THE USE OF LASER DOPPLER FLUXMETRY IN THE PRE - OPERATIVE ASSESSMENT OF AMPUTATION WOUND HEALING IN THE DYSVASCULAR PATIENT

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ABSTRACT

Amputation revision surgery in the dysvascular patient, is associated with high morbidity and mortality. The Durban Metropolitan Vascular Service uses the Transcutaneous Oxygen Pressure ($TcpO_2$) index of 0.55 to select the most distal amputation site which is likely to heal by primary intention. $TcpO_2$ measurement is time consuming and as a result not all patients are assessed. Laser Doppler Fluxmetry (LDF) has been proposed as a rapid test of amputation wound healing potential. The objective of this study was to evaluate the worth of LDF in pre-operative prediction of amputation wound healing in the dysvascular patient.

A validation study using the standard reactive hyperaemia experiment (Bircher *et al.*, 1994) assessed the repeatability and reproducibility of the LDF in 20 normal subjects. Repeatability coefficients (RC) and coefficients of variation (CV) were calculated for resting cutaneous blood flow variation over a two hour period (repeatability) and over five days (reproducibility). The results indicated large fluctuations in cutaneous blood flow over the five day test, with RC = 8.88 and CV = 14.4%.

$TcpO_2$ and LDF resting values were measured at the routine amputation sites in 60 patients with PVD requiring amputation using a non-heated LDF probe (n = 60), and with a heated LDF probe (n = 35). $TcpO_2$ absolute and index values were compared to the unheated, heated and vascular reserve LDF data, using a Spearman rank correlation test. The non-heated LDF probe was found to be of little use, while

the heated probe ($p<0.0001$) and vascular reserve ($p<0.0001$) values showed significant correlations with the $TcpO_2$ index. The most useful absolute, heated LDF (4.9 a.u.) and LDF vascular reserve value (3.5 a.u.) were calculated using the Receiver Operator Characteristic curve. These values produced a sensitivity of 82.76%, specificity of 97.56%, positive predictive value of 88.88%, negative predictive value of 96% and an overall accuracy for pre-operatively predicting wound healing potential of 91.43%.

The primary revision rate at King Edward VIII Hospital is 23%. Not all patients undergo $TcpO_2$ measurement due to time restraints. The results suggest that by using LDF provocative testing in a larger group of patients, that the primary revision rate may be reduced further. It is concluded that widespread implementation of the LDF will be as useful as limited use of $TcpO_2$ measurement for pre-operatively evaluating wound healing potential in the dysvascular patient.

PREFACE

This study represents original work by the author and has not been submitted in any form to another university or institution. Where use was made of the work of others it has been duly acknowledged in the text.

The research described in this thesis was performed in the Vascular Laboratory, King Edward VIII Hospital under the supervision of Professor M. Mars and Professor J. V. Robbs.

A M^{c} Kune

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CHAPTER ONE

1.0 INTRODUCTION

Peripheral Vascular Disease (PVD) is the commonest cause of lower limb amputation in the United States and Great Britain. More than 90% of the 60, 000 amputations performed in the United States each year are for ischaemic or infective gangrene (Krupski *et al.*, 1988). Similar statistics have been reported in Great Britain (Mc Coll, 1986). Amputations in these patients are associated with high morbidity and mortality (Mars *et al.*, 1993).

The problem facing the surgeon is the determination of the most distal site at which an amputation will heal. The more proximal the amputation, the more likely the chance of primary wound healing. Durham, (1995) states that in the past, amputations for PVD were performed at above-knee level in virtually all patients. Although healing rates at this level approached 100 percent, the overall rehabilitation potential of less than 30 percent was unacceptable. While this practice reduced initial morbidity, the additional energy cost required to use a larger and heavier prosthesis made total rehabilitation more difficult (Durham, 1995). Preservation of the knee or ankle joint improves the chances of successful rehabilitation, but increases the risk of delayed or failed wound healing, which requires subsequent operative revision to achieve healing. This latter approach is demoralising to the patient, results in increased morbidity and mortality, and may culminate in rehabilitation failure (Mars *et al.*, 1993; Durham, 1995).

The benefits to both the patients and hospital administrators of using routine pre-operative evaluation of amputation wound healing have been reported (Malone *et al.*, 1979

and Mars *et al.*, 1993). Despite this, routine evaluation of pre-operative wound healing potential has not been widely implemented. A reason for this is that there is no single investigation which has gained universal acceptance. This is not surprising as there are many factors in addition to the adequacy of regional blood flow which influence wound healing. Investigations such as Doppler ankle brachial pressure indices, $Xenon^{133}$ skin clearance, thermography, fluorescein dye angiography, photoplethysmography, skin perfusion pressure measurement, transcutaneous oxygen pressure ($TcpO_2$) measurement and laser Doppler fluxmetry (LDF) have been tried. While each test has its proponents, it has not always been possible to reproduce the results reported in different settings. Of these tests, $TcpO_2$ measurement is presently held to be the most useful investigation of pre-operative wound healing potential (Oishhi *et al.*, 1988; Wyss *et al.*, 1988; Padberg *et al.*, 1992; Mars *et al.*,1993). The test has also been plagued by inadequate discrimination at low levels of cutaneous oxygen (Tonnesen *et al.*, 1978; Franzeck *et al.*, 1982; Harward *et al.*, 1985; Malone *et al.*, 1987; Wyss *et al.*, 1988). The use of the ratio of $TcpO_2$ measurement obtained at the amputation level to that of the anterior chest wall, the $TcpO_2$ index, has improved the sensitivity and specificity of the test (Mars *et al.*, 1993). The index gives a better indication of oxygen delivery as it takes into account variation cause by central factors of oxygen delivery, such as cardiac and respiratory function. The Durban Metropolitan Vascular Service now uses the $TcpO_2$ index to select the most distal amputation site which will heal by primary intention. An amputation site with a $TcpO_2$ index of greater than 0.55 is considered likely to heal (Mars *et al.*, 1993).

There are however, various disadvantages when using $TcpO_2$ monitoring. Firstly, due to instrumental and methodological factors, $TcpO_2$ measurement is time consuming. The test involves heating the underlying skin to provide maximal vasodilation of the nutrient and thermoregulatory vessels of the skin. With the heating element of the probe set at 45°C hyperaemic stabilisation requires 20 minutes, after which readings can be taken. Routine measurements are taken at the mid dorsum of the foot, 10 cm below the knee over the

anterior compartment, 10 cm above the knee in the midline and over the anterior chest wall in the mid-clavicular line. With patient acclimatisation to the environmental temperature of the laboratory, probe calibration and measurement at the four sites, the average test time is approximately 2 hours.

Over the seven year period 1989 - 1995, 1209 amputations were performed for peripheral vascular disease at King Edward VIII hospital, an average of 173 per year. In those patients undergoing amputation at a site preselected using the $TcpO_2$ index, the revision rate is less than 5% (Mars *et al.*, 1993). The overall revision rate is however 23% because not all patients undergo pre-operative assessment. This is in part because the Vascular Laboratory cannot handle the additional time consuming workload. Under these circumstances a test is required which is as sensitive and specific as the $TcpO_2$ index, but which can be performed more rapidly.

Recently, a Laser Doppler Fluxmeter (LDF) was acquired by the University of Natal Medical School. Laser Doppler Fluxmetry is non-invasive, easy to use and provides rapid immediate recording of skin microcirculation perfusion data. The instrument has found a place in the evaluation of diabetic ulcers (Belcaro *et al.*, 1994). Theoretically, the instrument has been proposed to be able to improve detection of, and discrimination between, low levels of skin blood flow (Padberg *et al.*, 1992). It was therefore proposed as a test of amputation wound healing potential and researchers have attempted to pre-operatively predict lower limb amputation wound healing using LDF (Holloway *et al.*, 1983; Fairs *et al.*, 1986; Karanfilian *et al.*, 1986; Gebuhr *et al.*, 1989; Kram *et al.*, 1989; Lantsberg *et al.*, 1991; Padberg *et al.*, 1992; Adera *et al.*, 1995).

Results have been promising, however there are conflicting opinions about the usefulness of the LDF. These revolve around the following issues. There is controversy as to whether skin heating is required (Karanfilian *et al.*, 1986; Kram *et al.*, 1989; Kvernebo

et al., 1989; Castronuovo *et al.*, 1987). If the test is performed on unheated skin, readings can be obtained 3 minutes after application of the probe. Routine testing at the four sites would then take less than 20 minutes. However, (Holloway *et al.*, 1983; Matsen *et al.*, 1984; Allen *et al.*, 1987; Fairs *et al.*, 1987; Gebur *et al.*, 1989; Lantsberg *et al.*, 1991; Padberg *et al.*, 1992) maintain that to be clinically useful, vasodilation of the thermoregulatory circulation of the skin is required. In addition, several other possible shortcomings of LDF are documented (Belcaro *et al.*, 1994). These include problems with calibration, variations in biological zero and the various ways in which the output of the LDF is expressed, which range from mV, arbitrary units, flux units, to blood flow per cm^2. As a result, the role of LDF in amputation wound healing prediction has yet to be resolved. Mars (1995) has therefore suggested that in order to determine if an investigation is useful, each vascular laboratory should establish its own values, and its own standard operating procedure, including validation under specific practical conditions. Similarly, in 1983, Tenland *et al.* suggested the need for development of relevant and standardised provocative procedures, in order to fully utilise the inherent possibilities of, and overcome the problems associated with, the LDF. Through following the suggestions of Mars (1995) and Tenland *et al.*, (1983) the present study therefore aimed to provide initial data as to the usefulness of the LDF, as a non-invasive, pre-operative predictor of amputation wound healing, in the Vascular Laboratory at King Edward VIII Hospital.

