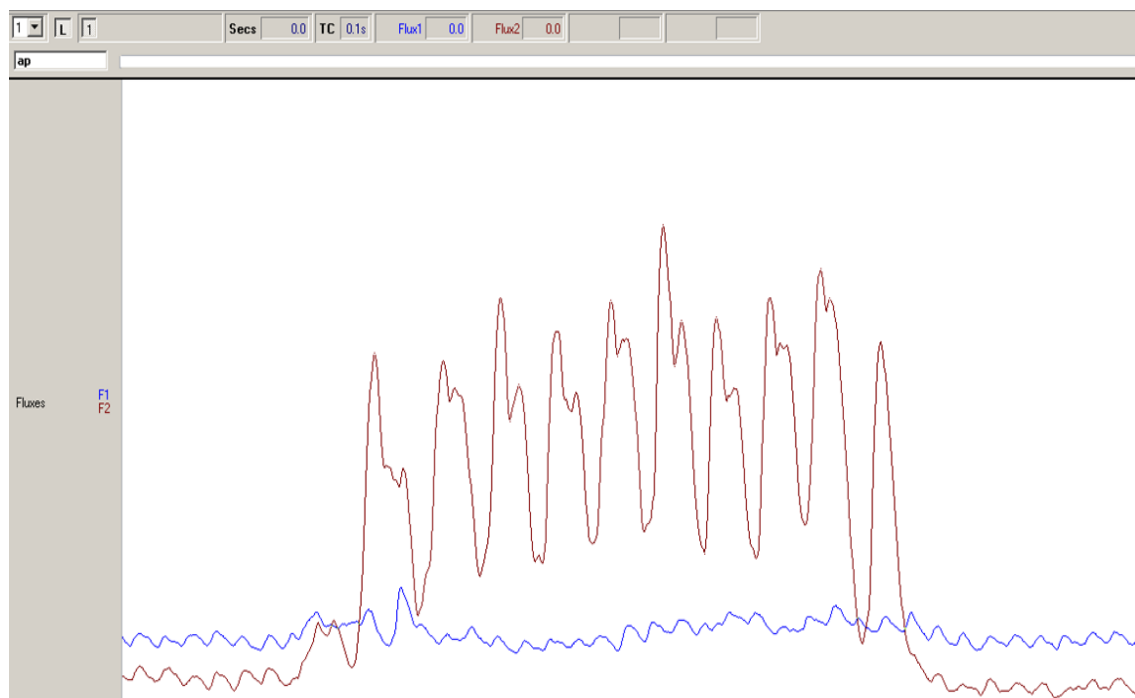


Figure 5.6: *LDF recording of the dorsiflexion exercise*

5.9 Transcutaneous PO₂ measurements

(**Figure 5.7**) shows transcutaneous PO₂ measurements of skin. This technique gives a quantitative measure for skin oxygenation (Franzeck, Haselbach, Speiser, & Bollinger, 1993). The device recorded delivery of oxygen to the skin, also it recorded skin blood flow, it gives an idea about the delivery of oxygen on a cellular level. Participant were in supine position

during recording. All measurements were taken before 10 am to avoid the effect of long standing or working and particularly to avoid the effect of oedema. Also measurements were taken in a controlled room temperature within the range of $22\pm1^{\circ}\text{C}$. tcPO_2 measurements reproducibility has been studied, and accuracy was within the 95% confidence (Gothgen & Jacobsen, 1978).



Figure 5.7: *Measuring tcPO_2*

Calibration was following the calibration recommendations by manufacturer. Site selection, ideal position would be located over a homogenous capillary bed without skin defects or hair. Probe applied on the gaiter area 2 cm from the edge of the ulcer, immediately after calibration, the area around the ulcer for about 5 to 8 cm where the probe is applied was prepared by stripping the skin 10-15 times with adhesive tape to remove the excessive cells, this will lead to improve oxygen diffusion and to standardize the measurements. The probe is sensitive for temperature variation, therefore the probe heated to 44°C . For the tcPO_2

readings to become stabilized, the measurements were taken after 20-25 minutes from applying the probe on the skin. This time allow O₂ diffusion through the probe membranes.

In order to increase the specificity and sensitivity of tcPO₂ recordings, a number of “sensitizing” procedures have been used, one of them is applying temporary pressure on the limb. We applied a cuff above the knee of the recorded limb and the cuff inflated rapidly to a pressure of 70 mmHg above the systolic pressure at the ankle for three minutes, at this point biological zero was taken.

All ulcers were clean at the time of measurements, not inflamed and no slough or necrosis and located at the internal perimalleolar area. Position, patient lie in a supine position and supported from sides to keep them comfortable as much as possible in order to decrease the effect of movement on recording.

Immediately after completion of calibration, the probe positioned within two cm from the edge of the ulcer mostly 1 cm from ulcer edge according to the manufacturer protocol. Approximately 20 minutes were required after the probe was placed on the site of the skin for stabilisation, followed by baseline recording. The intra-observer variation was (78 %, K = 0.56).

5.9.1 Factors influencing tcPO₂ value

- Probe temperature (due to the effect on microvasculature).
- Arterial PO₂.
- Metabolic consumption of O₂ by the dermal tissue.
- Oxygen consumption by the electrodes.

- Skin thickness and skin resistance to O₂ diffusion (more pronounced to dynamics).
- Capillary density and rate of cutaneous blood flow

5.10 Ulcer size

All venous ulcers were cleaned with normal saline to take off residual exudate and to provide a clear picture of the ulcer border. A metal ruler cleaned and used to measure the widest and longest dimensions of the ulcer area. The two maximal perpendicular diameters (in cm) were measured with ruler and multiplied. This method has been part of everyday clinical practice in primary care in the treatment of chronic ulcers. Therefore the surface area was determined by calculating the length and width of each ulcer by the Ruler.

**6 CHAPTER SIX: EVALUATION OF THE EFFECT OF
EXERCISE IN PATIENTS WITH VENOUS LEG ULCER
USING LASER DOPPLER FLOWMETRY**

6.1 Introduction

Previous studies demonstrate that in patients with chronic venous insufficiency, there is a delay in post hyperaemic response and increase in skin flux in the area of perimalleolar region, (Belcaro, Rulo, Vasdeski, Williams, & Nicolaides, 1989) (Butler & Coleridge smith, 1994). It has been suggested that these findings demonstrate the effect of venous hypertension, which manifests itself as a picture of microangiopathic changes of skin microcirculation. Practical findings of the studies reveal that the resting flux (RF) in the permalleolar region is higher than the normal flux unit in the healthy tissue. Also, there is a decrease in venoarteriolar response (VAR) (<30%) of normal value, which may explain the severity of microcirculatory abnormality.

Same findings were recorded in diabetes mellitus patients; where capillary hypertension and microvascular changes appear in the skin before the development of diabetic microangiopathy (Wahlberg, Jorneskog, Olofsson, Swedenborg, & Fagrell, 1990).

6.2 Aim and rationale

The aims of the clinical study as presented in this section include:

1. Evaluation of the effect of exercise on the healing of venous ulcer in chronic venous insufficiency patients.
2. Identifying if the Laser Doppler findings could be used as parameters for the healing of venous leg ulcer in chronic insufficiency patients.

6.3 Results

Before calculating the parameters given in (*Table 6.1*), all measurements were converted to perfusion units (10 mV = 1 PU, following the manufacturer's instructions), and averaged over 1sec. There were significant difference in resting skin flux (RF) in Group 4 (exercise and stocking group) before and after three months of regular exercise ($P < 0.001$). The RF is significantly decreased after 3 months of exercise compared to before exercise measurement, 8.39 ± 1.52 (before exercise) vs. 6.02 ± 1.6 sec (after exercise). However there is no significant difference in RF between pre exercise and post exercise in group1 and group 2, while there is difference in group 3, 7.87 ± 1.33 (before exercise) vs. 6.59 ± 1.53 sec (after exercise).

Patients who performed no exercise			
Group	Number of patients	RF at time 1 (sec)	RF at time 2 (sec)
Group 1 Control group	18	$8.66 \pm 2.06^*$	$8.67 \pm 1.98^*$
Group 2 Compression therapy	20	$8.25 \pm 1.86^*$	$8.23 \pm 1.70^*$
Patients who performed exercise			
Group 3 Exercise only	20	$7.87 \pm 1.33^*$	$6.59 \pm 1.53^*$
Group 4 Exercise and compression therapy	20	$8.39 \pm 1.52^*$	$6.02 \pm 1.62^*$

Table 6.1: LDF findings in the four groups of patients. *Values presented as mean $\pm$ SD

6.4 Statistical analysis

The methodology used was Linear Mixed Models for repeated measures adjusting for group, gender and age. Bonferroni correction was used for the comparisons between groups. Gender wasn't significant outcome measures.

Age was significant ($p < 0.001$) and the magnitude of its effect on RF is given by 0.102 meaning that a unit increase in age corresponds to a 0.102 increase in RF, these findings may be related to the dilation of the veins with age.

The difference between before and after varies across groups and this is shown in the table and graph below. The "Mean" column is the estimated RF mean for each group before and after adjusted for age (*Table 6.2*) and (*Figure 6.1*).G

Estimates^a

time	Group	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1	1	8.734 ^b	.263	8.209	9.259
	2	7.851 ^b	.253	7.348	8.355
	3	7.899 ^b	.250	7.401	8.397
	4	8.707 ^b	.252	8.205	9.208
2	1	8.739 ^b	.273	8.194	9.284
	2	7.837 ^b	.262	7.315	8.360
	3	6.624 ^b	.259	6.107	7.141
	4	6.333 ^b	.261	5.813	6.853

Table 6.2: RF level before and after exercise in the four groups

a. Dependent variable: RF

b. Covariates appearing in the model are evaluated at the following values:

Age = 65.218

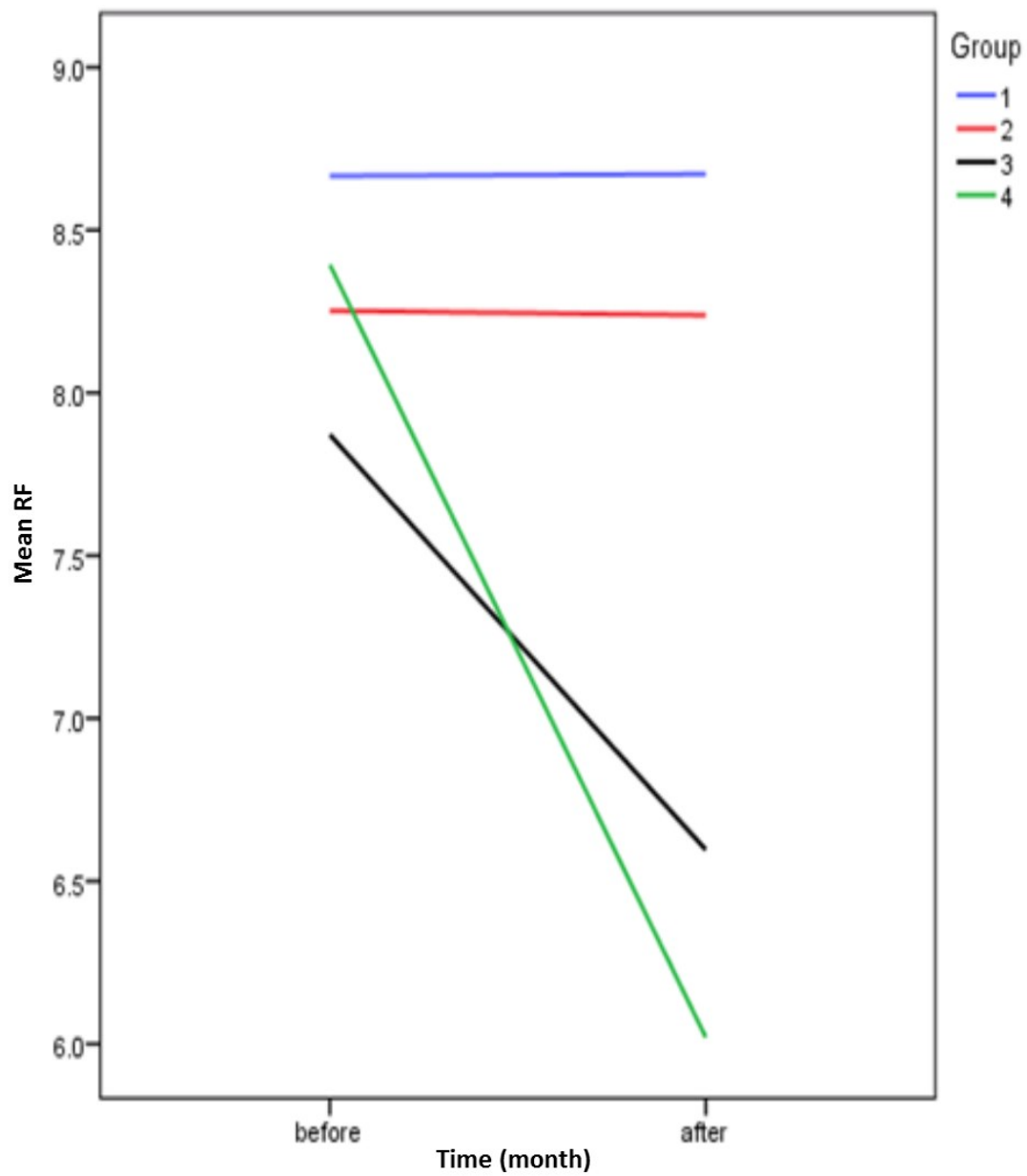


Figure 6.1: RF level in the four groups before and after the exercise

Time before: Measurements at the beginning of the study (month)

Time after: Measurements after 3 months (month)

Pairwise Comparisons^a

Group	(I) time	(J) time	Mean Difference (I-J)	p-value ^c	95% Confidence Interval for Difference ^c	
					Lower Bound	Upper Bound
1	2	1	.006	.964	-.236	.247
2	2	1	-.014	.904	-.243	.215
3	2	1	-1.275	.000	-1.504	-1.046
4	2	1	-2.373	.000	-2.603	-2.144

Table 6.3: Pairwise comparison between groups

a. Dependent variable: RF

c. Adjustments for multiple comparisons: Bonferroni

I time: Measurements at the beginning of the study

J time: Measurements after 3 months

Only groups 3 and 4 show a significant decrease - both with $p < 0.001$. In comparison between groups before and after. Before, there is no significant difference between groups. However after, group 4 is significantly lower than groups 1 ($p < 0.001$) and 2 ($p = 0.001$). Also group 3 is significantly lower than groups 1 ($p < 0.001$) and 2 ($p = 0.009$) (Table 6.3).

6.5 Discussion

Over the last thirty years, Laser Doppler flowmetry (LDF) has been reported to be a good indicator of skin microcirculation. LDF has been shown to record the movement of erythrocytes within a tissue blood volume (Haumschild, 1986). Researchers have also demonstrated that LDF is a convenient tool, which is used to measure microvascular changes in diabetic patients. (Leonardo, Arpaian, & Del Guercio, 1987), (Gasser, Muller, Mauli, & Staibli, 1992). Belcaro, et al., 1989, reported that LDF can be used to evaluate and quantify microangiopathy changes in patients, who have been diagnosed with chronic venous insufficiency and may be useful in separating venous leg ulcer of high potential for healing from the one presenting as low potential for healing (Belcaro, Rulo, Vasdeski, Williams, & Nicolaides, 1989).

These changes that are demonstrated in patients with venous leg ulcer are similar to those that have been recorded in diabetic patients with and without peripheral neuropathy (Rayman, Williams, & Spencer, 1986). According to Belcaro, et al., the increase in RF can be generally considered to be an index of venous hypertensive microangiopathy. He also considered the AVR to be a poor indicator of venous insufficiency due to the large overlap, and these findings were similar to the findings of this study.

Moreover, Maessen-Visch M. B et al., conducted a study to establish the difference in the microcirculation of chronic venous insufficiency (Atrophie Blanche) patients and healthy control individuals. They found that the RF in CVI individuals showed an increase when compared with normal skin, while $tcPO_2$ was low in CVI subjects when compared with healthy controls (Maessen-visch, Sommer, De Paepe, & Neeumann, 1998).

In addition, Knut Kvernebo, et al., reported a variation in the LDF readings too. They used Photoplethysmography (PPG), transcutaneous oxygen tensiometry (tcPO₂) and laser Doppler flowmetry (LDF) to measure perfusion changes of the lower limb of a pig. Knut Kvernebo, et al., concluded that tcPO₂, LDF and PPG could be used in combinations in order to improve the reliability of venous insufficiency diagnosis (Kvernebo, Megerman, Hamilton, & Abbott, 1989).

Other studies have reported a variability in LDF measurements. For instance, Fernando Morales, et al., conducted a multicentre study to establish analysis method for the post-occlusive reactive hyperaemia (PORH), using LDF. In the study, they examined 24 patients with Peripheral Vascular Disease and 30 healthy subjects. They found that there is no significant difference in the resting flux and maximum flux (RF and MF) measurements between the healthy control subjects and the PVD patients (Morales, et al., 2005). Similar findings were also recorded by other researchers, using various LDF generations, (Del Guercio, Leonardo, & Arpaia, 1986). Fernando Morales, et al., concluded that the differences among brands are unlikely to cause variability in the Flux values measurements.

It is observed that in diabetes mellitus, the diabetic patients show microvascular disease, which results in slow microvascular flow and a resting hyperaemia. Fernando Morales et al., also suggested that these features affect the laser Doppler flow response. In CVI and VLU patients, the picture is similar to diabetic patients, where there are hyperaemia and slow flow due to venous hypertension and venous stasis, therefore we can conclude that LDF is a good indicator for microvascular changes in VI. The current study demonstrates that there is a change in LDF parameters (RF) in patients with chronic venous insufficiency. The findings

from the current study are similar to those of previous studies. According to the findings of the current study, resting flux showed a significant decrease after 3 months of exercise in VLU patients group 4 (stockings and exercise). Additionally, there was a decrease in the RF but the study showed a lesser decrease in the group of patients, who were only exercising and not undergoing compression therapy. These results may give an indication for the healing of ulcer since it is known that RF increases with advanced stages of VLU and could be normal with healed VLU. Intra-observer agreement was (74 %, K = 0.54), which may represent one of the weaknesses of the study.

It can be inferred that the findings of the current study, may support the influence of exercise on ulcer healing as the LDF parameters, RF showed significant changes after 3 months of regular exercise. LDF measurements could be useful in the diagnosis of VLU; the study shows that RF measurements are high in venous insufficiency and VLU. However, these parameters decreased with the healing of venous ulcer. Although, the current study findings are similar to those of other studies, they may need to be confirmed by a more detailed multicentre randomised trial.

**7 CHAPTER SEVEN: t_cPO_2 MEASUREMENTS IN PATIENTS
WITH VENOUS LEG ULCER IN RELATION TO
EXERCISE**

7.1 Introduction

Transcutaneous measurement of PO₂ and PCO₂ provides an instant, precise and continuous information about delivery of O₂ to the skin and removal of CO₂ via the CVS (Hutch, Franzeck, Huch, & Bollinger, 1983).

The Transcutaneous oxygen measurement (tcPO₂), is a measurement of the gas tension of the tissue and not the measurement of arterial gas tension. If the haemodynamics of the body are stable, transcutaneous readings are known to correlate well with arterial values. However, this correlation does not mean that the measurements are identical. The metabolism in the skin consumes O₂ and produce CO₂ therefore, transcutaneous values differ from arterial values. PO₂ values in the skin are lower than in the arteries and PCO₂ measurements in the skin are a little higher than arteries (Kalani, Brismar, Fagrell, Ostergren, & Jorneskog, 1999). tcPO₂ measurement is a non-invasive technique, which is used widely in medicine for wound healing prediction, wound management, revascularisation and qualification for hyperbaric oxygen therapy.

Transcutaneous measurement of PO₂ is used in patients with CVI as a method to evaluate the functional changes of cutaneous microvasculature in venous disease. Researchers also found that tcPO₂ is reduced in chronic venous insufficiency and venous ulcer, while the tcPO₂ values could be normal in mild venous disease. These findings are different from studies which reveal that in arterial disease, low tcPO₂ can be found in a wider area (Mannarino, Pasqualini, & Maragoni, 1984) (Neuman, Van Leeuwen, & Van Den Broke, 1984) (Sindrup, Avnstrom, Steenfoss, & Kristensen, 1987). Moreover, Franzeck et al., recorded a direct correlation of tcPO₂ measurements with venous microvascular density. Additionally,

according to another study, on venous ulcer sites the tcPO₂ was unmeasurable, which indicates a very low tcPO₂ values in severe venous disease (Mossa, et al., 1987).

Other investigators also demonstrated that tcPO₂ measurements in CVI patients are different from one stage to another, based on the severity of the disease. For example, tcPO₂ in mild venous disease may be normal or very mildly affected and they recorded a number of (47.7±14.5 mm Hg) in patients without major trophic skin changes. Whereas, tcPO₂ values in areas of white atrophy were recorded at 0 mm Hg or close to 0 mm Hg (Franzeck, Haselbach, Speiser, & Bollinger, 1993).

In this clinical study, tcPO₂ measurements in four groups of venous leg ulcer patients were also recorded. During the study, measurements were recorded on two occasions, first one, at the beginning of three months period of regular exercise, and the second reading was recorded after the three months period of exercise. The details of the methodology used in the study analysis are discussed in the following chapter.

7.2 Aims and rationale

The aims of this experiment were:

1. To identify the effect of exercise on healing of venous ulcer in chronic venous insufficiency patients using tcPO₂ measurements.
2. To see if tcPO₂ values could be useful as a parameter for venous leg ulcer healing and follow up in chronic insufficiency patients.

7.3 Results

The four groups of subjects were comparable for age, sex and BMI, all subjects had ankle brachial pressure index >0.8 and less than 1.2. No adverse events (burns, blisters) were recorded. The results obtained in this study showed low tcPO₂ level in all venous ulcer patients on recruitment, however post exercise measurements were different (*Table 7.1*).

Patients who performed no exercise			
Group	Number of patients	tcPO ₂ at time 1 (mmHg)	tcPO ₂ at time 2 (mmHg)
Group 1 Control group	18	17.77±4.50*	17.82±4.70*
Group 2 Compression therapy	20	18.06±3.96*	19.50±4.98*
Patients who performed exercise			
Group 3 Exercise only	20	17.07±3.66*	22.40±5.51*
Group 4 Exercise and compression therapy	20	16.97±4.02*	33.01±10.08*

Table 7.1: tcPO₂ findings in the four groups of patients. *Values in mean±SD

Time 1: Measurements at the beginning of the study

Time 2: Measurements after 3 months.

In group 4 (stockings and exercise group), tcPO₂ level increased significantly ($p < 0.001$), it was 16.97 ± 4.02 before exercise and increased to 33.01 ± 10.08 post exercise (**Table 7.1**). In group 3 (exercise group only), there was increase in tcPO₂ level from 17.07 ± 3.66 (pre exercise) to 22.40 ± 5.51 (post exercise), while in group 1 and 2 (no exercise groups), participants tcPO₂ level not changed in response to exercise (**Figure 7.1**) and (**Figure 7.2**). These results suggest that PO₂ level in the venous ulcer patients skin is low and it will increase significantly or to the normal level if the patient perform regular exercise for three months. Moreover this increment in tcPO₂ measurements indicates the process of ulcer healing.

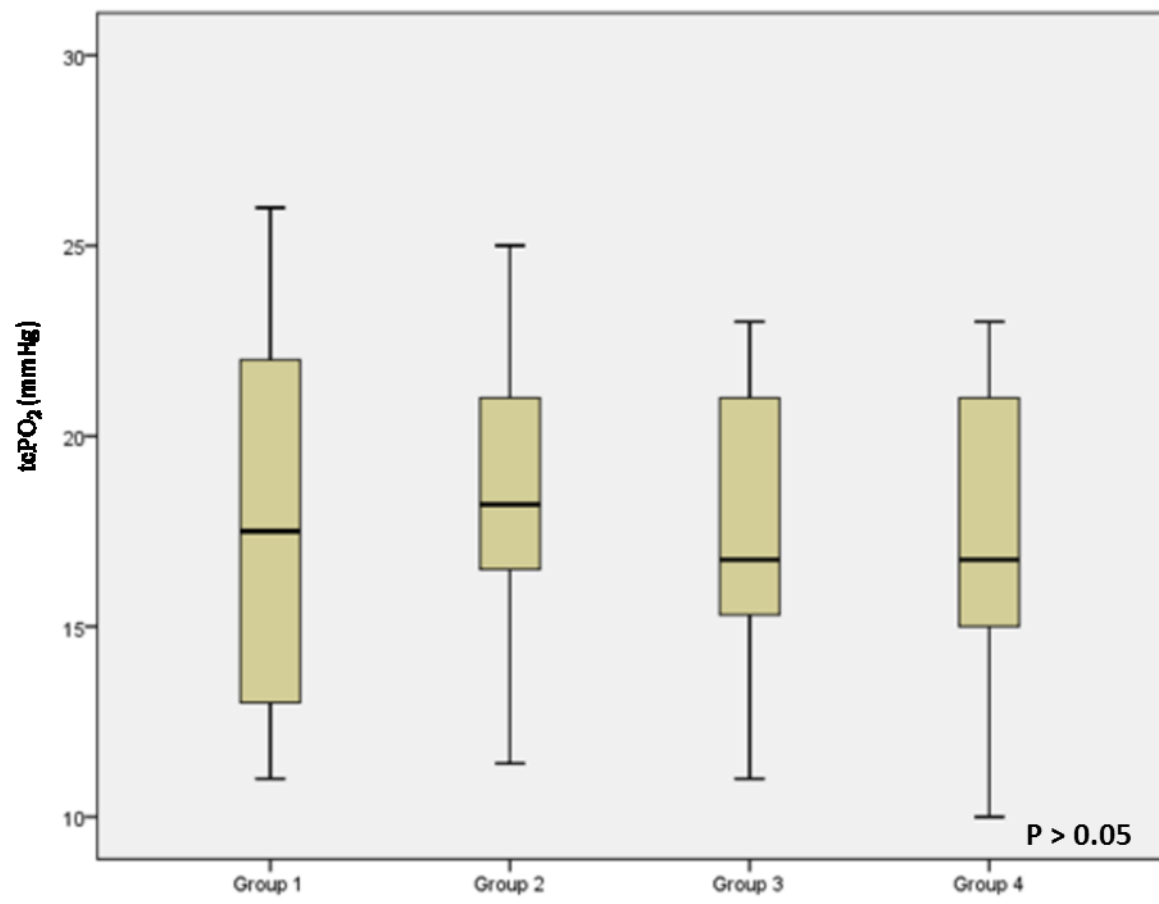


Figure 7.1: *tcPO₂ level (mmHg) in the four groups before exercise. There was no significant difference between the groups ($p > 0.05$)*

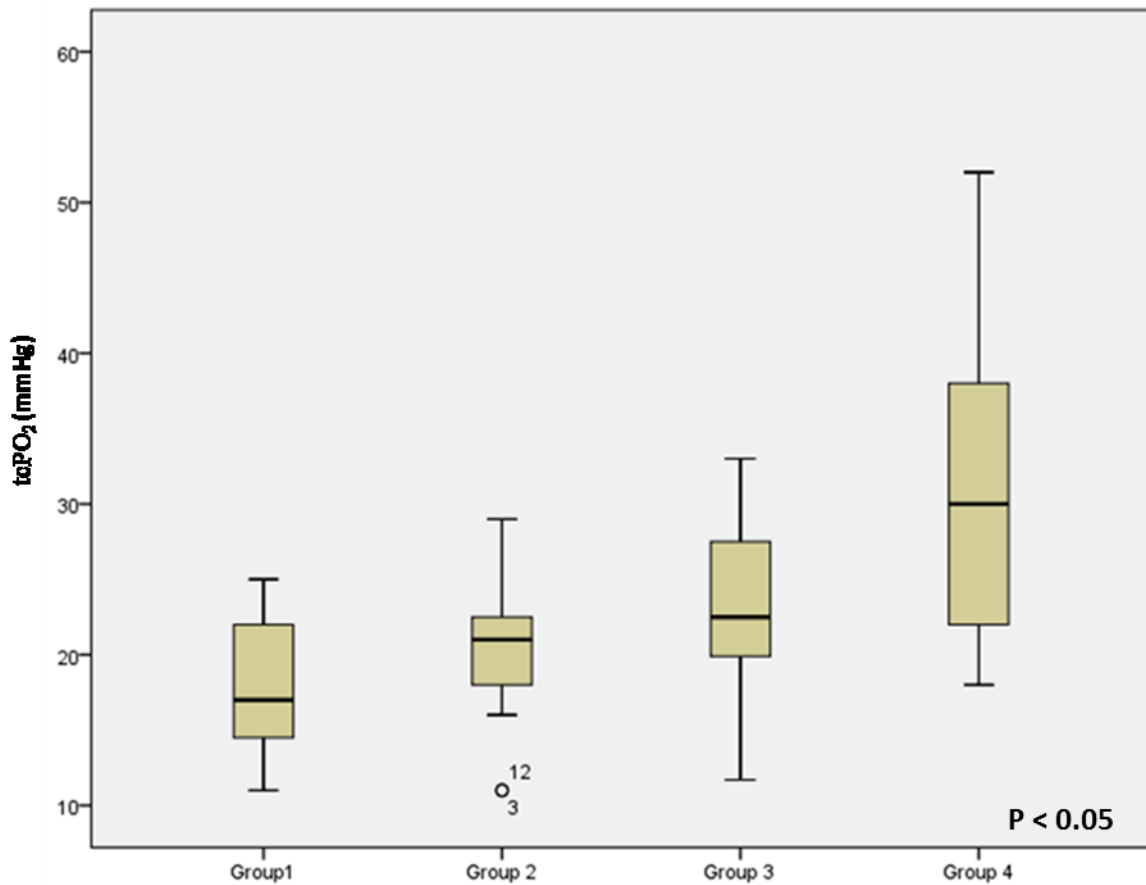


Figure 7.2: *tcPO₂ level (mmHg) in the four groups after exercise, there was a significant difference between the groups ($p < 0.05$).*

7.4 Statistical analysis

The methodology used was Linear Mixed Models for repeated measures adjusting for group, gender and age. Bonferroni correction was used for the comparisons between groups. Gender wasn't significant outcome measures.