1.1 Statement of the Problem

The problem addressed in this study was basically twofold:

1. The establishment of Laser Doppler Fluxmetry normal values for the King Edward VIII Vascular Laboratory.

2. The evaluation of the worth of Laser Doppler Fluxmetry in pre - operative prediction of amputation wound healing.

1.2 Null Hypothesis

Laser Doppler Fluxmetry with or without provocative testing is of no benefit in pre - operative prediction of amputation wound healing in patients with peripheral vascular disease.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 AN OVERVIEW OF THE PROBLEM

Mars *et al.*, (1993) argue that the incidence of PVD in the black population in KwaZulu Natal is steadily increasing. A feature of the disease in this population is the delay in presentation; with seventy - five to eighty percent presenting with established gangrene or infected tissue coupled with unreconstructable lower extremity arterial anatomy (Mars *et al.*, 1993). Many limbs are therefore unsalvageable and require amputation. The disease was generally thought to be uncommon in this population group. Data from the Natal Provincial Administration's computer records of all amputations performed at King Edward VIII Hospital between 1984 and 1995 revealed that PVD and Diabetes accounted for 60 percent of all lower limb amputations (Figure 1).

This high percentage may be due to the fact that South Africa is a developing country and Kostuik (1981) suggests that as developing countries acquire higher standards of living and populations survive longer, amputation for PVD increases as a percentage of all amputations. There may be another reason for the increase in the occurrence of lower limb amputations for PVD in the black population. This is related to their movement from the rural areas to the urban areas. They now have better access to hospitals where they can be assessed and treated for PVD. Burgess *et al.*, (1981) discuss numerous factors which are relevant to the increase in PVD in KwaZulu Natal. These include, an ageing population; a

high incidence of diabetes; air, water and food contamination; physical inactivity; poor diet; and tobacco-smoking.

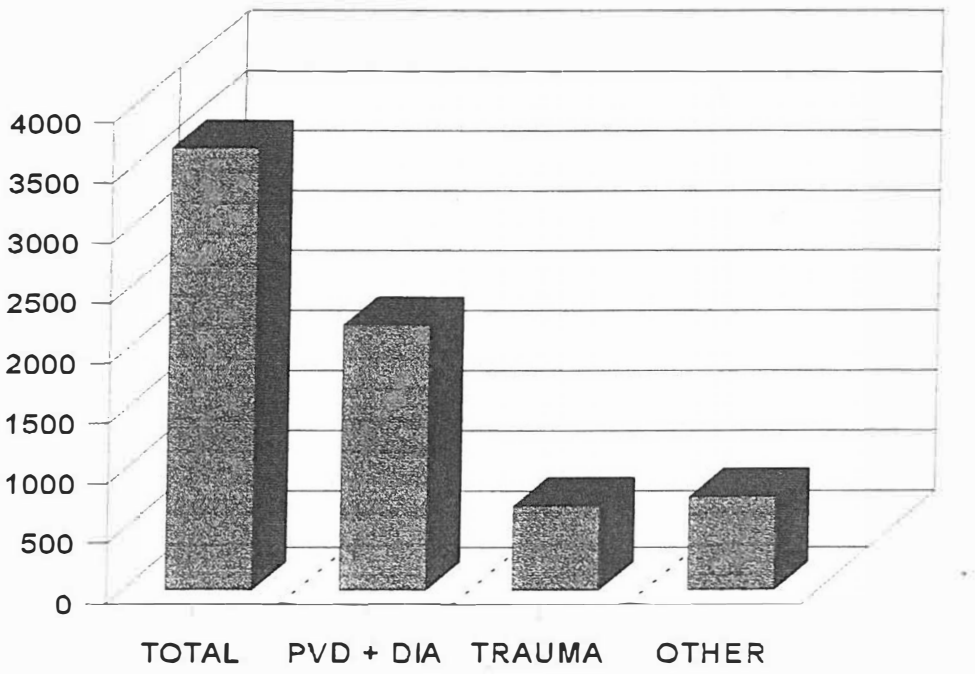

Figure 1: **Total number of patients undergoing lower limb amputation for PVD and Diabetes combined, trauma and other (1984 - 1995).**

In 1993, Mars *et al.*, addressed the problem of amputation revision surgery at King Edward VIII Hospital and outlined a programme involving pre-operative assessment of amputation wound healing potential that would save the hospital R1,07 million annually. In this study the authors reviewed the Natal Provincial Administration's centralised computer records of all patients admitted to King Edward VIII Hospital between 1984 and 1988. They found that during the 5-year period, 965 patients required 1563 lower limb amputations for PVD, 222 patients died in hospital. The primary revision rate, in other words, the number of first-time amputations that required revision, was 51%. The in-hospital mortality rate was 23,1%

and the mortality rate per amputation was 14,3%. They found that the primary revision rate was skewed due to the Vascular Services policy of performing an initial guillotine amputation and subsequent definitive amputation in patients with infected non-salvageable limbs (Desai *et al.*, 1986). Through taking this factor into account the primary revision rate was reduced to approximately 35%. They described the use of the $TcpO_2$ index which reduced the revision rate to 20,0% in 1989, and 8,2% in 1990. This percentage is not based on the entire amputee data from 1989 / 1990 but on 270 patients who met particular criteria for the study. Although $TcpO_2$ monitoring reduced the primary revision rate for these particular patients, through reviewing the amputee records between 1989 and 1995 it can be seen that there is still a high percentage of primary revision amputations at King Edward VIII Hospital.

Table I shows that during the seven-year period, 1989-1995, 1209 patients required 1824 lower limb amputations for PVD, 250 patients died in hospital. From the table primary revision rate, i.e. the number of first-time amputations that require revision, for PVD and Diabetes, in the seven-year period of investigation was 42.48%. The in-hospital mortality rate for the same patient group over the seven-year period was 20.94% while the mortality rate per amputation was 13.96%.

Once again, due to the Hospital's policy of performing an initial guillotine amputation, the primary revision rates presented above are skewed. Similarly to Mars *et al's.*, (1993) study, it was not possible to determine exactly how many guillotine amputations were performed in the PVD and Diabetic patients. The figure can, however, be approximated by counting the number of foot and below-knee amputations that were revised at the same level as the initial amputation. This reduces the primary revision rate in the patients who survived to approximately 23%. Analysis of several series reported by Hunter (1981) shows a 46% failure rate for toe amputations, and a combined 33% failure rate for transmetatarsal and Symes amputations. Warren *et al.*, (1968) reported a 32% failure rate in below-knee amputations in 127 patients and more recently Keagy (1986) reported a 19%

failure rate in 626 below-knee amputations. From this it can be seen that the revised data is in keeping with published figures. However, the revision rate is still too high when compared to the norm of 20% (Mars *et al.*, 1993) and compared to reports of revision rates as low as 10% (Burgess *et al.*, 1971) and less than 10% (Mars *et al.*, 1993). The findings from the amputee data review emphasises the need for a continued search for the most effective, reliable, non-invasive method for the assessment of wound healing potential.

Table I: Summary of PVD Amputee Data (1989 - 1995)

	89	90	91	92	93	94	95	Total
Total Number of Patients	253	149	153	188	149	136	181	1209
Total No. Pat. Requiring Rev.	114	49	82	80	65	55	71	516
Total Number of Amputations	386	203	243	293	240	202	257	1824
Primary Revision Rate (%)	45.06	32.88	53.59	42.55	43.62	40.44	39.22	42.48
Amps Revised at same level	49	25	34	39	20	33	41	241
Revised Revision Rate (%)	25.69	16.1	31.37	21.8	30.2	16.18	16.57	22.56
Deaths	37	33	32	49	30	25	44	250
In- Hospital Mortality (%)	14.62	22.15	20.92	26.06	20.13	18.38	24.31	20.93
Mortality per Amputation (%)	9.59	16.26	13.17	16.72	12.5	12.38	17.1	13.96

2.2 A reason behind the change in amputation philosophy, rehabilitation benefits

The success of rehabilitation following amputation is directly related to the level of limb ablation. Malone *et al.*, (1979) have stated that "correct choice of amputation level can make the difference between successful prosthetic rehabilitation and a bed-and-wheelchair

existence". This "correct choice" in terms of successful rehabilitation referred to the below-knee level. It is well documented that retention of the knee is especially important. A functional knee, will often allow an elderly person to walk, whereas he or she could not do so with an above-knee prosthesis. The reasons for this will be discussed in the next section. Assessments have shown that below-knee amputees have a greater degree of independence in daily living and vocational activities due to successful postoperative rehabilitation (Fisher *et al.*, 1978 and Kegal *et al.*, 1978).