Age was significant ($p < 0.001$) and the magnitude of its effect on tcPO₂ is given by -0.17 meaning that a unit increase in age corresponds to a 0.17 decrease in tcPO₂.

The difference between before and after varies across groups and this is shown in the table and graph below (*Table 7.2*) and (*Figure 7.3*). The “Mean” column is the estimated tcPO₂ mean for each group before and after adjusted for age.

Estimates ^a					
time	Group	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1	1	17.667 ^b	.829	16.015	19.319
	2	18.716 ^b	.796	17.130	20.302
	3	17.030 ^b	.786	15.463	18.597
	4	16.463 ^b	.792	14.885	18.042
2	1	17.717 ^b	1.426	14.876	20.559
	2	20.161 ^b	1.358	17.455	22.867
	3	22.360 ^b	1.352	19.665	25.056
	4	32.588 ^b	1.356	29.886	35.290

Table 7.2: tcPO₂ level before and after exercise in the four groups

a. Dependent variable: tcPO₂

b. Covariates in the model are evaluated at the following values: Age 65.218

Time 1: Measurements at the beginning of the study

Time 2: Measurements after 3 months

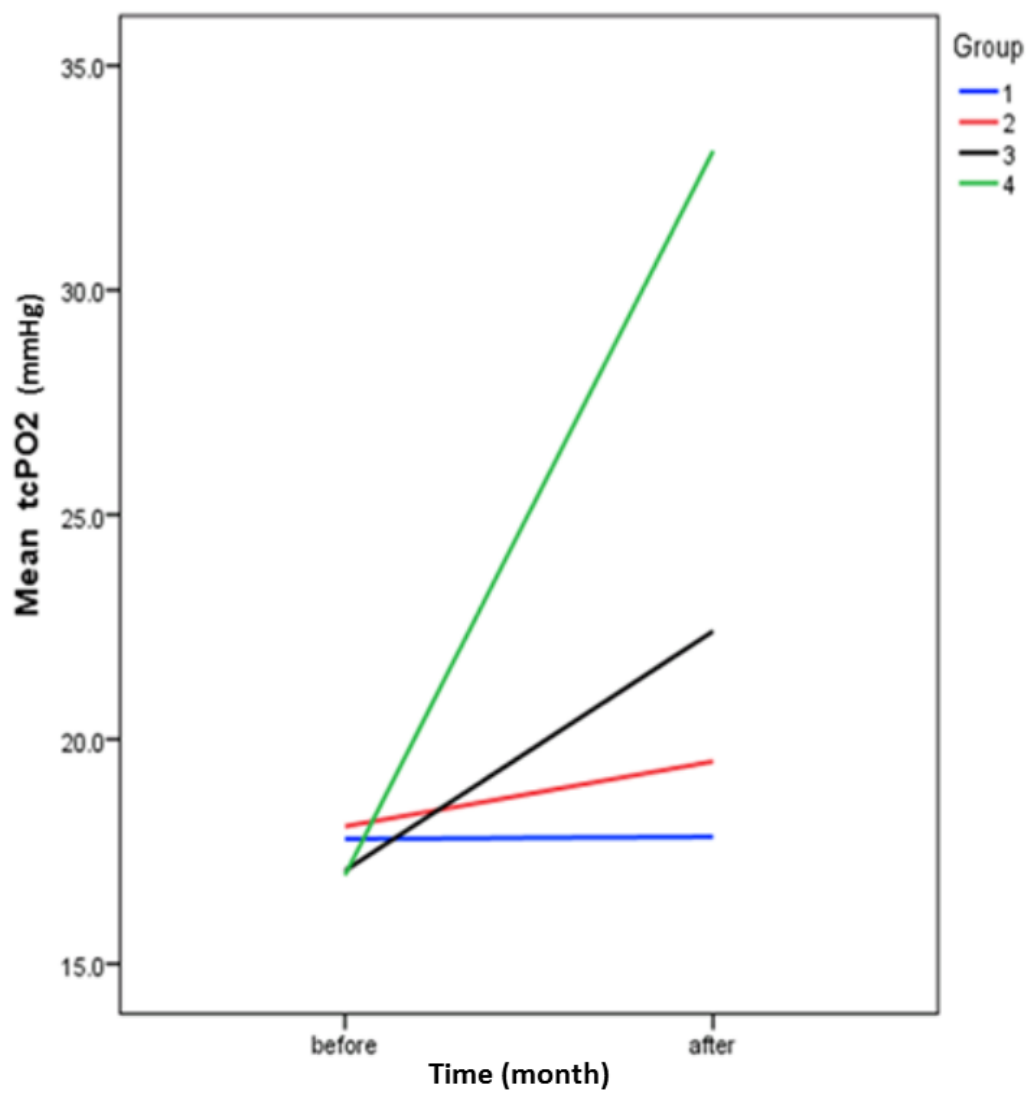


Figure 7.3: *tcPO₂ level (mmHg) before an and after exercise in the four group*

Time before: Measurements at the beginning of the study (month)

Time after: Measurements after 3 months (month)

The comparisons between groups before and after are below:

Pairwise Comparisons ^a						
Group	(I) time	(J) time	Mean Difference (I-J)	p-value ^c	95% Confidence Interval for Difference ^c	
					Lower Bound	Upper Bound
1	2	1	.050	.967	-2.323	2.423
2	2	1	1.445	.205	-.806	3.696
3	2	1	5.330	.000	3.079	7.581
4	2	1	16.125	.000	13.874	18.376

Table 7.3: Pairwise comparison between groups

Based on estimated marginal means

a. Dependent variable: $tcPO_2$

c. Adjustments for multiple comparisons: Bonferroni

I time: Measurements at the beginning of the study

J time: Measurements after 3 months

Only groups 3 and 4 show a significant increase - both with $p < 0.001$ (Table 7.3). Before exercise, there is no significant difference between groups. However after exercise, group 4 is significantly higher than groups 1, 2 and 3 and group 3 is higher than groups 1, 2 (all corresponding p-values < 0.001).

7.5 Discussion

In patients with chronic venous insufficiency (CVI), researchers also found that there is backflow of venous blood from diseased veins into dermal microcirculation, through incompetent valves. Additionally, in patients with CVI, low haemoglobin oxygen concentration in dermal microcirculation is considered to be the main factor for skin ulceration (Angel, Ramasastry, Swartz, Basford, & Futrell, 1987).

Studies show that when in standing or sitting position, the haemoglobin concentration (Chb) in dermal microcirculation is increased while haemoglobin oxygen saturation (Shb) is decreased and alternatively, when the body is active, like for example, during exercise the Chb is decreased and Shb is increased (Thum, Caspary, Creutzig, & Alexander, 1996). This alteration in the Chb and Shb is because, when the body is in sitting and standing position, the venous pump to the heart is lower as the calf muscle pump is not working, also venous pressure increases in the lower limbs in motionless individuals. Venous plexus filling leads to an increase in Chb. As a result, the venous plexus in legs with CVI are filled with deoxygenated blood while competent venous veins in healthy patients prevent an overfilling of dermal venous plexus with deoxygenated blood. These findings are supported by Franzeck et al., who further demonstrated a decrease in transcutaneous oxygen tension in venous ulcer patients, which can be significantly low (Franzeck, Haselbach, Speiser, & Bollinger, 1993).

Joachim, et al., suggested that sufficient haemoglobin oxygenation is maintained during walking and exercise only and the elimination of this factor whilst in the sitting position might contribute to the development of venous leg ulcer in CVI. Researchers have also suggested that venous ulceration is caused by microvascular ischemia, which may result from

more than one factor. This theory appears to be more logical and acceptable, and low tcPO₂ findings in venous ulcer could be a clear demonstration of microvascular ischaemia.

The findings of this study are consistent with the findings of researchers. The current study demonstrates that there are low tcPO₂ levels in patients, who are suffering from chronic venous insufficiency. These low values of tcPO₂ have been observed to improve significantly in participants, who had been exercising regularly for a period of three months. Patients, who performed regular exercise and wore compression bandaging, showed that their tcPO₂ measurements were slightly higher than participants who performed only exercise, both with p-values < 0.001. The explanation for that may be the additional effect of compression therapy. Also, the study presented no clear evidence whether the effect of exercise on the tcPO₂ measurements after 3 months is due to the effect of exercise in the last day or last few days or due to accumulation effect of the exercise during the whole 3 months. However, all the measurements were taken within one week of stopping the exercise. Therefore, the most acceptable explanation is that findings are a result of the accumulation effect of the exercise. Moreover, if the readings are presumed to be attributed to the immediate effect of the dorsiflexion, the findings would not reveal a significant difference in tcPO₂ level between the two measurements.

Thus, it can be inferred that regular exercise could improve venous circulation by regularly emptying the skin microcirculation from deoxygenated venous blood and replacing it with oxygenated arterial blood. This phenomenon might allow for an accelerated healing of venous ulcer in patients, who use compression stockings or bandaging and who also, at the same time, perform regular exercise. The findings also demonstrated that tcPO₂ values could

be a very useful measurement tool in following up patients with venous leg ulcer and for monitoring ulcer healing.

8 CHAPTER EIGHT: EFFECT OF EXERCISE ON ANKLE JOINT MOVEMENT IN VENOUS LEG ULCER PATIENTS

8.1 Introduction

Several studies have reported the effect of the range of ankle joint movement (ROM) on calf muscle function and eventually venous leg ulcer healing (Back, Padberg, Araki, Thompson, & Hosbon, 1995), (Yim, Vivas, Maderal, & Kirsner, 2014).

Effective calf muscle function depends mainly on the range of ankle joint movement. Studies show that normal function of calf muscle is associated with normal ankle joint movement especially dorsiflexion (Back, Padberg, Araki, Thompson, & Hosbon, 1995). During walking, the calf muscle veins stretch under the contraction of the calf muscle and this may be an important factor by which venous blood is squeezed up from lower limbs veins (Dix, Brooke, & Mc Collum, 2003).

The major two components that are responsible for calf muscle dysfunction in venous leg ulcer patients are reduction of ankle joint movement and wasting of the calf muscle. The normal range of ankle joint movement is around 55 degrees, while this range of ankle motion in chronic ulcer patients may drop down to 20 degrees (Back, Padberg, Araki, Thompson, & Hosbon, 1995).

8.2 Aims and rationale

1. Evaluation of the effect of exercise on ROM in venous leg ulcer patients.
2. Evaluation of the relationship between ROM and VLU healing.

8.3 Methods and materials

In the current study, forty patients of VLU patients underwent a 3 month trial of regular exercise. The details of the exercises are discussed in Chapter 5. These 40 patients were segregated in four groups. Two groups (Group 1 and Group 2) comprised of VLU patients, who performed exercise over three months and did not use compression stockings, while the other groups (Group 3 and Group 4) consisted of patients who are performed exercise and also used compression stockings throughout the 3 month period.

Group	Number of patients	Age (years)	M:F ratio	Ulcer size before exercise (cm)	BMI
Group 3 Exercise only	20	64.95±12.97*	10:10	3.27±2.70*	32.62±3.76*
Group 4 Exercise and compression therapy	20	62.15±14.05*	10:10	3.25±3.67*	30.85±4.28*

Table 8.1: Patient characteristics in group 3 and 4. *Values in mean±SD

All of the 40 participants, were checked for peripheral arterial occlusive disease, which was excluded by non-invasive tests (ankle pressure index (ABPI) >0.8 and < 1.2). In the study, participants were comparable at the baseline in terms of age, sex, body mass index and ulcer area (**Table 8.1**). Additionally, the colour duplex scan was performed for all examined subjects to demonstrate the presence of superficial, deep venous incompetence and absence

of proximal obstruction (*Table 5.1*). The inclusion and exclusion criteria for the subjects are discussed in Chapter 5.

During the study analysis, measurement of ROM (dorsiflexion and plantarflexion) was performed on two occasions. Once at the beginning of the trial and the second after 3 months of regular exercise. Measurement of ROM was performed with the use of a bi-plane goniometer, with the patient positioned in a sitting position with his or her knees, flexed at 90°. The participant's ankle joint was posited in a non-weight-bearing and neutral position. During measurements, the fulcrum was placed at the midpoint of the lateral malleolus, the stationary arm parallels the fibula and the moving arm was placed along the fifth toe. Measurements were taken at maximal dorsiflexion and plantarflexion, which the patient could achieve voluntarily. Those participants, who were able to move their ankle joints more or less with no pain or with little pain were included in the study and patients with severe arthritis or rheumatoid arthritis were excluded from the study. Each participant was given a phone call every 15 days, amounting to six times over the participation period to ensure that the participants strictly adhere to the exercise manual and to clarify any misunderstanding.

8.4 Results

Subjects maintained very good participation level, of the 40 patients participated in the study; all of them continued the study to the end.

Group	Number of patients	ROM At time 1	ROM At time 2
Group 3 Exercise only	20	20.28±2.63*	27.84±4.05*
Group 4 Exercise and compression therapy	20	20.51±2.67*	29.03±3.9*

Table 8.2: Range of ankle joint movement (ROM) findings. * Values in mean±SD

Time 1: Measurements at the beginning of the study

*Time 2: Measurements after 3 months.**

The median age of group 3 participants was 64.95±12.97 years, in group 4, median age was 62.15±14.05. Male to female ratio in the 2 groups were 10:10 (**Table 8.1**).

ROM for group 3 patients was 20.28±2.63 before exercise and 27.84±4.05 after exercise, an increase of 7.56 degrees ($p < 0.001$), in group 4 the ROM was 20.51±2.67 before exercise and 29.03±3.91 after exercise, an increase of 8.52 degrees ($p < 0.001$) (**Table 8.2**).

8.5 Statistical analysis

Trough out the report, time =1 is “before” and time =2 is “after”. The methodology used was Linear Mixed Models for repeated measures adjusting for group, gender and age. Bonferroni

correction was used for the comparisons between groups. Gender wasn't significant. Only the significant effects are showed.

The only significant effects were age and time. The magnitude of the effect of age on ROM is given by -0.15 meaning that with a unit increase in age corresponds to a 0.15 decrease in ROM (**Table 8.3**).

We can see from the table and graph below that there is a significant increase in ROM ($P < 0.001$) for both groups. However, there is no significant difference between groups either before or after (**Table 8.3**) and (**Figure 8.1**).

time * Group^a

time	Group	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1	3	20.530 ^b	.363	19.794	21.267
	4	20.328 ^b	.363	19.593	21.063
2	3	28.436 ^b	.647	27.126	29.747
	4	28.848 ^b	.637	27.557	30.139

Table 8.3: Comparison between groups

a. Dependent variable: ROM

b. Covariates in the model are evaluated at the following values: Age = 63.342

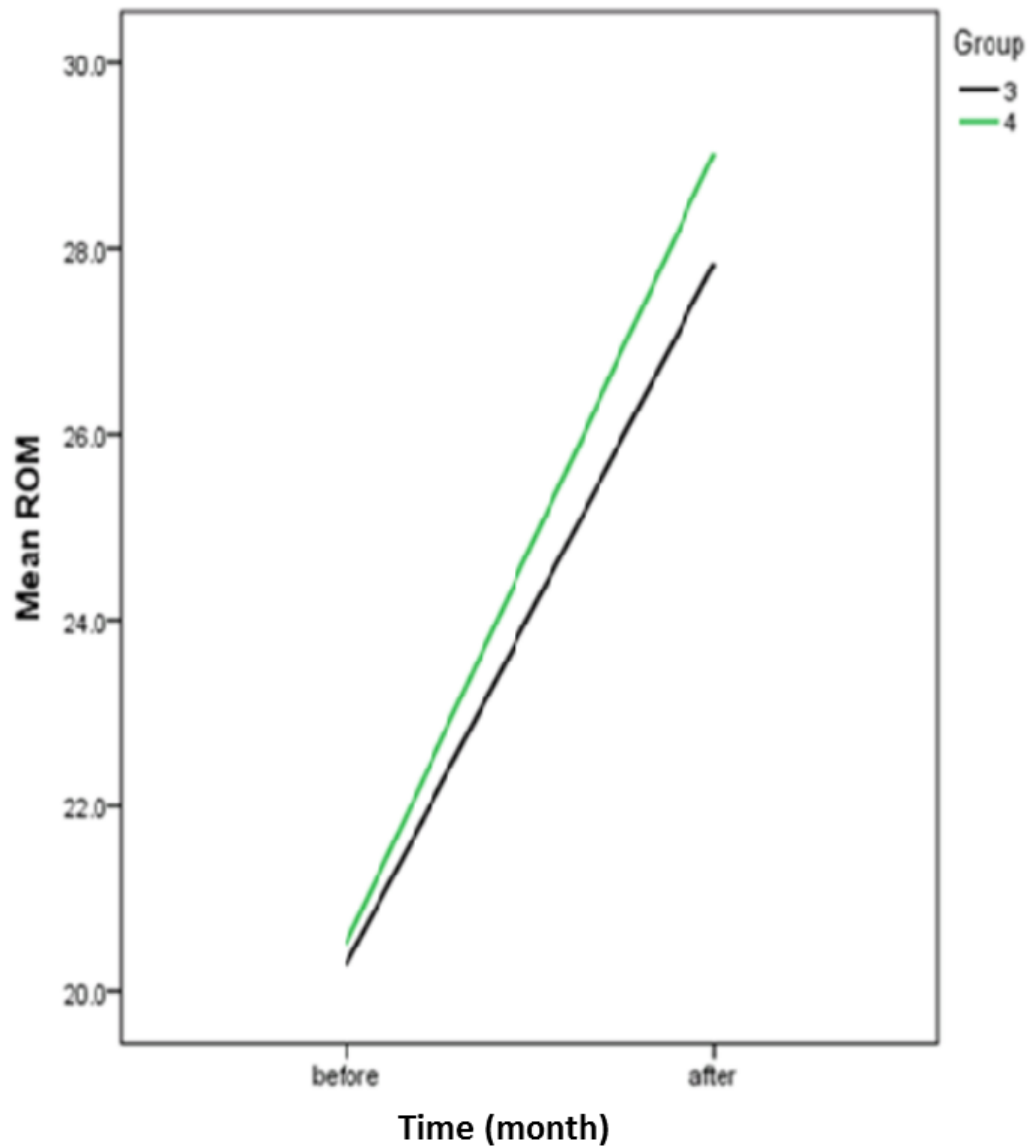


Figure 8.1: Mean ROM before and after exercise

Time before: Measurements at the beginning of the study (month)

Time after: Measurements after 3 months (month)

8.6 Discussion

Researchers demonstrated that exercise could increase ROM in VLU patients (Yim, Vivas, Maderal, & Kirsner, 2014). Davies JA, et al., demonstrated that home-based exercise programme can improve ankle joint movement significantly (Davies, Bull, Farrelly, & Wakelin, 2007). In their study, they recruited 11 patients with VLU and exercise consisted of movement of ankle joint against resistance, a thrice-weekly (5–10 min) for 24 weeks.

The current study involved recruitment of 40 patients with VLU and the exercise composed of 10 times dorsiflexion every hour during the waking hours of the patient. The researcher of the study decided that there is no need for resistance during exercise as it might cause injury to Achilles' tendon as most of VLU patients are elderly. Moreover, subjects were encouraged to perform rotational movements of ankle joint whenever possible. In addition, patients were advised about normal walking and heel to toe movement.

Researchers have demonstrated on various occasions that there is a strong association between ankle joint range of movement, calf muscle function and venous leg ulcer. Some also suggest that the limitation of ankle movement plays a significant part in venous leg ulcer development through deterioration of calf muscle function (Padberg, Johnston, & Sisto, 2004). Barwell and colleagues, in their study, demonstrated a significant improvement in venous ulcer healing in ankles showing a good range of ankle joint movement (Barwell, et al., 2001). Kugler, et al., also recognised in their study that artificial restriction of the ankle joint movement in healthy volunteers will affect calf muscle function (Kugler, Strunk, & Rudofsky, 2001).

Another interesting study by Tierney and colleagues demonstrates that significant dysfunction of calf muscle function lasts for 4 months after ankle joint fracture in the absence of any venous system impairment (Tierney, Burke, & Fitzgerald, 1993). Other clinical problems associated with ankle joint, include rheumatoid arthritis and osteoarthritis that could produce calf muscle pump dysfunction and venous ulcer. Gaylarde PM, et al., discussed these hypotheses in their study, however, randomised control study is not available to explore this concept (Gaylarde, Dodd, & Sarkany, 1990).

Researchers have also demonstrated that patients with varicose veins, who have no venous ulcer or lipodermatosclerosis may suffer from a significant reduction in ROM. whereas, improvement of ankle joint movement could improve venous function in those patients (Tierney, Burke, & Fitzgerald, 1993) (Dix, Brooke, & Mc Collum, 2003).

The above scientific discussion is corroborated by the fact that limitation of movement or lack of exercise may impair venous return mechanism because there is not enough blood being pumped to the heart, and at the same time, a lack of physical activity can lead to a dysfunctional calf muscle due to disuse of the muscle. In venous ulcer patients, the ulcer might get worse because there is not enough oxygenated blood circulating. Worsening of venous ulcer will have an additional impact on patient's quality of life as a VLU patient becomes more isolated, which leads to less mobility. This lack of mobility, subsequently leads to calf muscle dysfunction, making the venous ulcer worse. Therefore, it is can be safely inferred that exercise could cut this vicious cycle.

The study therefore comes to the conclusion that regular exercise in VLU patients increases the range of ankle joint movement (*Table 8.2*), and might play a significant role in venous ulcer healing (

Table 9.1). This improvement of the ankle movement could result in a greater impact on the patient's social life and make them more active and sociable. In fact, there is a good possibility that this outcome could easily be doubled if the exercise is performed through a group patient programme. Arranging patient group exercise through ulcer clinics or through community healthcare service could be more beneficial since it would provide the patients with an opportunity to overcome social isolation and exchange ideas and experience between patients.

Therefore, the current study showed that improvement of ankle joint movement in VLU patients is achievable by home-based exercise, which is demonstrated through a very good participation in a long three month period. Additionally, it also showed there is a positive relation between increase ROM and ulcer healing rate. The studies recommend that further studies should be conducted on this aspect of VLU patients and their recovery, which can be extended to be a multicentre randomised clinical trial.

9 CHAPTER NINE: EFFECT OF EXERCISE ON ULCER SIZE IN VENOUS LEG ULCER PATIENTS

9.1 Introduction

The main goal of VLU care is complete healing of the ulcer. Venous leg ulcer healing parameters are many, and ulcer area may be the most important parameter. It is difficult to measure the healing of ulcer unless the size of the ulcer decreases or the ulcer area disappears completely (Skene, Smith, Dore, Charlett, & Lewis, 1992) (Oien, Hakansson, Hansen, & BJellerup, 2002).

VLU is a chronic problem with very complicated pathophysiology background. Also, shrinkage of the ulcer takes a very long time as most of VLU patients are elderly with many comorbidities and limitation of physical activity. Moreover, VLU ulcer healing can recur and frequently take up the form of a vicious circle of healing and recurrence. Consequently, for such complicated ulcer, it is essential to identify reliable and easily measurable parameters of healing. It is proposed that measuring ulcer area may be the most reliable parameter to assess VLU healing, as ulcer size can be measured with the use of different tools. The advantage of this parameter is that it can be recorded precisely at the measurement and is easier to follow up.

Methods for assessing ulcer area may vary from simple measurement with a simple ruler (Ruler-based assessment) to transparency tracings, photographic methods, video image analysis, stereophotogrammetry, and a sophisticated computer programme. Choosing the suitable method of ulcer size measurement is dependent on certain factors such as the needs and resources available, the volume of the ulcer and irregularity of the ulcer (Tallman, Muscare, Carson, Eaglstein, & Falanga, 1997).

9.2 Aims and rationale

1. To evaluate the effect of exercise on ulcer size in venous leg ulcer patients.
2. To evaluate the relationship between ulcer healing and exercise.

9.3 Results

During the study period, the ulcer area reduced in size in group 3 (exercise only) and group 4(exercise and compression treatment) (

Table 9.1). Group 3: there was reduction in ulcer size from 3.27 ± 2.70 before exercise to 2.56 ± 2.34 after exercise. Group 4: The reduction is more significant than group 3, from 3.25 ± 3.67 before exercise to 1.59 ± 3.11 after exercise. There is no real difference in ulcer size in group 1 and group 2.

Patients who performed no exercise			
Group	Number of patients	Ulcer size at time 1 (cm)	Ulcer size at time 2 (cm)
Group 1 Control group	18	2.70 (5.25-1.29)*	3.20 (5.21-2.55)*
Group 2 Compression therapy	20	2.52 (4.18-0.63)*	2.52 (3.34-0.63)*
Patients who performed exercise			
Group 3 Exercise only	20	2.63 (4.02-1.35)*	2.50 (4.12-0.48)*
Group 4 Exercise and compression therapy	20	2.39 (3.51-1.16)*	0.72 (1.17-0.12)*

Table 9.1: Ulcer size findings. *Values presented as median

Time 1: Measurements at the beginning of the study

Time 2: Measurements after 3 months.

9.4 Statistical analysis

The methodology used was Linear Mixed Models for repeated measures adjusting for group, gender and age. Bonferroni correction was used for the comparisons between groups. Gender wasn't significant in any of the four outcome measures.

This outcome measure is quite skewed so the log transformation was used to obtain reliable p-values. Age wasn't significant ($p < 0.001$).

The difference between before and after varies across groups and this is shown in the table and graph below. The "Mean" column is the estimated Size (logarithm of size) mean for each group before and after adjusted for age (*Table 9.2*), (*Figure 9.1*).

Estimates^a

time	Group	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
1	1	1.020	.221	.581	1.460
	2	.453	.209	.036	.870
	3	.906	.209	.489	1.323
	4	.752	.221	.312	1.192
2	1	1.199	.257	.687	1.712
	2	.463	.244	-.023	.949
	3	.389	.244	-.097	.875
	4	-.467	.263	-.991	.058

Table 9.2: The difference in ulcer size between groups

a. Dependent variable: Ulcer size

Time 1: Measurements at the beginning of the study

Time 2: Measurements after 3 months

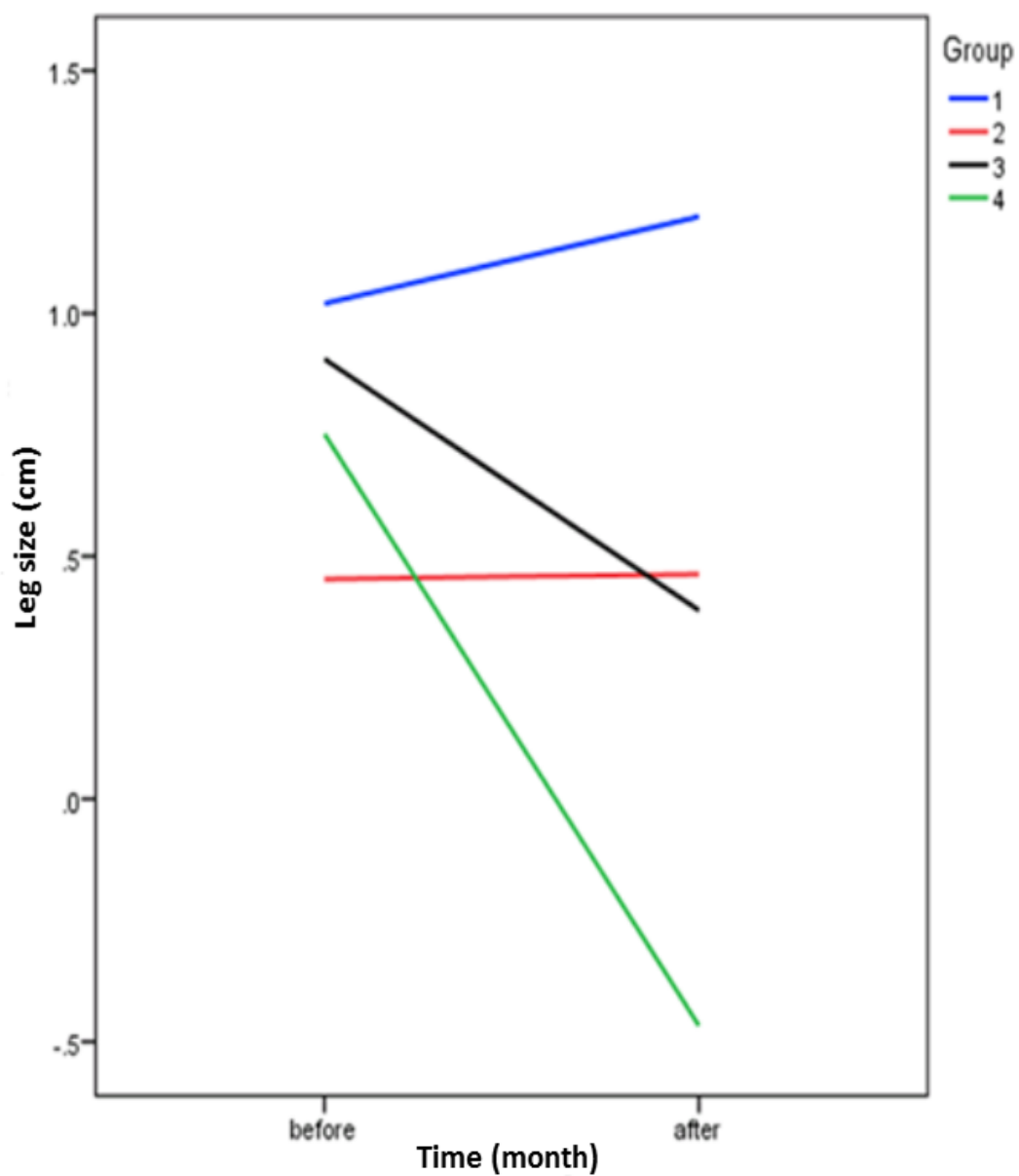


Figure 9.1: The difference in ulcer size between groups (cm), *values in mean

Only groups 3 and 4 show a significant decrease - with p-values = 0.001 and < 0.001 respectively (*Table 9.2*).