2.2.1 Advantages of below-knee amputations

The most obvious advantage of a below-knee amputation compared to an above-knee amputation is biomechanical in nature. The gait of a below-knee amputee is more efficient than an above-knee amputee in terms of energy expenditure. Ambulation requires a 10 to 40 per cent increase in energy expenditure for a unilateral below-knee prosthesis compared with a 50 to 70 per cent increase for a unilateral above-knee prosthesis (Waters *et al.*, 1976). Successful rehabilitation is achieved in about 70% of all below-knee amputees but in only 10 to 30 percent of all patients following above knee amputation (Couch *et al.*, 1977; Roon *et al.*, 1977; Steinberg *et al.*, 1985). Malone *et al.*, (1979) have found that geriatric patients who undergo unilateral below-knee amputations show a greater than 90% success rate of rehabilitation to ambulation compared to a success rate of 30% or less for a similar group of above-knee amputees. Preservation of the knee joint is therefore extremely important for geriatric patients, many of whom have severe coronary artery disease or severe chronic obstructive pulmonary disease. It is, therefore, critical that the surgeon be able to assess accurately the viability of the limb so that amputation can be performed at the lowest reasonable level (Burgess *et al.*, 1981).

2.3 Pre-operative assessment methods of amputation wound healing potential in peripheral vascular disease

2.3.1 Conventional Methods

This involves clinical judgement which involves the evaluation of the skin or muscle colour, capillary refill, and bleeding characteristics (Jones 1984) as well as the presence of pulses in the affected extremity, and non instrumentally assessed measurement of skin temperature. With the exception of pulse measurement the other methods are subjective, relying on the experience of the observer as well as the variable ambient lighting (Furnas *et al.*, 1991). Malone *et al.*, (1984) state that one physical finding that has some value in identifying proposed amputation levels is the presence of dependent rubor. They state that skin which develops dependent rubor is clearly ischaemic, and that skin with dependent rubor, like gangrenous tissue, is an absolute contraindication to amputation at that level. However, they conclude by stating that the absence of dependent rubor does not necessarily ensure healing ability. With regard to performing amputations at the site of the most distal palpable pulse these authors argue that it results in a high healing rate. However many patients who have healing potential at a more distal level are thus denied the benefits of an optimum amputation. In their final analysis on the conventional methods Malone *et al.*, (1984) conclude that "none of the methods based on clinical judgement or the results of physical examination have a consistent enough correlation with amputation healing to provide a sound basis for clinical decision making".

2.3.2 Non-invasive / Relatively Non-invasive Methods

The high morbidity and mortality rates, as shown by the King Edward VIII amputee data, and poor rehabilitation results due to incorrect level selection have resulted in the

development of numerous non-invasive or relatively non-invasive methods for the pre-operative evaluation of wound healing potential.

Sarin *et al.*, (1991) state that the number and diversity of these various methods indicate that absolute determination of perfusion is not a simple problem. Different techniques measure different physiological parameters of cutaneous blood flow, physical movement, heat transport and oxygen content (Swain *et al.*, 1989).

The movement of blood to clear away a tracer substance is used in the dermofluorometric and radionuclide techniques, whilst the measurement of this movement is detected using ultrasound and photoplethysmography. As the blood flows through the skin surface it transports heat and oxygen to the area, so temperature and oxygen content measurement are relevant parameters. Only two methods, thermography and fluorescein staining, give an indication of regional flow. The remaining methods look at small volumes of tissue of different areas and at different depths (Swain *et al.*, 1989). The following section discusses the investigations which have been used to try to assess wound healing potential.

2.3.2.1 Thermography

This was one of the earliest methods for evaluating skin blood flow. The method is simple and inexpensive. A surface thermometer is placed on the skin at the proposed level of amputation, and the temperature is compared with temperatures at other levels. Unfortunately, the temperature difference between skin with blood flow that was adequate for healing and skin with blood flow not adequate for healing was not broad enough to identify a clear point for level selection (Malone *et al.*, 1987). The method is reported as "relatively" reliable for differentiating between above-knee and below-knee amputation levels (Golbranson *et al.*, 1982; Spence *et al.*, 1984). Malone *et a*l., (1987) concluded that the

ultimate role of skin temperature measurement for selecting amputation level in the performance of Syme's, transmetatarsal, or toe amputation awaits further evaluation.

2.3.2.2 Doppler Segmental Pressure

The measurement of systolic blood pressure at the ankle, calf and popliteal sites, is one of the most widely used techniques for determining the circulatory status of an extremity. A correctly sized blood pressure cuff is placed about the limb at the level where the pressure is to be measured and is briefly inflated to more than the patient's systolic pressure. The cuff pressure is then progressively reduced to the point at which distal blood flow recommences. This pressure is recorded as the segmental systolic blood pressure.

The onset of distal blood flow is indicated by the return of a Doppler signal from a superficial artery. A number of investigators have advocated segmental pressure measurements as a predictor of the success of an amputation (Carter, 1973; Dean *et al.*, 1975; Barnes *et al.*, 1976; Verta *et al.*, 1976; Baker *et al.*, 1977; Wagner, 1977; Towne *et al.*, 1979; Mehta *et al.*, 1980). Some authors have recommended comparing the segmental pressure with that of the brachial artery, reporting that the amputation is likely to succeed if the segmental pressure in the lower extremity is 35 to 45% of the brachial systolic pressure (Verta *et al.*, 1976; Wagner, 1977). Others have stated that an amputation is likely to be successful when the segmental pressure exceeds a certain absolute value. However, this so-called critical value has ranged widely from forty to seventy millimetres of mercury (Carter, 1973; Dean *et al.*, 1975; Barnes *et al.*, 1976; Verta *et al.*, 1976; Baker *et al.*, 1977; Wagner, 1977; Towne *et al.*, 1979; Mehta *et al.*, 1980).

Use of the segmental blood-pressure technique is based on several assumptions: (1) the principal arteries beneath the cuff are compressible; (2) the pressure in the cuff is transmitted effectively to the artery; and (3) the major arterial systolic pressure is the primary

determinant of wound-healing. Thus, erroneous information about the local circulatory status is obtained if the principal arteries are less compressible than normal. In many patients it may be impossible to tell if arterial incompressibility is a significant source of error, in others it may be obvious, as when the measured pressure exceeds 300 millimetres of mercury. In addition to this Malone *et al.*, (1987) state that systolic pressure measurements in patients with diabetes may be falsely elevated because of medial calcinosis of the popliteal and tibial vessels. Measurements may fail to correlate with healing if the local anatomy is not conducive to the uniform distribution of applied pressure (for example, at the ankle or foot where the bones may "shield" the arteries from the cuff pressure), if significant collaterals exist in the presence of major arterial occlusion, or if factors other than segmental arterial pressure significantly alter the metabolic potential of the skin at the intended site of amputation.

In large patient populations it appears that a correlation does exist between segmental pressure and the success of amputation. This is not an absolute correlation, however, since there is a significant incidence of failure of healing in the presence of high pressures and of successful healing in the presence of extremely low or undetectable segmental pressures (Burgess *et al.*, 1981). Unfortunately, the ankle pressure gives little, if any, guidance as to the possibility of healing an amputation in the below knee position. Some studies suggest an ankle systolic pressure of 30mmHg or more will give below knee healing (Nicholas *et al.*, 1982), but Clyne's (1991) experience agrees with many others, in that many patients with indeterminate ankle pressures went on to heal below-knee amputations (Ratliff *et al.*, 1984). The Doppler method although cheap and rapidly performed, suffers from the problems of all preoperative predictive tests for selection of amputation level i.e. it will predict with accuracy nearly all those amputations that will heal, but it will suggest that a number of below-knee amputations should not be performed in patients who would ultimately be perfectly capable of healing amputations at below-knee level. Hence, if surgeons relied on these tests entirely, an excess of above-knee amputations would be performed.

2.3.2.3 Fluorescein Dye Angiography

The measurement of skin fluorescence for determining amputation levels has been shown to hold promise (Swain *et al.*, 1989; Malone *et al.*, 1984). Following an intravenous infusion of sodium fluorescein, the fluorescein diffuses freely into the extracellular fluid of perfused tissue. This extracellular fluorescein can then be made to produce a yellow green fluorescence by exposure of the skin to ultraviolet or blue light. This fluorescence can then be quantified by instruments such as the dermofluorometer developed by Silverman *et al.*, (1985). This technique is more invasive than Doppler segmental pressure measurements (Malone *et al.*, 1984). These authors go on to state that flurometry may be advantageous in the context of marginal limb perfusion because it facilitates easy assessment at multiple sites on the same limb. Techniques such as LDF and $TcpO_2$ are less suited for evaluation of multiple sites. The primary problem with the use of fluorescence for amputation level selection has to do with its safety (Malone *et al.*, 1984). Side effects of fluorescein include nausea, vomiting, hypotension, and (rarely) anaphylaxis (Malone *et al.*, 1984; Furnas *et al.*, 1991).

2.3.2.4 Xenon^{133} Skin Blood Flow Measurement

There has been extensive research on the use of Xenon^{133} skin clearance for amputation level selection (Moore 1973; Daly *et al.*, 1980). The technique involves the intradermal injection of Xenon^{133} in saline which diffuses into the capillaries; the rate of removal of the Xenon^{133} is proportional to the skin blood flow, calculated from the clearance slope as measured by gamma camera (Sarin *et al.*, 1991). The major difficulty with this method is the lack of reproducibility of the results (Malone *et al.*, 1984). This fact may be due to two reasons. Firstly, the technique is greatly dependent on the accurate siting of the intradermal injection. If the injection is too deep (subcutaneous) then the skin blood flow readings will be falsely low, while if the injection is too superficial a falsely high figure will be

obtained (Sarin *et al.*, 1991). The second reason, suggested by Malone *et al.*, (1984) is that the quality of $Xenon^{133}$ in the form of gas dissolved in saline can vary. The product is usually manufactured by local nuclear medicine departments, and such local production probably accounts for the variability of published results. Other problems with using this technique include its affinity for adipose tissue and its biphasic clearance (Sarin *et al.*, 1991). The current status on the usefulness of $Xenon^{133}$ is reflected by Malone *et al.*, (1987) who, despite early enthusiasm, conclude that it is not statistically reliable as a selection method for amputation level determination.

2.3.2.5 Skin Perfusion Pressure (SPP)

The use of SPP for the evaluation of amputation wound healing potential has been well documented (Holstein, 1973). The technique involves an intradermal injection of a radioactive tracer mixed with histamine with the washout being measured with a scintillation counter. A blood pressure cuff is applied over the injection site and the pressure in it increased in steps until washout stops. This cuff pressure is taken as the SPP (Sarin *et al.*, 1991). Researchers have found, similarly to other techniques, that the SPP technique is useful for determining a cut-off point for the prediction of amputation healing (Sarin *et al.*, 1991). However, reviews on this technique fail to mention a cut-off point for wound healing failure and it is ultimately this elusive value that needs to be identified. Sarin *et al.*, (1991) stated that this method does have its disadvantages. These authors conclude that it is time consuming, especially if the SPP is to be determined at more than one site, and it is painful enough to require analgesia.

Two non-invasive methods for determining SPP have been found to be useful for predicting amputation wound healing. The earlier method introduced by Nielsen *et al.*, (1973) involved the use of a photodetector applied to the skin connected to a plethysmograph. External counter-pressure was then applied over this using a blood pressure

cuff. The cuff is slowly deflated and the SPP taken as the external counter pressure at the point when capillary blood flow recommences. Authors such as Ovesen *et al.*, (1984) and Van den Broek *et al.*, (1988) found this technique to be less reliable than other non-invasive methods.

Recently, Adera *et al.*, (1995) suggested a second method. They reported the use of a laser Doppler instrument for determining skin perfusion pressure. The technique involves the use of a specially designed blood pressure cuff, inside of which a laser Doppler probe is placed. The probe connected to the laser Doppler instrument is used in the same way as a photodetector connected to a plethysmograph, and that is to detect the pressure when capillary blood flow returns. The technique was found to be useful for evaluating wound healing potential in major amputations however the authors suggested that further study is required to assess the techniques ability to predict minor amputation wound healing.

2.3.3 Transcutaneous Oxygen Pressure Measurement

$TcpO_2$ measurement was initially developed for monitoring perfusion in neonates. The method has been adapted for predicting the outcome of amputation (Ratliff *et al.*, 1984). The test involves heating the underlying skin to provide maximal vasodilation of the nutrient and thermoregulatory vessels of the skin. The instrument uses a modified Clark type polarographic electrode - three 15 platinum cathodes encircled by a silver ring anode. The electrolytic solution bathing the electrode is covered by a thin Teflon membrane. Within the probe, a heating coil lies adjacent to the anode. This is controlled by a thermistor which constantly monitors skin temperature. The probe is attached to the skin by means of a double sided adhesive ring, over a drop of contact solution. The skin is heated to 45 degrees to achieve maximal vasodilation of the cutaneous vessels (Mars, 1995). Oxygen that is not taken up to meet the metabolic demands of the skin diffuses out of the skin (Figure 2), through the membrane, and is measured as the current generated across the electrodes (Allen

et al., (1987). Approximately 20 minutes is required before hyperaemic stabilisation is reached. Once this has been achieved the 95% response time is about 10 seconds (Mars 1995). The resulting signal is recorded as the transcutaneous oxygen tension in mmHg. According to Allen *et al.*, (1987) and Mars (1995) the instrument has a number of limitations with various physiological, morphological and methodological factors affecting $TcpO_2$ measurements.

2.3.3.1 Physiological and Morphological Variables in relation to $TcpO_2$ measurement

The major physiological factors are cardiac output and sympathetic tone, with red blood cell concentration and haemoglobin saturation affecting readings to a lesser extent. A further variable includes arterial pO_2. When blood flow is adequate, $TcpO_2$ has been shown to reflect changes in arterial pO_2. When blood flow is low, $TcpO_2$ follows changes in blood flow (Mars, 1995). Under these circumstances $TcpO_2$ measurements depend on arterio-venous gradients and cutaneous vascular resistance. This relationship is unfortunately non-linear at very low flow states (Matsen *et al.*, 1984; Mars, 1995).

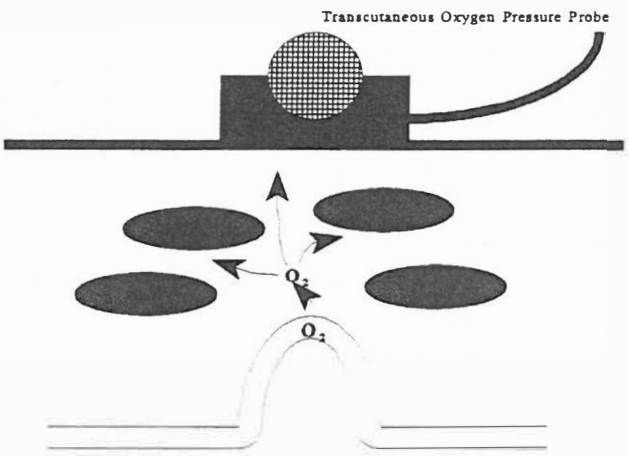

Figure 2: **Oxygen that is not used by cells outside the capillaries diffuses through the skin and is taken up by the $TcpO_2$ probe.**

Morphological variables include the thickness of the skin and changes in vascular morphology with increasing age (Mars, 1995). Another variable which is physiological and morphological in nature is the effect that oedema has on $TcpO_2$ readings. Sindrup *et al.*, (1987) found that increased oedema paralleled a decreased $TcpO_2$. Oedema increases the distance that oxygen must travel to reach the probe. In addition, the volume in which oxygen must reach equilibrium, is increased by oedema, thereby lowering oxygen concentration and partial pressure.

2.3.3.2 $TcpO_2$ Methodological Variables

Firstly, the semipermeable membrane was designed for neonatal skin, and is perhaps too thin for accurate readings in adults. Secondly, both the membrane and the electrolyte solution around the electrode must be changed regularly, and the well must be fixed with an air-tight seal to the skin. Other variables include electrode oxygen consumption and electrode response times in machines with membranes of different thickness (Allen *et al.*, 1987; Mars, 1995). Calibration against a zeroing solution is a simple procedure, and the instrument must be calibrated before it can be used on each occasion. Skin heating to achieve hyperaemic vasodilation requires 20 - 30 min per reading (Allen *et al.*, 1987).

2.3.4 Laser Doppler fluxmetry

Sarin *et al.*, (1991), state that the ideal test for selecting amputation levels would require no additional personnel or equipment, take little time to do and have a high sensitivity and specificity. Furnas *et al.*, (1991) states that a monitoring system must fulfil several requirements before it can be used universally. The system should "respond rapidly and deliver continuous data....The equipment should be simple, safe, inexpensive and portable...". The LDF has been proposed as a simple, rapid test of skin microcirculatory blood flow (Holloway *et al.*, 1977; Nilsson *et al.*, 1980a; Nilsson *et al.*, 1980b). It involves quantitative

evaluation of skin perfusion via the optical analogue of ultrasound Doppler measurement - Laser Doppler Fluxmetry (LDF).

Most clinicians are aware of the principles and use of Doppler ultrasound for measurement of pressure indices in the investigation of peripheral vascular disease. Sound waves are directed towards an advancing column of blood in a major axial artery and are reflected back to the probe, undergoing a Doppler shift. The laser Doppler, as its name implies, works on the same principle of measuring the Doppler shift, but uses photons of light in place of sound (Nilsson *et al.*, 1980a and Nilsson *et al.*, 1980b).