Before, there is no significant difference between groups. However after, group 4 is significantly lower than groups 1 ($p < 0.001$) and it is borderline lower than group 2 ($p = 0.069$).

9.5 Discussion

The measurement of ulcers size is essential to evaluate or assess the effectiveness of treatment of VLU. Several methods have been described by researchers in studies to measure venous leg ulcers; however, no single method is able to provide a precise measurement of ulcer size (Coleridge smith & Scurr, 1989,).

VLU measurement techniques can be divided into two groups, namely, contact and non-contact techniques. Recently non-contact techniques have become more popular because they offer a reduced or no wound contamination and cause less discomfort to the patients. However, concerns have been raised about the use of these methods and some researchers have argued that non-invasive techniques can be expensive, time-consuming and impractical from a clinical perspective (Brown, 2003).

In general, venous leg ulcer is difficult to measure as its border is characterised by irregularity. The tracing and counting squares from graph paper technique is another option that can be considered. Some investigators, however, believe that there are a number of serious errors that are associated with this technique, especially when measuring large and irregular ulcers (Brown, 2003). Photography is a non-contact technique of measuring ulcers;

however, it is surrounded by controversy. Although it provides a visual recording of the ulcer and can provide more information about the wound such as the base, type of tissue and wound margin, there might be problems with the camera resolution. Moreover, photography consumes time, is expensive and requires a skilled medical photographer to take the most accurate photographs but unfortunately, most clinicians do not possess such photography skills.

The ruler method provides a simple, easy to perform technique that does not demand specific skills to administer and is suitable for use in the clinical settings. Although, some researchers believe that the real value may be overestimated (Oien, Hakansson, Hansen, & BJellerup, 2002). During the current study analysis, it was found that the method of ruler measurement is reliable, easy to use and also provides a capability to fulfil the aims of the study. It is able to give a clear idea about the effect of exercise on ulcer size.

Therefore, this study concludes that exercise has a real effect on VLU healing as the size of the ulcer was seen to have decreased significantly in patients, who had performed exercise for a 3 month period (*Figure 9.2*), (*Figure 9.3*).



Figure 9.2: Venous leg ulcer before and 3 months after exercise (exercise group only)



Figure 9.3: Venous leg ulcer before and 3 months after exercise (exercise and compression group)

In conclusion exercise was seen to have a significant effect on VLU healing, especially if used in conjunction with compression stockings. It would be advisable that our findings be confirmed through further multicentre randomised clinical trials.

10 CHAPTER TEN: DISCUSSION

10.1 Introduction

Chronic venous insufficiency is associated with venous hypertension that leads to abnormal microvascular alteration. This is presented clinically, as skin changes, in the form of oedema and lipodermatosclerosis and eventually venous leg ulcer.

The treatment of venous leg ulcer has not changed since the last 2000 years (Jonathan & John, 2007). The current treatment consists of the compression therapy coupled with bandaging of the diseased limb; however, the compression therapy is not a suitable option for all patients with venous ulcer. Such as, for those patients with the peripheral vascular disease, where it could have developed into ischemia of the lower limbs due to the compression of arteries. Additionally, it is seen that some patients could develop itchiness and discomfort with compression and bandaging, especially in warmer weather. Moreover, most of the venous ulcer patients are elderly and suffer from other comorbidities such as osteoarthritis and other debilitating illnesses. Therefore, this projects a need for more experienced staff to help patients, and it is observed that in most circumstances they are not always available. This leads to noncompliance, which is very common with noncompliance rates varying from 9.7% to 80% (Moffat, Kommala, Dourdin, & Choe, 2009). Moreover, the recurrence rates are as high as 70% with such modality of treatment (Barwell, et al., 2004).

Many modalities of treatment have been used to treat VLU, however, there is no single effective treatment and therefore, compression therapy remains the standard treatment worldwide. The next most popular treatment is surgery, and mainly conventional surgery, although endogenous surgery has become more popular recently. However, the surgical intervention is not without pitfalls. Firstly, the percentage of people who present with

superficial saphenous and ankle perforating veins incompetence constitute only one-third of venous ulcer patients (Darke & Penfold, 1992). Secondly, many patients with VLU are not fit for surgery and are not willing to undergo surgical procedures, mainly due to the fact that majority of VLU patients are elderly and have other comorbidities (Warwick, Thomas, & Dood, 2007).

Therefore, there is a real need for simple effective treatment either as a part of a multidisciplinary approach or as a preventive measure or both. There is a growing awareness that a suitable exercise programme could help in decreasing venous hypertension with consequent improvement in ulcer healing.

This study aims to investigate the relationship between exercise and healing of venous leg ulcer by identifying the level of tcPO₂, LDF blood flow measurement, changes in the range of ankle joint movement and ulcer size in the venous ulcer area before and after a period of regular exercise. In addition, the study records and documents the changes that occur on the microvascular level in the skin in patients performing the exercise as compared to those who were not performing any exercise. The work described in this thesis has been achieved by a series of experimental clinical studies, involving the gaiter area, on the perimalleolar region, which is the area most frequently involved with a venous ulcer.

In chapter 6, the laser Doppler Flowmetry measurements (RF) were measured in the four groups of patients at the beginning of the study and again at the end. These measurements were compared within the groups themselves, before and after exercise, also the LDF measurements were compared between the different groups in the study. In a comparison between the groups before and after exercise, the LDF readings showed significant

differences between (group 1 and 2), those not performing exercise, and (group 3 and 4), where exercise was being performed. RF showed a significant decrease in group 3 and 4, mainly group 4 while there was no change in groups 1 and 2. This indicates that regular exercise resulted in changes on microvascular level in VLU patients, whilst at the same time it also indicates healing of the venous ulcer since the RF decrease with the healing of wounds.

In the following study (chapter 7), the results show that tcPO₂ measurements increased dramatically in patients, who were performing the exercise while tcPO₂ readings remained nearly the same in subjects, who were not performing the exercise. The findings also revealed low tcPO₂ levels in all patients who were involved with VLU. These results indicated that tcPO₂ levels are low in venous ulcer area, which is coherent with several other previous studies. It also showed that tcPO₂ records changed in response to the external effect of exercise. Moreover, the study reveals a negative relationship between the tcPO₂ level and ulcer size indicating that the presence of high or normal tcPO₂ levels is related to VLU healing (decrease in ulcer size). In conclusion, the tcPO₂ level is a good indicator for microvascular changes in venous ulcer area and can be used as a parameter for ulcer healing.

The range of ankle joint movement measurements (chapter 8) indicates a parallel relationship between exercise and ROM. To arrive at the findings, the study compared ROM in two groups of VLU participants group 3 and 4, (those individuals who performed the exercise). No significant difference was presented between the two groups before starting the exercise and the same was applicable for post-exercise measurements. Alternatively, a significant difference was seen within each group between pre-exercise time and post-exercise time. In

addition, ulcer size was seen to have a negative relationship with ROM in both groups, as the ulcer size decreased dramatically in both groups while ROM increased significantly in those groups. If the improvement of ROM played a role in decreasing ulcer size and healing or if this decrease in the ulcer size was due to exercise only, needs further investigations.

In chapter 9, the study evaluates the effect of regular exercise on venous ulcer size. Ulcer size is compared within groups between pre-exercise time and post-exercise time. The findings are similar in the control group and the compression therapy group, participants in both groups did not perform the exercise. In both of the groups, where exercise was performed, the ulcer size is revealed to decrease significantly ($p < 0.001$) after 3 months of exercise. The decrease in ulcer size in participants, who wore compression stockings during the exercise, was more significant compared with those performed the exercise only, the explanation for this may be due to the additional effect the compression stockings had. The result of this study has shown that exercise had a significant effect on VLU size and that this effect may be enhanced further with compression therapy.

In conclusion, the study reveals that exercise has a demonstrable effect on VLU healing parameters and these parameters may be seen as a useful tool when evaluating the microvascular changes in venous ulcer area and can be used as a measurement for healing and follow-up of venous leg ulcer.

10.2 Future work

The results described in these clinical studies have revealed that exercise does have an influence on the venous ulcer healing parameters. The study has also demonstrated the need for more research work in this domain. Future work is important for the standardisation of ulcer healing parameters in different groups of patients, at different centres throughout the world and with a larger number of participants.

In addition, the influence of exercise on VLU needs to be investigated in other groups of patients that have not been included in this study, such as diabetes mellitus, PVD and CVD individuals. It is evident that these patients would have other comorbidities, which might affect the physiological measurements, whilst at the same time these patients do constitute a significant number of VLU patients.

Futuristic research work could focus on examining and evaluating the effect of exercise on VLU through histopathological studies, which might provide a better understanding of the influence of exercise on VLU healing. Also, it would be beneficial to compare the changes in microcirculatory parameters with those of histological changes and these findings might subsequently help in designing of standard evaluation protocols.

The work that is needed should consider and incorporate other types of treatments, such as, surgery or sclerotherapy. Future studies could aim at investigating the effect of exercise on patients with previous venous surgery as a preventive measure for post-surgical recurrence.

The study shows that it is important to evaluate the effect of exercise on the quality of life in VLU patients, as the majority of VLU patients are elderly and suffer from comorbidities, varying disabilities and social isolation.

Finally, the positive effect of exercise on human beings, whether from a physical or a social point of view is well recognised and further work to establish exercise as an integral part of leg ulcer treatment is well justified.

REFERENCES

- Abbade, L., & Lastoria, S. (2005). International Journal of Dermatology. *Venous ulcer: epidemiology, physiopathology, diagnosis and treatment*, 44, 449–456.
- Abramson, D., & Fierst, S. (1946). Arterial blood flow in extremities with varicose veins. *Arch Surg*, 964.
- Albrechtsson, U., & Olsson, C. (1976). Thrombotic side effects of lower limb phlebography. *Lancet*, 723-724.
- Angel, M., Ramasastry, S., Swartz, W., Basford, R., & Futrell, J. (1987). The causes of skin ulcerations associated with venous insufficiency; a unifying hypothesis. *Plast Reconstr Surg*, 97(2), 289-297.
- Araki, C., Back, T., Padberg, F., Thompson, P., Jamil, Z., Lee, B., . . . Hobson, R. (1994). The significance of calf muscle pump function in venous ulceration. *J Vasc Surg*, 20(6), 872–877.
- Arnold, M., & Barbu, A. (2006). Nutrition and wound healing. *Plast Reconstr Surg*, 117(7S), 42S-58S. doi:10.1097/01.prs.0000225432.17501.6c
- Aronson, D., & Thomas, D. (1985). Experimental studies on venous thrombosis: effect of coagulants, procoagulants and vessel contusion. *Thromb Haemost*, 866-870.
- Back, T., Padberg, F. J., Araki, C., Thompson, P., & Hosbon, R. (1995). Limited range of motion is a significant factor in venous ulceration. *J VAsc SURG*, 22(5), 519-523.

- Barwell, J., Davies, C., Deacon, J., Harvey, K., Minor, J., Sassano, A., . . . Poskitt, K. (2004). Comparison of surgery and compression with compression alone in chronic venous ulceration (ESCHAR study): randomised controlled trial. *Lancet*, 363(9424), 1854-1859.
- Barwell, J., Taylor, M., Deacon, J., Davies, C., Whyman, M., & Poskitt, K. (2001). Ankle motility is a risk factor for healing of chronic venous leg ulcers. *Phlebology*. *Phlebology*, 16, 38–40.
- Bazigou, E., Lyons, O. A., Smith, A., Venn, G. E., Cope, C., Brown, N. A., & Makinen, T. (2011). Genes regulating lymphangiogenesis control venous valve formation and maintenance in mice. *J Clin Invest*, 121, 2984-2992.
- Beavers, K., Hsu, F., Isom, S., Kritchevsky, S., Church, T., Goodpaster, B., . . . Niklas, B. (2010). Long-term physical activity and inflammatory biomarkers in older adults. *Med Sci Sports Exerc*, 42(12), 2189-2196. doi:10.1249/MSS.0b013e3181e3ac80
- Belcaro, G. (1997). *Venous hypertensive microangiopathy: Evaluation with laser Doppler flowmetry*. London: University of London.
- Belcaro, G., Rulo, A., Vasdeski, S., Williams, M., & Nicolaides, A. (1989). Combined evaluation of postphlebotic limbs by laser Doppler flowmetry and trans-cutaneous PO₂/PCO₂ measurements. *VASA*, 17(4), 259-261.
- Bergan, J. J., Schmid-schonbein, G. W., Coleridge-smith, P. D., Nicholaides, A. N., Boisseau, M. R., & Eklof, B. O. (2006). Chronic Venous Disease. *N engl j med*, 355, 488-498. doi:10.1056/NEJMra055289

- Bergan, J., Murray, J., & Greason, K. (1996). Subfascial endoscopic perforator vein surgery: a preliminary report. *Ann Vasc Surg*, 10(3), 211-219.
- Bianchi, J., Zamiri, M., Mc Intosh, H., Dawe, R., & Douglas, W. (2008). Pulse oximetry index: a simple arterial assessment for patients with venous disease. *J Wound Care*, 17(6), 253–258.
- Bishop, A. (2008). Role of oxygen in wound healing. *J Wound Care*, 17(9), 399-402.
doi:<http://dx.doi.org/10.12968/jowc.2008.17.9.30937>
- Blalock, A. (1929). Oxygen content of blood in patients with varicose veins. *Arch Surg*, 19, 898-905.
- Bloemenkamp, K., Rosendaal, F., Helmerhorst, F., Buller, H., & Vandenbroucke, J. (1995). Enhancement by factor V Leiden mutation of risk of deep-vein thrombosis associated with oral contraceptives containing a third-generation progestagen. *Lancet*, 346, 1593–1596.
- Brem, H., Kirsner, R., & Falanga, V. (2004). Protocol for the successful treatment of venous ulcers. *American Journal of Surgery*, 1S–8S.
- Briggs, M., Nelson, E. A., & Martyn-St James, M. (2012). *Cochrane Database of Systematic Reviews*, CD001177. doi:10.1002/14651858.CD001177.pub3
- Broussard, C. (2004). Hyperbaric oxygenation and wound healing. *J Vasc Nurs*, 22(2), 42–48. doi:10.1016/j.jvn.2004.03.001
- Brown, D. (2003). Comparing different ulcer measurement techniques: a pilot study. *Primary Intention*, 11(3), 132-134.