The foundation for the development of the laser Doppler was laid in 1958 with the development of a highly coherent monochromatic light source (laser) by Schawlow *et al.* In 1964 Cummins *et al.*, suggested that the laser could detect the movement of macromolecules if a proper heterodyning technique (mixing of two close frequencies and using their difference) was used while in the same year Yeh and Cummings documented that even very low flow velocities could be detected (+/- 0.07 mm sec) with this approach. LDF was directly applied in experimental invasive microcirculatory research by Einav *et al.*, (1975). They examined microvascular bloodflow using a special microscope system. The use of the laser Doppler technique for blood flow measurements was first presented by Riva *et al.*, (1972), studying retinal blood flow. Stern (1975) described the first non-invasive application of the Laser Doppler technique to monitor blood flow while the first portable clinical instrument was developed by Holloway *et al.*, (1977). In 1980, Nilsson *et al.* demonstrated that LDF equipment *in vitro* gives a linear response to flow for low and moderate erythrocyte velocities and erythrocyte volume fractions.

2.3.4.1 Theory

2.3.4.1.(i) Scattering of light within the tissue

Within the tissue, photons of light are randomly scattered by both moving red blood cells as well as stationary tissue cells. The majority of photons are scattered from stationary tissue in a predominantly forward direction according to the Rayleigh - Debye theory, with less than 0.1% backscattered by moving red blood cells (Bonner, 1981). This scattering of light in the forward direction results in a significantly lower average frequency shift of the light compared with backscattering. In other words the photons scattered by the moving red blood cells undergo Doppler shifts in frequency while those scattered by non-moving cells show a minimal shift in frequency or none at all. The light being scattered from moving red blood cells will undergo a frequency shift according to the Doppler equation : $f = (2vf\theta \cos \text{theta}) / c$. Where **v** is the velocity of the red blood cell, **fθ** is the frequency of the incident laser light, **c** is the speed of light in tissue and **theta** is the angle between the incident ray of light and the direction of motion of the red blood cell. The absolute value of the Doppler shift can only be calculated if **theta** is known.

2.3.4.2 (ii) Laser

Bircher *et al.*, (1994) states that the traditional fluxmeters were constructed with a helium-neon laser tube with a wavelength of 632.8nm. Recently, fluxmeters based on a laser diode with a wavelength of 780 nm have appeared on the market. Belcaro *et al.*, (1994) suggest that such wavelengths are not readily absorbed by tissue pigments, so that measurements can be made even on the darkest tissue. They also state that the use of longer wavelengths increases the measurement volume because of the greater penetration depth. The laser diode emits only one mode of laser light whereas the tube may switch between

different modes, particularly when warming up. Change of laser mode may be rhythmical. The diode may be mounted directly in the probe, and therefore an optical fibre cable can be avoided. In helium-neon lasers the laser light is led from the tube to the skin via optical fibres. Fibres create optical noise and artefacts, with a reduction in the signal-to-noise ratio. More recently fibres of smaller diameter and less noise have been constructed, and the problem of fibre artefact has more or less been overcome (Gush *et al.*, 1987; Newson *et al.*, 1987).

2.3.4.1 (iii) Photodetector

The source of the infared light (wavelength = 760 - 800 nm) in the Laser Doppler used in the present study was a low-power (2 milliwatt), solid-state laser diode. The beam produced has a low tissue penetration and is the same type of laser commonly used in the telecommunications and video disk industries. This type of laser light does not damage the tissues under evaluation or produce an increase in tissue temperature. The light is delivered via a fibre optic cable to a probe which is placed on the sample tissue being monitored.

2.3.4.1 (iv) Signal Processing

The problem faced in analysis of the signal is three-fold. Firstly, in tissue with a high concentration of red blood cells light may be scattered by more than one moving cell and hence theta may vary between 0 and 180 degrees for each scattering event. Secondly, the anatomical arrangement of the microvasculature of the skin needs to be accounted for. Unlike the use of the Doppler ultrasound in which the sound is beamed at an advancing column of blood, the photons of light from the laser Doppler are reflected from blood in the capillary loops moving both towards, and away from the light source, and from blood in the deep plexus moving at right angles to the light source (Figure 3). The situation is analogous to swarming flying ants attracted to a street light.

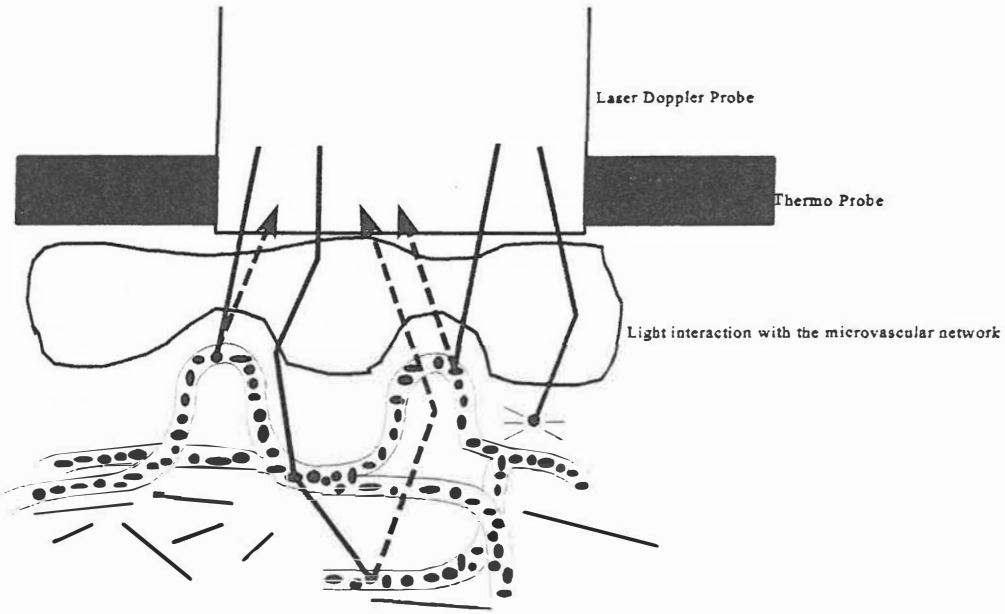

Figure 3: **LDF light interaction with the microvascular network.**

At any one moment, the velocity of all the flying ant must be computed, their number counted, and correction made for the direction in which they are moving (Mars, 1995). This issue caused Stern (1975) to state that it is practically impossible to theoretically calculate the Doppler spectrum due to multiple scattering. However, in practice the measured frequency shift is derived from an average value of theta, the magnitude of which is unknown. Thirdly, some components of the electrical signal are caused by external biologic or instrumental elements (e.g. vibration) and some by internal factors (mainly electronic noise) (Bircher *et al.*, 1994).

The scattered light is collected by receiving optical fibers in the probe and returned to the surface of a photodetector (located in the monitor) where the photons mix (heterodyne) generating an electrical signal which is then analysed through mathematical filtering in the digital signal processor. This filtering occurs via the use of various algorithms which make major

computational changes taking the concentration / theta angle relationship, and anatomical arrangement of the microvasculature into account, as well as distinguishing between the noise and the true signal due to blood cell motion.

Due to the fact that the magnitude of the Doppler frequency shift depends on the velocity of the red blood cells (Mars, 1995), the signal processor computes the mean Doppler frequency shift and scales the value accordingly, with the output being expressed in terms of cell velocity (the mean velocity of the moving cells); cell concentration (represents the mean concentration of moving cells in the tissue study area) and the flow or flux (the product of the number of moving cells and their mean velocity). This relationship is valid irrespective of how the product of red cell density and velocity is obtained. However, its validity has been questioned in very highly perfused tissue (Tenland, 1982).

2.3.4.2 Terminology

Unlike the relatively well defined geometry of the vessels ordinarily studied with ultrasound, the cutaneous vasculature consists of a complex network of interlacing vessels. Arterioles feed hairpin-like systems of capillaries that rise vertically from the papillae of the corium and return to the subpapillary venous plexus, whereas the larger vessels in the deeper dermis parallel the skin surface. The incident laser beam therefore intersects the flow vectors at multiple angles. More over, the light beam is scattered by the tissues both in transmission and again after it is reflected back to the receiving system. As a result, the frequency shifts represent a composite of the various velocities and angles. Along this line of thinking, Bircher *et al.*, (1994) states firstly, that the expression of blood flow per gram of tissue therefore represents an average (qualitative information) and is not truly quantitative, because of the complex microanatomy of the cutaneous vasculature.

Due to the issue of the nature of the signal discussed above, the output from the LDF has been referred to as flux instead of flow. This causes some confusion and misunderstanding since flux is not a word in common usage. The difference in terminology is best explained by Almond (1994). If blood is replaced with saline that does not contain large scattering particles. A method that measures volume flow of the fluid, such as venous occlusion plethysmography, would measure approximately the same value of flow as if blood were present. With saline, a LDF would give a zero output because there are no scattering particles to produce a Doppler shift. Thus the LDF does not measure blood volume flux (flow) as such but rather blood cell flux (Almond, 1994). However, in most physiologic and clinical situations even with a variable relationship between the cellular and fluid elements, LDF flux and volume flow have a good correlation. Almond (1994) goes further to state that it is arguable that LDF flux is a more useful measurement since it is the flow of red blood cells that is important for maintaining the delivery of oxygen to the tissue rather than the flow of fluid. If the local blood haematocrit remains constant then flux and flow will be directly related. However, if blood cells are not homogeneously distributed within the plasma then the flux of red cells and blood flow will have a variable relationship (Almond, 1994).

Additional terms used to express output signal such have been volt or mV and arbitrary units (a.u.). Bircher *et al* (1994) states that the results of LDF are essentially arbitrary and recommends that perfusion values be expressed as a.u. They suggest therefore that a.u. should be the common standard among manufacturers. The term flow may be used to express a concept that is more familiar to most physicians when referring to flow in certain contexts and considering the limitations. The European Laser Doppler Users Group (ELDUG) has proposed the use of the term Laser Doppler Perfusion to describe the output of the instrument thus avoiding the use of the words flux or flow (ELDUG, London, 1992). Perfusion is defined as the product of red blood cell local absolute velocity and concentration.

2.3.5 Evaluation of laser Doppler fluxmetry

The Laser Doppler is easy to use and is capable of making multiple rapid measurements at different locations and has a continuous monitoring capability. It is non-invasive, and hence does not interfere with the microcirculation when measuring local blood flow. These measurements are impossible to obtain with any other non-invasive technique. These advantages of the instrumentation promised rapid acceptance. The latter capability has made the LDF particularly useful for prolonged monitoring of tissue viability, such as after plastic surgery or to record skin flow perfusion during sleep or in the new born. LDF, has found a place in the evaluation of diabetic ulcers (Belcaro *et al.*, 1989). It has also been proposed as a test of amputation wound healing potential (Holloway *et al.*, 1983; Fairs *et al.*, 1986; Karanfilian *et al.*, 1986; Gebuhr *et al.*, 1989; Kram *et al.*, 1989; Lantsberg *et al.*, 1991; Padberg *et al.*, 1992; Adera *et al.*, 1995). Researchers have still not been able to establish the LDF as a diagnostic tool and hence LDF methodology has yet to find a niche in the armamentarium of the vascular laboratory. The technique is influenced by many instrumental, methodological, individual, and environmental variables, which need to be taken into account for meaningful interpretations. These will be discussed in the next section.

2.3.5.1 Practical Limitations

Belcaro *et al.*, (1994) state that there are various issues that must be addressed in the application of LDF. They argue that these become the concern of the instrument user, although in many cases the user has very little control over them or ability to mitigate their impact on measurement results.

2.3.5.2 Instrumental Variables

2.3.5.2 (i) Measuring Depth

Johansson (1991) states that a well-defined measuring depth is of vital importance for the interpretation of results obtained by any method designed to measure local microcirculation. The measuring depth depends on biophysical factors such as the optical properties of the tissue. These properties include the surface properties of the skin, blood content of the vessels, and the composition of the skin tissue.

2.3.5.2 (ii) Surface Properties

The outer layer of the skin varies in humans from one area to another, and also from normal to diseased states. Patients with vascular disease often have hyperkeratosis of the skin, which changes the penetration depth in relation to normal skin. Also hyperpigmentation may affect the penetration depth, and by this the sample volume also (Fagrell, 1994).

2.3.5.2 (iii) Composition of Skin Tissue

Not only the blood content, but also the composition of the skin influences how the light is spread in the tissue. The fat content for example may vary considerably in the same area from one subject to another. Besides this, patients with PVD often have ischaemic oedema, hyperkeratosis etc., which must affect the sample volume of the instrument (Fagrell, 1994).

2.3.5.2 (iv) Blood Content

During normal conditions, the skin microvessels fill with blood in a rhythmic fashion caused by pressure and flow changes due to cardiac action and by vasomotion. Variations in the amount and the movement of blood in the measuring volume, influence the penetration depth of the monochromatic light that is directed onto the skin surface by the LDF and consequently, the sample volume shows up as continuous variations in the strength of the LDF signal (Fagrell, 1994).

2.3.5.3 Instrumental Design Factors

These include the optical fibre configuration of the transmitting and receiving fibres of the probe, as well as the optical wavelengths. Due to the diverse optical properties of different tissues and the various alternative probe designs, the measuring depth has so far been difficult to ascertain. Consequently, comparison of results obtained by different groups may be ambiguous, in spite of the fact that the blood perfusion has been recorded in the same type of tissue. A well-defined measuring depth is, therefore, most important for the interpretation of the results

By using the so-called Monte Carlo calculations (Weis *et al.*, 1989) the theoretical mean measuring depth in the skin has been estimated to be 0.14 mm but in an artificial model this value has been calculated to be approximately 1.5mm (Tamaru and Oberg, 1988). It has been postulated that the measuring depth is shallow, when using probes comprised of small core diameter fibres (0.062-0.1 mm). The use of probes with large core diameter fibres (0.7mm) has given LD-signal values that are linearly related to the total blood flow of deeper sites. (Shepherd *et al.*, 1990).

Fagrell (1994) suggested that penetration depth varies from one subject to another, and from one moment to another. They concluded that measuring depth is not a fixed value for a certain tissue, but most probably is a continuous variable in all living tissues. The fibre separation distance in the LDF used in the present study is approximately 0.5 millimetres. In most biological tissues this yields a measurement depth of 1.0 millimetre. Specifically this means that 80% of the blood flow information is generated in the top 1.0 millimetre layer of tissue. Approximately 95% of the blood flow information is generated in the top 1.5 millimetre layer of tissue. The consequence is that at least 95% of the LDF output signal is made up of information gained from the deep thermo-regulatory plexus while only approximately 5% of the signal being derived from the nutritive layer (Mars, 1995) (Figure 4). The LDF signal that is recorded is therefore one in which the thermoregulatory flow component is predominant and the nutritional flow is a small component. For this reason the LDF technique cannot be used for evaluating skin capillary circulation, but only the total skin microcirculation in humans. New microprobes may reduce the measuring depth and provide a means of selective evaluation of the most relevant superficial nutritional capillaries, but clinically relevant data are not yet available. These new probe involve using polycarbonate (Delrin) spacers between the probe and the skin surface. The spacers have the same optical density as the skin and may be useful to separate the most superficial skin flux from the total skin flux, which is obtained by placing the probe directly on the skin (Fagrell, 1994)

Increasing the wavelength also provides a proportional increase in the average measurement depth. For example, an optical wavelength of 780 nanometers would have approximately 23% greater measurement depth than a wavelength of 633 nanometers (Vasamedics, 1995)

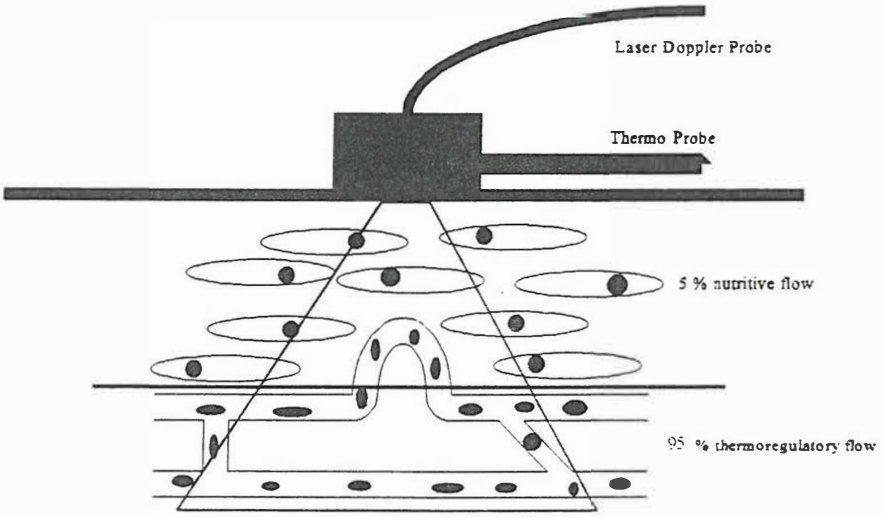

Figure 4: Depth and area of LDF light penetration.

2.3.5.3(i) Measurement Area

Belcaro *et al.*, (1994) state that for most instruments the sample volume is about one cubic millimeter and that variations in the number of capillaries per unit volume of tissue are evident even on this scale of sizes. Problems may be overcome by multiple recording within the study region, and averaging. Bircher *et al.*, (1994) suggested that the construction of integrated probes, special probe holders and scanners may also overcome this problem. Salerud *et al.*, (1986) developed a probe which integrated signals from seven optical fibres in order to try and reduce spatial and temporal variability. Theoretically this would reduce the variability by the factor of 7. In their experimental setups, the spatial variation was significantly reduced, but the modification appeared to emphasize the temporal variation. Wahlberg *et al.*, (1994), are one group of only a few who have compared a multiprobe (using 7 fibres) to a standard probe. These probes were placed between the knee and the first toe. The sites were chosen to fit a future study on amputation levels. They found that spatial variation in widely separated probe sites is reduced with the multi-probe. However, the temporal variation was not found to be reduced. In 1993, Wardell *et al.*, reported the use of laser Doppler perfusion imaging (LDPI)

(PIM 1.0, Lisca Development AB, Sweden). This equipment involves a laser beam which successively scans the tissue and the backscattered and Doppler shifted light is recorded in several thousand measurement points. After a scan is completed all measurements are compiled to form a colour coded image of the tissue perfusion. Bircher *et al.*, (1994) argues that these new laser Doppler modifications require further research. These new instruments need to be validated and documented versus standard recording, and to be shown to be a significant improvement.