- Browse , M., & Burnand, K. (1982). The cause of venous ulceration. *Lancet*, 243-245.
- Browse, N., Burnand, K., Irvine, A., & Wilson, N. (1999). *Disease of the veins* (2nd ed.). London/New York: Arnold.
- Bucalo, B., Eaglstein, W., & Falanga, V. (1989). The effect of chronic wound fluid on cell proliferation in vitro. *J Invest Dermatol*, 92, 539.
- Burnand, K., Whimster, I., Naidoo, A., & Browse, N. (1982). Pericapillary fibrin in the ulcer-bearing skin of the leg: the cause of lipodermatosclerosis and venous ulceration. *Br Med J*, 285(6348), 1071-1072.
- Burton, D., Stokes, K., & Hall, G. M. (2004). Physiological effects of exercise. *Critical Care & Pain*, 4(6), 185-188. doi:10.1093/bjaceaccp/mkh050
- Butler, C., & Coleridge smith, P. (1994). Microcirculatory aspects of venous ulceration. *J Dermatol Surg Oncol*, 20(7), 474-480.
- Caggiati, A., & Bertocchi, P. (2001). Regarding fact and fiction surrounding the discovery of the venous valves. *J Vasc Surg*, 33(6), 1317.
- Caggiati, A. (2013). The venous valves of the lower limbs. *Phlebology*, 20(2), 87-95.
- Callam, M. J., Ruckley, C. V., Hrper, D. R., & Dale, J. J. (1985). Chronic ulceration of the leg: extent of the problem and provision of care. *BMJ*, 290(6485), 1855-1856.
- Cheatle, T., Sarin, S., Coleridge Smith, P., & Scurr, J. (1991). The pathogenesis of Skin Damage in Venous Disease: A review. *Eur J Vasc Surg*, 5(2), 115-123.

- Cheryl, M., Michael, D., Hughes, B. R., & Robert, W. (2006). Accelerated Wound Healing: Multidisciplinary Advances in the Care of Venous Leg Ulcers. *Angiology*, 57, 139-144.
- Cina, C., Katsamouris, A., Megerman, J., Brewster, D., Strayhorn, E., Robison, J., & Abott, W. (1984). Utility of transcutaneous oxygen tension measurements in peripheral arterial occlusive disease. *J Vasc Surg*, 1(2), 367-371.
- Clyne, C., Ramsden, W., Chant, A., Webster, & JH. (1985). Oxygen tension on the skin of the gaiter area of limbs with venous disease. *Br J Surg*, 72(8), 644-647.
- Cockett, F. (1956). The pathology and treatment of venous ulcers of the leg. *Br J Surg*, 43, 260-278.
- Colbert, L., Visser, M., Simonsick, E., Tracy, R., Newman, A., Kritchevsky, S., . . . Harris, T. (2004). Physical activity, exercise, and inflammatory markers in older adults: findings from the Health, Aging and Body Composition Study. *J Am Geriatr Soc*, 52(7), 1099-1104.
- Coleridge smith, P., & Scurr, J. (1989,). Direct method of measuring venous ulcers. *Br J Surg*, 76(7), 689. doi:10.1002/bjs.1800760712
- Coleridge Smith, P., Thomas, P., Scurr, J., & Dormandy, J. (1988). Causes of venous ulceration: a new hypothesis. *Br MED J*, 296(6638), 1726-1727.
- Coleridge-Smith, P. D. (2009). Leg ulcer treatment. *J Vasc Surg*, 49(3), 804-808.
- Coleridge-smith, P., Hasty, J., & Scurr, J. (1990). Venous stasis and vein lumen changes during surgery. *Br J Surg* 1990, 77(9), 1055–1059.

- Collins, L., & Seraj, S. (2010). Diagnosis and treatment of venous ulcers. *American Family Physician*, 8, 989–996.
- Comerota, A., Stewart GJ, Alburger, P., Smalley, K., & White, J. (1989). Operative venodilation: a previously unsuspected factor in the cause of postoperative deep vein thrombosis. *Surgery*, 106(2), 301–309.
- Conconi MT, B. S. (2003). Effects of hyperbaric oxygen on proliferative and apoptotic activities and reactive oxygen species generation in mouse fibroblast 3T3/J2 cell line. *J Invest Med*, 51, 227–232.
- Darke, S., & Penfold, C. (1992). Venous ulceration and saphenous ligation. *Eur J Vasc Surg*, 6(1), 4–9. doi:10.1016/S0950-821X(05)80086-6
- Davies, J., Bull, R., Farrelly, I., & Wakelin, M. (2007). A home-based exercise programme improves ankle range of motion in long-term venous ulcer patients. *Phlebology*, 22(2), 86–89.
- De Borst, G., & Molll, F. (2012). Percutaneous venous valve designs for treatment of deep venous insufficiency. *J Endovasc Ther*, 19(2), 291–302.
- De Carvalho, M. (2015). Comparison of outcomes in patients with venous leg ulcers treated with compression therapy alone versus combination of surgery and compression therapy: a systematic review. *J Wound Ostomy Continence Nurs*, 42(1), 42-46. doi:10.1097/WON.0000000000000079

- Del Guercio, R., Leonardo, G., & Arpaia, M. (1986). Evaluation of postischemic hyperemia on the skin using laser Doppler velocimetry: study on patients with claudicatio intermittens. *Microvasc. Res*, 32(3), 289–299.
- Delis, K. T., Husman, M., Kalodiki, E., Wolfe, J. H., & Nicolaides, A. N. (2001). In situ hemodynamics of perforating veins in chronic venous insufficiency. *J Vasc Surg*, 33(4), 773–782.
- Dell, J. J., Callam, M. J., Ruckley, C. V., Harper, D. R., & Berrey, P. N. (1983). Chronic ulcers of the leg: a study of prevalence in a Scottish community. *Health Bull Edinb*, 41, 310-314.
- Dix, F., Brooke, R., & Mc Collum, C. (2003). Venous disease is associated with an impaired range of ankle movement. *Eur J Vasc Endovasc Surg*, 25(6), 556-561.
- Dow, J. (1952). The venographic diagnosis of the method of recurrence of varicose veins. *Br J Radiol*, 25, 382.
- Dumville, J., Desphande, S., O'Meara, S., & Speak, K. (2013). Foam dressings for healing diabetic foot ulcers. *Cochrane Database Syst Rev*, 9, CD009111.
- Dustman, R., Rushling, R., Russell, E., Shearer, D., Bonekat, H., Shigeoka, J., . . . Bradford, D. (1984). Aerobic exercise training and improved neuropsychological function of older individuals. *Neurobiol Aging*, 5(1), 35–42.
- Eberhardt, R. T., & Raffetto, J. D. (2014). Chronic Venous Insufficiency. *Circulation*, 333-346. doi:10.1161/01.CIR.0000164199.72440.08

- Eberhardt, R., & Faffetto, J. (2005). Contemporary Reviews in Cardiovascular Medicine; Chronic Venous Insufficiency. *Circulation*, 2398-2409.
- Eickhoff, J., & Jacobsen, F. (1980). Correlation of transcutaneous oxygen tension to blood flow in heated skin. *Scand J Clin Lab Inves*, 40, 761-765.
doi:10.3109/00365518009095593
- Emery, C., & Blumenthal, J. (1991). Effects of physical exercise on psychological and cognitive functioning of older adults. *Ann Behav Med*, 13(3), 99-107.
- Emery, C., Kiecolt-Glaser, J., Glaser, R., Malarkey, W., & Frid, D. (2005). Exercise accelerates wound healing among healthy older adults: a preliminary investigation. *J Gerontol*, 60(11), 1432- 1436. doi:10.1093/gerona/60.11.1432
- Fagrell, B. (1982). Microcirculatory disturbances-the final cause for venous ulceration. *VASA*, 11, 101-103.
- Fairey, A., Courneya, K., Field, C., Bell, G., Jones, L., Martin, B., & Mackey, J. (2005). Effect of exercise training on C-reactive protein in postmenopausal breast cancer survivors: a randomized controlled trial. *Brain Behav Immun*, 19(5), 381-388.
- Falanga V & Eaglstein WH. (1993). The trap hypothesis of venous ulceration. *Lancet*, 341, 1006-1008.
- Falanga, V., Moosa, H., Nemeth, A., Alstadt, S., & Eaglstein, W. (1987). Dermal pericapillary fibrin in venous disease and venous ulceration. *Arch Dermatol*, 123(5), 620-623.

- Fernando Moralesa, R. G., Moralesa, F., Graaff, R., Smit, A., Bertuglia, S., Petoukhova, A., . . . Rakhorst, G. (2005). How to assess post-occlusive reactive hyperaemia by means of laser Doppler perfusion monitoring: Application of a standardised protocol to patients with peripheral arterial obstructive disease. *Microvascular Research*, 69(1-2), 17-23.
- Finlayson, K., Edwards, H., & Courtney, M. (2011). Relationships between preventive activities, psychosocial factors and recurrence of venous leg ulcers: a prospective study. *J Adv Nurs*, 67(10), 2180-2190.
- Franklin, D., Scheegel, W., & Rushmer, R. (1961). Blood flow measured by Doppler frequency shift of back scattered ultrasound. *Science*, 134, 564-565.
- Franklin, K. (1987). Comparative study of the distribution of venous valves in the lower extremities of black Africans and Caucasians: pathogenetic correlates of prevalence of primary varicose veins in the two races. *Anat Rec*, 217, 407-412.
- Franzeck, U., Bollinger, A., Hutch, R., & Hutch, A. (1984). Transcutaneous oxygen tension and capillary morphologic characteristics and density in patients with chronic venous incompetence. *Circulation*, 806.
- Franzeck, U., Haselbach, P., Speiser, D., & Bollinger, A. (1993). Microangiopathy of cutaneous blood and lymphatic capillaries in chronic venous insufficiency. *J of biology and Medicine*, 66, 37-46.

- Fries , R., Wallace, W., Roy, S., Kuppusamy, P., Bergdall, V., Gordillo, G. M., . . . Sen, C. K. (2005). Dermal excisional wound healing in pigs following treatment with topically applied pure oxygen. *Muta Res*, 579(1-2), 172-181.
- Gasser, P., Muller, P., Mauli, D., & Staibli, C. (1992). Evaluation of reflex to cold provocation by laser-Doppler flowmetry in clinically healthy subjects. *Angiology*, 43(5), 389-394. doi:10.1177/000331979204300503
- Gaylarde, P., Dodd, H., & Sarkany, I. (1990). Venous leg ulcers and arthropathy. *Br J Rheum*, 29(2), 142-144. doi:10.1093/rheumatology/29.2.142
- Geffken, D., Cushman, M., Burke, G., Polak, J., Sakkenen, P., & Tracy, R. (2001). Association between physical activity and markers of inflammation in a healthy elderly population. *Am J Epidemiol*, 153(3), 242-250. doi:10.1093/aje/153.3.242
- Gibbins, I., Wattchow, D., & Coventry, B. (1987). Two immunohistochemically identified populations of calcitonin gene related peptide (CGRP)-immunoreactive axons in human skin. *Brain Res*, 414(1), 143–148.
- Gibbs, N. M. (1957). Venous thrombosis of the lower limbs with particular reference to bed-rest. *Br J Surg*, 45(191), 209-236. doi:10.1002/bjs.18004519102
- Gielen, S., Schuler, G., & Adams, V. (2010). Cardiovascular effect of exercise training: molecular mechanism. *Circulation*, 122, 1221-1238.
- Gloviczki , P., Bergan, J., Rhodes, J., Canton, L., Harmsen, S., & Ilstrup, D. (2002). The North American Study Group. Mid-term results of endoscopic perforator vein interruption for chronic venous insufficiency: lessons learned from the North

- American subfascial endoscopic perforator surgery registry. *J Vasc Surg*, 29(3), 489-502.
- Gloviczki, P. (2001). *Handbook of venous disorders* (2nd ed.). New York: Arnold Publisher.
- Gomez-cabrera, M., Domenech, E., & Vina, J. (2008). Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. *Free Radic Biol Med*, 44(2), 126-131. doi:10.1016/j.freeradbiomed.2007.02.001
- Goodman, A., Guyton, A., Drake, R., & Loflin, J. (1974). A television method for measuring capillary red cell velocities. *J Appl Physiol*, 37(1), 126-130.
- Gordillo, G., & Sen, C. (2003). Revisiting the essential role of oxygen in wound healing. *Am J Surg*, 186(3), 259–263.
- Gosain, A., & DiPietro, L. (2004). Aging and wound healing. *World J Surg*, 28(3), 321-326. doi:10.1007/s00268-003-7397-6
- Gothgen, I., & Jacobsen, E. (1978). Transcutaneous oxygen measurement I. Age variation and reproducibility. *Acta Anaesthesiol Scand*, 67, 66-70.
- Graham , I. D., Harrison, M. B., Nelson, A. E., Lorimer, K., & Fisher, A. (2003). Prevalence of lower-limb ulceration: a systematic review of prevalence studies. *Advances in Skin and Wound Care*, 16(6), 305-316. doi:10.1177/026835550101600106
- Gschwandtner, M., & Ehringer, H. (2001). Microcirculation in chronic venous insufficiency. *Vasc Med*, 6, 169–179.
- Guo, S., & Dipietro, L. (2010). Factors affecting wound healing. *J Dent Res*, 89(3), 219-229. doi:10.1177/0022034509359125

- Halabi-Twail, M., Lionnet, F., Girot, R., Bachmeyer, C., Levy, P., & Aractingi, S. (2008). Sickle cell leg ulcers: a frequently disabling complication and a marker of severity. *Br J Dermatol*, 158(2), 339–344.
- Hampson, N., & Atik, D. (2003). Central nervous system oxygen toxicity during routine hyperbaric toxicity during routing hyperbaric oxygen therapy. *Undersea Hyperb Med*, 30(2), 147–53.
- Hardy, S., Riding, G., & Abidia, A. (2004). Surgery for deep venous incompetence. *Cochrane Database Syst Rev*, CD001097.
- Haumscchild, D. (1986). Microvascular blood flow measurement by. Laser Doppler Flowmetry. *TSI Application Note*, 1-8.
- Heinen, M., Borm, G., Van der Vleuten, C., Evers, A., Oostendorp, R., & van Achterberg, T. (2012). The Lively Legs self-management programme increased physical activity and reduced wound days in leg ulcer patients: results from a randomized controlled trial. *Int J Nurs Stud*, 49(2), 151-161.
- Heit, J. A., Mohr, D. N., Silverstein, M. D., Petterson, T. M., O'Fallown, W. M., & Melton, L. J. (2000). Predictors of recurrence after deep vein thrombosis and pulmonary embolism: a population-based cohort study. *Arch Intern Med*, 160(6), 761-768.
- Heit, J., Silverstein, M., Mohr, D., Petterson, T., O'Fallon, W., & Melton, L. (1999). Predictors of survival after deep vein thrombosis and pulmonary embolism: a population-based, cohort study. *Arch Intern Med*, 159(5), 445-453.

- Hiet, J. A. (2008). The epidemiology of venous thromboembolism in the community. *Arteriosclerosis, Thrombosis and Vascular Biology*. *Arteriosclerosis, Thrombosis and Vascular Biology*, 28(3), 370-372. doi:10.1161/ATVBAHA.108.162545
- Hojensgard, I. C., & Stump, H. (1949). Venous pressure in primary and postthrombotic varicose veins. *Acta Chir Scand*, 133-153.
- Homans, J. (1917). The aetiology and treatment of varicose ulcer of the leg. *Surg Gynecol Obst*, 24, 300-311.
- Huch, R., & Lubbers, D. (1969). Quantitative polarographische sauerstoffdruckmessung auf der kopfhaut des neugeborenen. *Arch Gynaekol*, 207, 443-451.
- Huisman, L. C., Baker, D. C., & Wittens, C. H. (2013). Microcirculatory changes in venous disease. *Phlebology*, 28, 73–78.
- Hunt, T., Zederfeldt, B., & Goldstick, T. (1969). Oxygen and healing. *Am. J.Surg.*, 118(4), 521–525.
- Hutch, A., Franzeck, U., Huch, R., & Bollinger, A. (1983). A transparent cutaneous oxygen electrode for simultaneous studies of the skin capillary morphology, flow dynamics and oxygenation. *Int J Microcirc*, 2(2), 103.
- Jonathan, J., & John, A. (2007). *The evidence for vascular surgery* (2nd ed.). Shrewsbury: tfm Publishing limited.
- Jones, S., Bowler, P., Walker, M., & Parsons, D. (2004). Controlling wound bioburden with a novel silver-containing hydrofiber dressing. *Wound Repair Regen*, 12(3), 288–294. doi:10.1111/j.1067-1927.2004.012304.x

- Jull, A., Waters, J., & Arroll, B. (2002). Pentoxifylline for treating venous leg ulcers. *Lancet*, 359, 1550-1553.
- Kahn, S. R., Kearon, C., Julian, J., Mackinnon, B., Kovacs, M. J., & Wells, P. (2005). Predictors of the post-thrombotic syndrome during long-term treatment of proximal deep vein thrombosis. *J Thromb Haemost*, 3(4), 718-723.
- Kahn, S. R., M'Land, C. E., Lamping, D. L., Kurz, X., Berard, A., & Abenhaim, L. (2004). The influence of venous thromboembolism on quality of life and severity of chronic venous disease. *J Thromb Haemost*, 2(12), 2146-2151.
- Kahn, S., & Ginsberg, J. (2004). Relationship between deep venous thrombosis and the postthrombotic syndrome. *Arch Intern Med*, 164(1), 17-26.
- Kakkar, W., Howe, C., Flanc, C., & Klarke, M. (1969). Natural history of postoperative deep-vein thrombosis. *Lancet*, 230-232.
- Kalani, M., Brismar, K., Fagrell, B., Ostergren, J., & Jorreskog, G. (1999). Transcutaneous oxygen tension and toe blood pressure as predictors for outcome of diabetic foot ulcers. *Diabetes Care*, 22(1), 147-151.
- Kalliainen, L., Gordillo, G., Schalanger, R., & Sen, C. (2003). Topical oxygen as an adjunct to wound healing: a clinical case series. *Pathophysiology*, 7, 81-87.
- Kampmeier, O., & Birch, L. (1927). The origin and development of the venous valves, with particular reference to the saphenous district. *Amer J Anat*, 38, 451-499.
- Keith, M. L. (2014). *Moore clinical oriented anatomy* (7th ed.). Philadelphia: Lippincott Williams& wilkins.

- Keylock, K., Vieira, V., Walling, M., DiPietro, L., Schrementi, M., & Woods, J. (2008). Exercise accelerates cutaneous wound healing and decreases wound inflammation in aged mice. *Am J Physiol Regulatory Integrative Comp Physiol*, 294(1), 179-184. doi:10.1152/ajpregu.00177.2007
- Kim, J., Choi, W., & Jeong, S. (2013). The effects of physical activity on breast cancer survivors after diagnosis. *J Cancer Prev*, 18(3), 194-200.
- Kohner, E., Hamilton, A., Saunders, S., Sutcliffe, B., & Bulpitt, C. (1975). The retinal blood flow in diabetes. *Diabetologia*, 11(1), 27-33.
- Kolari, P., Pekamaki, K., & Pohjola, R. (1988). Transcutaneous oxygen tension in patients with post-thrombotic leg ulcers: treatment with intermittent pneumatic compression. *Cardiovasc Res*, 22(2), 138-141. doi:http://dx.doi.org/10.1093/cvr/22.2.138
- Kugler, C., Strunk, M., & Rudofsky, G. (2001). Venous pressure dynamics of the healthy human leg: role of muscle activity, joint mobility and anthropometric factors. *J Vasc Res*, 38(1), 20-29.
- Kvernebo, K., Megerman, J., Hamilton, G., & Abbott, W. (1989). Response of skin photoplethysmography, laser doppler flowmetry and transcutaneous oxygen tensiometry to stenosis-induced reductions in limb blood flow. *Eur J Vasc Surg*, 3(2), 113-120.
- Kyrle, P. A., Minar, E., Bialonczyk, C., Hirschl, M., Weltermann, A., & Eichinger, S. (2004). The risk of recurrent venous thromboembolism in men and women. *N Engl J Med* 2004, 350(25), 2558-5263.

- Labropoulos, N., Giannoukas, A. D., Delis, K., Mansour, M. A., Kang, S. S., Nicolaides, A. N., . . . Baker, W. H. (1997). Where does venous reflux start? *J Vasc Surg*, 26(5), 736–742.
- Laing, W. (1992). *Chronic venous diseases of the leg*. London: Office of Health Economics.
- Lansdown, A. G. (2004). Nutrition 2: a vital consideration in the management of skin wounds. *Br J Nurs*, 13(20), 1199-1210.
- Layer, G., Stacey, M., & Burnand, K. (1986). Stanazolol and the treatment of venous ulceration - an interim report. *Phlebology*, 1, 197-203.
- Lazareth, I., Taieb, J., Michon-pasturel, U., & Priollet, P. (2009). Ease of use, feasibility and performance of ankle arm index measurement in patients with chronic leg ulcers. *J Mal Vasc*, 34(4), 264–271. doi:10.1016/j.jmv.2009.02.006
- Lazarus, G., Mahmud, M., Umair, Q., & Nisa, M. (2014). Chronic venous leg ulcer treatment: Future research needs. *Wound Rep Reg*, 22, 34–42.
- Leach, R., & Browse, N. (1981). Lymph fibrinogen in long- and shortterm venous hypertension in the hind leg of the dog. *Br J Surg*, 354.
- Leavitt, J. W. (1990). Medicine in Context: a review essay of the history of medicine. *The American Historical Review*, 95(5), 1471-1481.
- Lee, A. J., Evans, C. J., Allan, P. L., Ruckley, C. V., & Fowkes, F. G. (2003). Lifestyle factors and the risk of varicose veins: Edinburgh Vein Study. *J Clin Epidemiol*, 56(2), 171-179.

- Leonardo, G., Arpaian, M., & Del Guercio, R. (1987). A new method for the quantitative assessment of arterial insufficiency of the limbs: cutaneous postischemic hyperemia test by Laser Doppler. *Angiology*, 38(5), 378-385. doi:10.1177/000331978703800505
- LePage , P., Villavicencio, J., Gomez, E., Sheridan, M., & Rich, N. (1991). The valvular anatomy of the iliac venous system and its clinical implications. *J Vasc Surg*, 678-683.
- Levick J. (2010). *An introduction to cardiovascular physiology* (5th ed.). London: Butterworth Heinemann.
- Lumpkin, E., & Caterina, M. (2007). Mechanisms of sensory transduction in the skin. *Nature*, 858–865.
- Maartini, F. H. (2006). *Fundamentals of Anatomy & Physiology*. new york: Benjamin Cummings.
- Maessen-visch, M. B., Sommer, A., De Paepe, J. A., & Neeumann, H. A. (1998). Changes in microcirculation in patients with atrophie blanche visualized by laser doppler perfusion imaging and transcutaneous oxygen measurements. *Phlebology*, 13(2), 245-249. doi:DOI: 10.1177/026835559801300203
- Mannarino, E., Pasqualini, L., & Maragoni, G. (1984). Chronoic venous incompetence and transcutaneous oxygen pressure : a controlled study. *VASA*, 17(3), 213-219.
- Marsden, G., Perry, M., Kelley, K., & Davies, A. H. (2013). Diagnosis and management of varicose veins in the legs: summary of NICE guidance. *BMJ*, 347:f4279.

- Martin, P., D'Souza, D., Martin, J., Grose, R., Cooper, L., Maki, R., & Mckercher, S. (2003). Wound healing in the PU.1 null mouse—tissue repair is not dependent on inflammatory cells. *Curr Biol*, 13(13), 1122-1128.
- Masuda, E., & Kistner, R. (1994). Long-term results of venous valve reconstruction: a four- to twenty-one-year follow-up. *J Vasc Surg*, 19(3), 391- 403.
- Mathieu, D. (2006). Non-healing wounds. In *Handbook on hyperbaric medicine* (pp. 401-427). Netherlands: Springer.
- Mc Gregor, I., & Morgan, G. (1963). Axial and random pattern flaps. *Br J Plast Surg*, 26(3), 202.
- Mc Mullin, G. (1990). Surgical treatment of the postphlebitic limb. *Vasc Med*, 69-71.
- Meagher, H., Ryan, D., Clarke-Moloney, M., O'laighin, G., & Crace, P. A. (2012). An experimental study of in the management of venous leg ulcers. *journal of wound care*, 21(9), 421-430.
- Meissner, M. H., Moneta, G., Burnand, K., Gloviczki, P., Lohr, J. M., Lurie, F., . . . Sumner, D. S. (2007). The hemodynamics and diagnosis of venous disease. *J Vasc Surg*, 46(6), 4S–24S. doi:10.1016/j.jvs.2007.09.043
- Michel, C. (1990). Oxygen diffusion in odematous tissue and through pericapillary cuffs. *phlebology*, 5(4), 223-230. doi:10.1177/026835559000500403
- Moffat, C., Kommala, D., Dourdin, N., & Choe, Y. (2009). Venous leg ulcers: patient concordance with compression therapy and its impact on healing and prevention of recurrence. *Int Wound J*, 6(5), 386–893. doi:10.1111/j.1742-481X.2009.00634.x.

- Moffatt, C. J., Franks, P. J., Doherty, D. C., Martin, R., Blewett, R., & Ross, F. (2004). Prevalence of leg ulceration in a London population. *QJM*, 97(7), 431–437.
- Mogford, J., Tawil, N., Chen, A., Gies, D., Xia, Y., & Mustoe, T. (2002). Effect of age and hypoxia on TGFbeta1 receptor expression and signal transduction in human dermal fibroblasts: impact on cell migration. *J. Cell. Physiol.*, 190(2), 259–265. doi:10.1002/jcp.10060
- Mohr, D., Silverstein, M., Heit, J., Petterson, T., O'fallon, W., & Melton, L. (2000). The venous stasis syndrome after deep venous thrombosis or pulmonary embolism: a population-based study. *Mayo Clin Proc*, 75(12), 1249-1256.
- Moore, K., Hall, V., Paull, A., Morris, T., Brown, S., Mc Culloch, D., . . . Harding, K. (2010). Surface bacteriology of venous leg ulcers and healing outcome. *J Clin Pathol*, 63(9), 830-834. doi:10.1136/jcp.2010.077032
- Morales, F., Graaff, R., Smit, A., Bertuglia, S., Petoukhova, A., Steen bergan, W., . . . Pakhorst, G. (2005). How to assess post-occlusive reactive hyperaemia by means of laser doppler perfusion monitoring; application to patients with peripheralarterial occlusive disease. *Microvascular Research*, 69, 17-23.
- Mossa, H., Falananga, V., Steed, D., Makaroun, M., Peitzman, A., Eaglstien, W., & Webster, M. (1987). Oxygen diffusion in chronic venous ulceration. *J Cardiovasc Surg*, 28(4), 464-467.

- Moyses, C., Cederholm-Williams, S., & Michel, C. (1987). Haemoconcentration and accumulation of white cells in the feet during venous stasis. *Int J Microcirc Clin Exp*, 5(4), 311-320.
- Mustoe, T., & Gurjala, A. (2011). The role of the epidermis and the mechanism of action of occlusive dressings in scarring. *Wound Repair Regen*, 19, 16-21.
- Nayman, J. (1974). The use of the ultrasonic flow meter in peripheral vascular disease. *Aust NZJ Surg*, 44(2), 157-166.
- Neglen, P., Thrasher, T. L., & Raju, S. (2003). Venous outflow obstruction: An underestimated contributor to chronic venous disease. *J Vasc Surg*, 38(5), 879–885.
- Nelson , E., Hillman, A., & Thomas, K. (2014). Intermittent pneumatic compression for treating venous leg ulcers. *Cochrane Database Syst Rev*(5), CD001899. doi:10.1002/14651858.CD001899.pub4
- Nelson, E. A., Harper, D. R., Prescott, R. J., Gibson, B., Brown, D., & Ruckley, C. V. (2006). Prevention of recurrence of venous ulceration: Randomized controlled trial of class 2 and class 3 elastic compression. *Journal of Vascular Surgery*, 44, 803–808.
- Neuman, H., Van Leeuwen, M., & Van Den Broke, M. (1984). Transcutaneous oxygen tension in chronic venous insufficiency syndrome. *VASA*, 13, 213-119.
- Nicholas, A., Andrea, D., & Alejandra, C. (2013). Evidence based management of common chronic lower extremity ulcers. *Dermatologic Therapy*, 26, 187–196.
- Nicolaidis, A. (2000). Investigation of chronic venous insufficiency: A consensus statement. *Circulation*.

- Nicolaides, A., Hussein, M., Szendro, G., Christopolous, D., Vasdekis, S., & Clarke, H. (1993). The relation of venous ulceration with ambulatory venous pressure measurements. *Journal of Vascular Surgery*, 17(2), 414-419.
- Obermayer, A., & Garzon, K. (2010). Identifying the source of superficial reflux in venous leg ulcers using duplex ultrasound. *J Vasc Surg*, 52(5), 1255-1261.
- O'brien, J., Finlayson, K., Kerr, J., & Edwards, H. (2014). Testing the effectiveness of a self-efficacy based exercise intervention for adults with venous leg ulcers: protocol of a randomised controlled trial. *BMC Dermatol*, 14-16. doi:10.1186/1471-5945-14-16
- O'Donnell TF. (2010). The role of perforators in chronic venous insufficiency. *Phleb*, 25(1), 3-10. doi:10.1258/phleb.2009.009071.
- Oien, R., Hakansson, A., Hansen, B., & BJellerup, M. (2002). Measuring the size of ulcers by planimetry: a useful method in the clinical setting. *J Wound Care*, 11(5), 165-168. doi: <http://dx.doi.org/10.12968/jowc.2002.11.5.26399>
- O'toole, E., Marinkovich, M., Peavy, C., Amieva, M., Furthmayr, H., Mustoe, T., & Woodley, D. (1997). Hypoxia increases human keratinocyte motility on connective tissue. *J Clin Invest*, 100(11), 2881-2891. doi:10.1172/JCI119837
- Padberg, F. J., Johnston, M., & Sisto, S. (2004). Structured exercise improves calf muscle pump function in chronic venous insufficiency: a randomized trial. *J Vasc Surg*, 39(1), 79-87.
- Palfreyman, S., Nelson, E., Lochiel, R., & Michaels, J. (2006). Dressings for healing venous leg ulcers. *Cochrane Database Syst Rev*, CD001103.