2.3.5.3(ii) Motion Sensitivity

Tenland (1982) found that any movement, within the area illuminated by the laser, will cause the light to be spectrally broadened by the Doppler effect. Clinton (1991) found that erroneously high flow values can be the result of tissue motion. Tissue motion may be extrinsic (room vibration) or intrinsic (muscle fasciculation, respiration, or excessive patient movement). Patient movement can result in unnaturally high readings for several seconds. It is therefore critical that the patient realises from the beginning of measurement that he or she should be as relaxed and comfortable as possible in order to avoid movement of the limbs or a change in the blood flow which could affect the readings.

2.3.5.3(iii) Calibration

There are three reasons why calibration of the LDF is problematical. Firstly, The range of fluxmetry instruments (there are at least eight), their different principles, and the range of applications and modifications, together with the popularity of the method, make it impossible to develop one universal standard (Bircher *et al.*, 1994). The LDF is not directly quantitative and hence cannot be calibrated in absolute units with each type of LD fluxmeter giving its own readings. This makes it difficult to compare results from different manufacturers (Mars, 1995).

Secondly, there is no gold standard against which to compare LD readings particularly in the skin. Although many studies have demonstrated a good correlation between LDF measurements and other methods of blood flow measurements. In these studies cutaneous blood flow measurement accuracy was comparable to xenon (Engelhart *et al.*, 1983), plethysmography (Johnson *et al.*, 1984), and video microscopy (Tyml *et al.*, 1985), in human subjects. Calibration however, has not been reported using techniques that more appropriately reflect capillary perfusion, such as microspheres or hydrogen clearance. However, this correlation that does exist with many other methods is not surprising since in most cases an instrument that measures flow in one vascular compartment will probably on average correlate with another method even if it measures flow in a different way (Almond, 1994).

Almond (1994) goes further to argue that the validity of a universal calibration factor for the LDF in all tissues is questionable. The calibration will be influenced by uncontrollable variables such as the heterogeneity of the distribution of red blood cells in the tissues and by factors affecting light penetration in the tissue such as pigmentation or epidermal thickness. In addition, at high red blood cell concentrations the response of the flowmeter may be non-linear. Because of the local nature of the measurement, recording flow in absolute units is not that meaningful since this may not accurately reflect the global tissue blood flow.

Bircher *et al.*, (1994) suggest that a standard, made of a colloidal suspension of microspheres can be used on a temporary basis, but the suspension is not stable over longer periods, and tends to flocculate. They therefore see sophisticated calibration procedures as not being useful or realistic for everyday use. A third difficulty linked to calibration is the difference between instrumental and biological zero. Tenland *et al.*, (1983), Caspary *et al.*, (1988) and Wahlberg *et al.*, (1994) have reported that the biological zero is another important factor that influences the laser Doppler output variability.

2.3.5.(iv) The Biological Zero

The instrumental flux zero baseline is obtained by positioning the probe against a white surface (white porcelain is recommended by Bircher *et al.*, (1994). The biological zero is defined as the background value measured from a defined anatomical site after the arterial supply has been blocked with a cuff inflated to a pressure at least 25mm Hg over systolic pressure (Tenland *et al.*, 1983; Caspary *et al.*, 1988; Bircher *et al.*, 1994; Wahlberg *et al.*, (1994) and Mars, (1995). The biological zero is higher than the instrumental zero (Mars, 1995 and Bircher *et al.*, 1994) (Figure 6).

The origin of the signal responsible for the biologic zero is not clearly understood for it varies from region to region and also from organ to organ. Caspary *et al.*, (1988) and Mars (1995) suggest that it is probably produced by Brownian Motion while Fagrell (1994) states that it is generated by flow independent movements such as vasomotion activity in tissue. When blood flow to the skin is completely abolished, the LDF signal decreases to 20% to 50% of the normal tissue flux measurement while in situations of inflammation or edema formation it increases up to 70% of the normal flux. Caspary *et al.*, (1988) found that the biological zero could amount up to 80% of the total LDF signal in PVD patients. These authors also showed in their study, which involved measuring LDF in excised tissue, that an elevated baseline from the instrumental zero existed, even several hours after excision. However, this elevated biologic zero disappeared after a few days. Recently it has also been shown that the value of biological zero could vary from one moment to another in the same area. Sometimes it can be significantly increased in the skin of diabetic ischaemic feet encompassing the majority of the recorded signal (Caspary *et al.*,1988; Wahlberg *et al.*, 1992).

The clinical implications of these findings are still not clear, and further studies are required. The biological zero seems to be composed of a mixture of different parameters that

differ from one situation to another. Some researchers have therefore suggested that in the practical clinical evaluation of limbs, particularly in low perfusion states, the biological zero, should be recorded and presented and subtracted from the total LDF signal achieved (Caspary *et al.*, 1988 and Bircher *et al.*, 1994). Fagrell (1994) states that if this is not done it is a great risk that, at least in some tissues, the interpretations of the results may be wrong. Caspary *et al.*, (1988) concluded that although the biological zero phenomenon seems negligible under good perfused conditions, in cases of low perfusion (e.g. PVD patients) it should be taken into account when evaluating measurements, especially relative signal changes following provocative tests. Finally, Bircher *et al.*, (1994) suggest that values can either be expressed as the value directly read from the display or as the difference from biological zero, and that it must be made clear in publications which mode of expression is being used.

2.3.5.4 Individual Related Variables

2.3.5.4(i) Age

Huether *et al.*, (1986) found no significant difference between laser Doppler flux in any of six skin sites tested in 51 healthy volunteers, age range 20-53 years. Similarly, De Boer, (1989) studied the volar aspects of the forearms of 156 healthy volunteers (age range 17-63 years) and found that LD flux was not age-related. Suichies (1990) however, found a difference in normal neonates. The LD flux decreased in the first week after birth, due to the further development of the capillary network in this period, resulting in an increase in the microvasculature exchange area in the skin. The above studies were all conducted without heating the skin. The issue on whether to heat the skin will be discussed in detail later in the chapter. The effect of temperature on cutaneous blood flow has been studied by placing the subject's forearm in a hot water bath, and a decreased response of the cutaneous microvasculature to thermal stimuli was found with increasing age. The studies have

however led Bircher *et al.*, (1994) to conclude that for the major part of the age range, excluding the neonatal period, LD flux is not age dependent.

2.3.5.4(ii) Sex and Race

Huether (1986); Agner (1991); and Bircher *et al.*, (1994), conclude that there appears to be no major sex LD flux differences. However, in an experiment with 27 healthy subjects, LD flux on the forearms was significantly higher in males than in females (De Boer, 1989). Thus, at present the findings as to relationship between sex and LD flux are contradictory. Nevertheless, there appears to be no major difference in cutaneous blood flow between the sexes. A study by Berardesca *et al.*, (1988) comparing white and Hispanic and white and black subjects showed no significant differences in resting LD flux between the groups. However, Karanfilian *et al.*, (1986) and Padberg *et al.*, (1992) have reported that dark skin pigmentation prevented assessment of wounds by LDF. Further studies are therefore required.

2.3.5.4(iii) Intra-and Inter-individual Variation

Bircher *et al.*, (1994) states that controversial results on the repeatability of red blood cell flux have been reported. They suggest that one reason for conflicting results may be the small size of the measuring area and the problem of exact repositioning of the probe. Almond (1994) states that if blood flow varies significantly over the region of interest then a single spot measurement of LDF output may not be representative. Making several measurements over the region and computing an average value may help to resolve this problem. Braverman *et al.*, (1990) found that flux values measured by LDF varied by more than 100%, depending on the type of blood vessels in the skin tissue volume measured (this issue which relates to variation in anatomical microvasculature will be discussed in the next section). Agner *et al.*, (1990) measured resting flux bilaterally on the upper arm of 20

healthy subjects, and found that the intraindividual coefficient of variation was 25% as compared to an interindividual coefficient of variation of approximately 50%. Sundberg (1984) found that the forehead yielded the lowest coefficient of variation, but for continuous monitoring (e.g. physiologic testing, drug evaluation), suggested that even the forearm skin can serve as a suitable testing site. Kvernebo (1988) demonstrated that although the median values are satisfactorily reproducible for a given group of control subjects, this did not hold true for individual subjects.