- Pappas, P., Duran, W., & Hobson, R. (2001). Pathology and cellular physiology of chronic venous insufficiency. In Y. J. Gloviczki P, *Handbook of Venous Disorders* (2nd ed., pp. 58–67). New York: Arnold Publisher.
- Partsch, H., Flour, M., & Smith, p. (2008). Indications for compression therapy in venous and lymphatic disease consensus based on experimental data and scientific evidence. Under the auspices of the IUP. *Int Angiol*, 27(3), 193-219.
- Pence, B., DiPietro, L., & Woods, J. (2012). Exercise speeds cutaneous wound healing. *Advances in wound care*, 3(1), 1846-1854. doi:10.1089/wound.2012.0377.
- Percival, N. (2002). Classification of wounds and their management. *Surgery*, 20(5), 114–117.
- Persoon, A., Heinen, M. M., Van Der, V. C., De Rooij, M., Van De KerKhof, P., & van Achterberg, T. (2004). Leg ulcers: a review of their impact on daily life. *Journal of Clinical Nursing*, 341–354.
- Phillips, T., Stanton, B., Provan, A., & Lew, L. (1994). A study of the impact of leg ulcers on quality of life: financial, social, and psychological implications. *J Am Acad Dermatol*, 31, 49-53.
- Piazza, G., & Goldhaber, S. Z. (2006). Acute pulmonary embolism: Part II treatment and prophylaxis. *Circulation*, 114, e42-e47.
- Piulachs, P., & Vidal-Parraquer, F. (1995). Pathogenic study of varicose veins. *Angiology*, 4, 59.

- Pollack, A., & Wood, E. (1949). Venous pressure in the saphenous vein at the ankle in man during exercise and changes in posture. *J Appl Physiol*, 1, 649-662.
- Prandoni, P., Lensing, A., Cogo, A., Cuppini, S., Villalta, S., Carta, M., . . . Prins, M. (1996). The long-term clinical course of acute deep venous thrombosis. *Ann Intern Med*, 125(1), 1-7.
- Prandoni, P., Bernardi, E., Marchiori, A., Lensing, A. W., Prins, M. H., & Villalta, S. (2004). The long term clinical course of acute deep vein thrombosis of the arm: prospective cohort study. *BMJ*, 329(7464), 484-485.
- Purwins, S., Herberger, K., Debus, E. S., Rustenbach, S. J., Pelzer, P., Rabe, E., . . . Augustin, M. (2010). Cost-of-illness of chronic leg ulcers in Germany. *Int Wound J*, 7(2), 97-102. doi:10.1111/j.1742-481X.2010.00660.x.
- Radek, K., Baer, L., Eckhardt, J., DiPietro, L., & Wade, C. (2008). Mechanical unloading impairs keratinocyte migration and angiogenesis during cutaneous wound healing. *J Appl Physiol*, 104(5), 1295-1303. doi:10.1152/japplphysiol.00977.2007
- Rayman, G., Williams, S., & Spencer, P. (1986). Impaired microvascular hyperaemic response to minor skin trauma in type one diabetes. *Br Med J*, 292(6531), 1295–1298.
- Rhoades, R., & Pflanzer, R. (2003). *Human Physiology* (4th ed.). USA: Thomson Learning, Inc.
- Richmond, N. A., & Maderal, A. D. (2013). Evidence based management of common chronic lower extremity ulcers. *Dermatologic Therapy*, 26(3), 187–196. doi:10.1111/dth.12051

- Robertson, L., Eans, C., & Fowkes, F. G. (2008). Epidemiology of chronic venous disease. *Phlebology*, 23, 103-111.
- Rodriguez, P., Felix, F., Woodley, D., & Shim, E. (2008). The role of oxygen in wound healing: A review of the Literature. *Dermatol Surg*, 34(9), 1159–1169. doi:10.1111/j.1524-4725.2008.34254.x
- Rojas, A., & Phillips, T. (1999). Patients with chronic leg ulcers show diminished levels of vitamins A and E, carotenes, and zinc. *Dermatol Surg*, 25(8), 601-604.
- Rosales, A. (2015). Valve reconstructions. *Phlebology*, 30, 50–58.
- Rosaria Cesarone, G., Belcaro, G., Errichi, S., Peiiegri, L., Ledda, A., Vinciguerra, G., . . . Corsi, M. (2008). Microcirculatory effects of Viatromb spray gel heparin in chronic venous insufficiency: evaluation of TcPO₂ and PCO₂- a product evaluation study. *Angiology*, 59(4), 519.
- Saumet, J., Kellogg, D. J., Taylor, W., & Johnson, J. (1988). Cutaneous laser-doppler flowmetry: influence of underlying muscle blood flow. *J Appl Physiol*, 65(1), 478-481.
- Schmid-Schonbein, G., Coleridge-Smith, P., Nicolaides, A., Boisseau, M., Eklof, B., & Bergan Jawad. (2006). Chronic venous disease. *N Engl J Med*, 355, 488-498.
- Sen, C., Gordillo, G., Roy, S., Kirsner, R., Lambert, L., Hunt, T., . . . Longaker, M. (2009). Human skin wounds: A major and snowballing threat to public health and the economy. *Wound Repair Regen*, 17(6), 763-77. doi:10.1111/j.1524-475X.2009.00543.x.

- Seraj, S., & Collins, L. (2010). Diagnosis and Treatment of Venous Ulcers. *American Family Physician.*, 81(8), 989-996.
- Shami, S., Sarin, S., & Scurr, J. (1997). Chronic Venous Insufficiency Disease. *International Journal of Angiology*, 6, 30-48.
- Shin, Y., Lee, J., Song, W., & Jun, T. (2008). Exercise training improves the antioxidant enzyme activity with no changes of telomere length. *Mech Ageing Dev*, 129(5), 254-260. doi:10.1016/j.mad.2008.01.001
- Siddiqui, A., Galiano, R., Connors, D., Gruskin, E., Wu, L., & Mustoe, T. (1996). Differential effects of oxygen human dermal fibroblasts: acute versus chronic hypoxia. *Wound Repair Regen*, 4(2), 211–218. doi:10.1046/j.1524-475X.1996.40207.x.
- Simon, D., Dix, F., & Mc Collum, C. (2004). Management of venous leg ulcers. *British Medical Journal*, 328, 1358–1362.
- Sindrup, J., Avnstrop, C., Steenfoss, H., & Kristensen, J. (1987). Transcutaneous PO₂ and laser doppler blood flow measurements in 40 patients with venous leg ulcers. *Acta Derm Venereol*, 67(2), 160-163.
- Skene, A., Smith, J., Dore, C., Charlett, A., & Lewis, J. (1992). Venous leg ulcers: a prognostic index to predict time to healing. *BMJ*, 305(6862), 1119-1121.
- Soo-ampon, S., Vongsavan, N., Soo-ampon, M., Chuckpaiwong, S., & Mathews, B. (2003). The sources of laser Doppler blood-flow signals recorded from human teeth. *Arch Oral Biol*, 48(5), 353-360. doi:10.1016/S0003-9969(03)00011-6

- Spiet, G., Denong, C., & Radermacher, P. (2002). Genotoxicity of hyperbaric oxygen. *Mutat Res*, 111–119.
- Stacey , M., Burnand, K., Pattison, M., Thomas, M., & Layer, G. (1987). Changes in the apparently normal limb in unilateral venous ulceration. *Br J Surg*, 74(10), 936-939.
- Stegall, F. (1966). Muscle pumping in the independent leg. *Circ Res*, 180-190.
- Steins, A., Hafher, H., Hahn, M., & Junger, M. (2002). Microcirculation in Chronic Venous Insufficiency. *Phlebology*, 17, 115-120. doi:10.1177/026835550201700307.
- Stonebridge, P., Chalmers, N., Beggs, I., Bradbury, A., & Ruckley, C. (1995). Reccurent varicose veins: a varicographic analysis leading to a new practical classification. *Br J Surg*, 82(1), 60-62.
- Strandness, D., & Sumner, D. (1976). *Hemodynamics for surgrons*. New York: Grune and Stratton Inc.
- Struckmann, J., Christensen, S., & Lendorf, A. (1986). Venous muscle pump improvement by low compression elastic stockings. *Phlebology*, 1(2), 97-103.
- Szpaderska, A., Zuckerman, J., & DiPietro, L. (2003). Differential injury responses in oral mucosal and cutaneous wounds. *J Dent Res*, 82(8), 621-626.
- Tallman, P., Muscare, E., Carson, P., Eaglstein, W., & Falanga, V. (1997). Initial rate of healing predicts complete healing of venous ulcers. *Arch Dermatol*, 133(10), 1231-1234.
- Tandara, A., & Mustoe, T. (2004). Oxygen in wound healing- more than a nutrient. *World J Surg*, 28(3), 294-300. doi:10.1007/s00268-003-7400-2.

- Taylor, G., & Pan, W. (1998). Angiosomes of the leg: anatomic study and clinical implications. *Plast Reconstr Surg*, 102(3), 599-616.
- Thelesius, O. (1992). Capillaroscopy with fiberoptic video microscope. *VASA*, 21(1), 87-88.
- Thomas, P., & Roerts, H. (1997). Hypercoagulability in venous and arterial thrombosis. *Ann Intern Med*, 126, 638-644.
- Thomas, P., Nash, G., & Dormandy, J. (1988). White cell accumulation in dependent legs of Patients with venous hypertension: a possible mechanism for trophic cchanges in the Skin. *Br MED J*, 296(6638), 1693-1695.
- Thum, J., Caspary, L., Creutzig, A., & Alexander, K. (1996). A new method for the assessment of tissue haemoglobin oxygenation in patients with chronic venous insufficiency. *Micro Vas Research*, 51(3), 317-326.
- Tiep, B., Carter, R., Nicotra, B., Berry, J., Phillips, R., & Ostap, B. (1987). Demand oxygen delivery during exercise. *Chest*, 91(1), 15-20.
- Tierney, S., Burke, P., & Fitzgerald, P. (1993). Ankle fracture is associated with prolonged venous dysfunction. *BJS*, 80(1), 36-38.
- Torrey, L. A., & Deepi, G. G. (2004). Pulmonary embolism: an unsuspected killer. *Emerg Med Clin N Am*, 961-983.
- Tzu-fei, W., Catherine, A., Paul, E. M., Mark, S. T., Keith, F. W., & Brian, F. G. (2014). Risk factors for inpatient venous thromboembolism despite thromboprophylaxis. *Thrombosis Research*, 25-29.

- Uhl, J., & Gilot, C. (2012). Anatomy of the foot venous pump: physiology and influence on chronic venous disease. *phlebology*, 27(5), 219-230.
- Valluru, S., & Babji, K. (2015). A clinical study on technique of subfascial endoscopic perforators surgery: a recent advancement. *Int J Sci Stud*, 10(2), 74-78.
- Van Den Bos, R., Arends, L., Kockaert, M., Neumann, M., & Nijsten, T. (2009). Endovenous therapies of lower extremity. *J Vasc Surg*, 49, 230-238. doi:10.1016/j.jvs.2008.06.030.
- Van Gent, W. B., Wilschut, E. D., & Wittens, C. (2010). Management of venous ulcer disease. *BMJ*, 341, 1092-1096. doi:10.1136/bmj.c6045.
- Van Gent, W., Catarinella, F., Lam, Y., Nieman, F., Toonder, I., van der Ham, A., & Wittens, C. (2015). Conservative versus surgical treatment of venous leg ulcers: 10-year follow up of a randomized, multicenter trial *Phlebology*. *Phlebology*, 30(1), 35–41. doi:10.1177/0268355514568848.
- Vasxdekis, S., Clarke, G., & Nicolaides, A. (1989). Quantification of venous reflux by means of duplex scanning. *J Vas surg*, 10(6), 670–677.
- Verdaet, D., Dendale, P., De Bacquer, D., Delanghe, J., Block, P., & De Baker, G. (2004). Association between leisure time physical activity and markers of chronic inflammation related to coronary heart disease. *Atherosclerosis*, 176(2), 303-310.
- Wahlberg, E., Jorneskog, G., Olofsson, P., Swedenborg, J., & Fagrell, B. (1990). The influence of reactive hyperemia and leg dependency on skin microcirculation in

- patients with peripheral arterial occlusive disease (PAOD) with and without diabetes. *VASA*, 19, 301-306.
- Walker , A., & Loncland, C. (1950). Venous pressure measurement in the foot in exercise as an aid to investigation of venous disease in the leg. *Clin. Sci.*, 101-114.
- Warwick, A., Thomas, C., & Dood, P. (2007). The impact of the ESCHAR trial on clinical practice. *Australian and New Zealand Journal of Surgery*, 77, 100. doi:10.1111/j.1445-2197.2007.04134_13.x.
- White , R., Nolan, L., Harley, D., Long, J., Klein, S., Tremper, K., . . . Shoemaker, W. (1982). Noninvasive evaluation of peripheral vascular disease using transcutaneous oxygen tension. *AM J Surg*, 144(1), 68-75.
- Widmaier, E. (2014). *Vander's Human Physiology*. (13th ed.). New York: McGraw-Hill Companies, Inc.
- Wiliam, L. J. (2001). *Human Embryology* (3rd ed.). philadelphia: Churchill Livingstone.
- Wilkinson, I., & Webb, D. (2001). Venous occlusion plethysmography in cardiovascular research: methodology and clinical applications. *Br J Clin Pharmacol*, 52(6), 631-646. doi:10.1046/j.1365-2125.2001.01495.x.
- Williams, P., Warwick, R., Dyson, M., & Bannister, L. (1989). *Gray's anatomy*. Edinburgh: Churchill Livingstone.
- Wu, L., Xia, Y., Roth, S., Gruskin, E., & Mustoe, T. (1999). Transforming growth factor-beta1 fails to stimulate wound healing and impairs its signal transduction in an aged

ischemic ulcer model: importance of oxygen and age. *Am. J. Pathol*, 154(1), 301–309.

doi:10.1016/S0002-9440(10)65276-5.

Yarwood-Ross, L., & Haigh, C. (2012). Managing a venous leg ulcer in the 21st century, by improving self-care. *British Journal of Community Nursing*, 17(10), 460-465.

Yim, E., Vivas, A., Maderal, A., & Kirsner, R. (2014). Neuropathy and ankle mobility abnormalities in patients with chronic venous disease. *JAMA Dermatology*, 150, 385-389.

Yong, K., & Khwaja, A. (1990). Leukocyte cellular adhesion molecules. *Blood Rev*, 4(4), 211- 25.