2.3.5.4(iv) Spatial Variations

Sundberg (1984), states that considerable regional variation LDF readings between different parts of the body can be attributed to the significant regional differences in anatomy and function as well as the fact that the cutaneous vasculature is highly responsive.

Braverman *et al.*, (1990) and Colantuoni *et al.*, (1994) discuss Sundberg's (1984) first point in detail. They found that variations in LDF readings were due to the structure of the anatomical microvasculature under the probe. Specifically, that the characteristics of the ensuing signals were directly related to the activity of the vessel from which they originated. Colantuoni *et al.*, (1994) argue that in practice the diameter of the LDF probe is too wide to record flow from individual vessels, and the ensuing LDF flow measurement averages signals from many vessels. The LDF output could therefore be seen to be a composite of the net flow through the tissue with the variability of the signal being determined by the activity of the different microvessels. They stated that although the LDF signal is representative of tissue flow, it is primarily an expression of motion of blood in the tissue, regardless of its origin. They found that the mean flow values of larger venular vessels are always greater than the corresponding arteriolar order values, and hence concluded that the clinically used LDF signal, assumed to be representative of tissue perfusion, is mostly influenced by venular

flow. They went on further to state that the signal also includes non-perfusion-related components associated with flow variability.

In an early report which discussed spatial variance in blood flux, Tenland (1982) found wide variations between adjacent skin sites on the forearms. Later Tur *et al.*, (1983) reported that the lower legs and sides of the trunk showed low perfusion, but the hands, fingers and face had a high perfusion in healthy volunteers. They did find however that flux was equal in contralateral sites. DeBoer, (1989) studied 27 healthy volunteers, and found a significant difference between the proximal and distal regions of the volar aspects of the forearms with the distal values being higher. Kvernebo *et al.*, (1988) reported that the highest LDF output was obtained from the pulp of the toe. This was most likely due to high concentration of arteriovenous shunts, a fact to be considered when nutritional flow conditions are the focus of an investigation. From these few studied it can be seen that spatial variation of blood flux is considerable and needs to be taken into account. Sundberg (1984) stated that the cutaneous vasculature is highly responsive. The reason being that it is under the influence of constant and dynamic regulation. This regulation causes temporal variation in blood flux readings, a further methodological difficulty (Bircher *et al.*, 1994). Spatial variation is discussed further in Chapter Four, in relation to the results of the study.

2.3.5.4(v) Vasomotion

Short-term variations in red blood cell flux have been recorded due to the fact that LDF allows continuous recording. These variations are rhythmical and there is great intra- and interindividual variety in their magnitude (Salerud *et al.*, 1983). The rhythms include those due to the cardiac cycle as well as slower rhythms, which are unrelated to the respiratory or cardiac cycles and independent of the autonomic nervous system. (Salerud *et al.*, 1983; Tenland *et al.*, 1983; Engelhart *et al.*, 1986; Wilkin, 1987). The cycles only disappear under anaesthesia (general or local) (Salerud *et al.*, 1983). This fact indicates that

these variations are vasomotor waves which are an expression of local arteriolar autoregulatory mechanisms independent of the central nervous system (Mars, 1995)

Bircher *et al.*, (1994) states that this autoregulation of the microcirculatory blood flow is still not fully understood. Vasomotion is accentuated under conditions of reduced perfusion but diminishes with severe ischaemia, a condition during which LDF-monitored vasomotion also disappears. Colantuoni *et al.*, (1994); Meyer *et al.*, 1988; and Schmidt *et al.*, 1993 have studied vasomotion and have found that some experimental studies show that oscillatory flow behaviour is present, starts or stops, in different situations, including hypertension, hypovolemic shock, and peripheral vascular insufficiency. However, LDF measurements from the skin of healthy subjects and patients with ischaemia present a variability of patterns and are obtained using different procedures. This variability, coupled with the lack of uniform criterion for the analysis of time-dependent LDF signals, is in part the reason that this methodology does not allow to unequivocally discern between normal and pathological perfusion conditions (Fagrell, 1984; Wilkin, 1986; Creutzig *et al.*, 1987). Mars (1995) concludes this argument suggesting that at present analysis of vasomotion has not been found to be of clinical benefit.

2.3.5.4(vi) Temporal Variation

Tenland *et al.*, (1983) found large day-to-day variations in flux during a reproducibility study which took repetitive recordings at different skin regions. However, Sundberg, (1984) found no significant variation in flux at 2-hour intervals and in 5 day-to-day measurements. Reproducible results of flux were also obtained in a large group of individuals where measurements were taken 1 week apart (Zeghal *et al.*, 1986).

Ducloux *et al.*, 1989 found resting flux to remain stable in a short-term experiment (60-90 min) while Muller *et al.*, (1987) found that resting flux and flux induced by various

stimuli of cutaneous vasomotor reflexes were not significantly different when measured in the morning and in the afternoon. Therefore, no major changes, in cutaneous blood flow during the day have been observed, however Bircher *et al.*, (1994) have suggested that long-term studies are performed with repetitive measurements at the same time of day. Bircher *et al.*, (1994) also suggest that in the comparison of values at different sites or over periods of time it is important to examine individuals in the same position, as flux is dependent on posture. Mean values in the supine position have been reported to be higher than those measured sitting (Sundberg, 1984). Creutzig *et al.*, (1987) found in healthy subjects, that leg flux values decrease on dependency, but not on elevation above heart level.

2.3.5.4(vii) Physical and Mental Activity

Exercise has a considerable influence on cutaneous blood flux. Hatanaka *et al.*, (1984) showed that a slow exercise climbing two steps forty times, significantly increased fingertip flux while Ducloux *et al.*, (1989) reported a significant increase of flux in areas of muscular activity between athletes and untrained individuals. Bircher *et al.*, (1994) states however that pre-experimental effort influence of resting flux is of a short duration and can be prevented by an adaptive / acclimatization phase before the experiment. Mental stress (Elam *et al.*, 1987) and performance of mathematical calculations (Wilkin *et al.*, 1987) both have an influence on flux in areas rich in arteriovenous shunts and should therefore be avoided during LDF measurements. Also skin vasomotor reflexes such as Valsalva manoeuvres (Low *et al.*, 1983; Muller *et al.*, 1987;), deep inspirations (Muller *et al.*, 1987) and hyperventilation (Smits *et al.*, 1987) have a transient influence on flux, especially in areas with arteriovenous shunts. Bircher *et al.*, (1994) therefore recommend the performance of measurements in a quiet environment in the absence of powerful audio-visual and other mental stimuli.

2.3.5.4(viii) Food, Drugs and Nicotine

Hatanaka *et al.*, (1984) reported that fingertip flux was not influenced by food intake. Bircher *et al.*, (1994) states that all potentially vasoactive agents, such as vasodilating and antinflammatory drugs, as well as the consumption of nicotine, caffeine and alcohol should be avoided when measuring resting flux. Sundberg, (1984) and Stevenson *et al.*, (1987) found that nitroglycerine and Sundberg, (1984) found that prazosine had prolonged influences on LDF flux. Waeber *et al.*, (1984) reported that smoking reduced forearm LDF flux and other authors found that alcohol ingestion increased flux, especially in subjects with a history of flushing with alcohol (Wilkin *et al.*, 1985; Wilkin., 1986).

2.3.5.5 Environment-related Variables

2.3.5.5(I) Air Convection, Ambient and Local Temperature

A resting individual loses heat to the environment in 4 ways: the most important is radiation, followed by evaporation, convection, and conduction (Nilsson *et al.*, 1986). Among the factors which influence laser Doppler flux are therefore ambient temperature, humidity and air movements. The anatomical location is of importance here since arteriovenous shunts play an important role in thermoregulation; they react quickly to temperature changes and may considerably influence flux.

One of the most important factors which influences cutaneous flux is skin temperature, which is dependent on local or environmental temperature changes. The ambient air temperature influences the skin temperature directly by the mechanisms mentioned above and indirectly by central thermoregulatory effects (Bircher *et al.*, 1994). Hatanaka *et al.*, (1984) found that within a certain range, ambient temperature had little influence on laser Doppler flux. These authors showed that measurements taken at the

fingertip at room temperatures between 4 to 38°C showed stable flux values between 17 and 28°C, a significant decrease below 17°C and a trend towards an increase at 38°C. Winsor *et al.*, (1989) found similar results at the big toe. They reported no significant effect on flux at room temperatures from 24 to 30°C, while from 30 to 36°C a large temperature-related increase in flux was observed. Sundberg, (1984); Hassan *et al.*, (1988) and Richardson, (1989) reported that direct forced local cooling or warming (using a heated laser Doppler probe) decreases or increases laser Doppler flux respectively.

Bircher *et al.*, (1994) states that cool air currents significantly lower skin temperature and flux, though only if they are applied over a time period. Nilsson, 1987 and Nilsson *et al.*, (1986) found that forced convective cooling reduced LD flux and skin temperature significantly, whereas radiative cooling decreased skin temperature but did not effect laser Doppler flux.

Bircher *et al.*, (1994) therefore suggest that it is advisable to measure LD flux at constant environmental conditions. They state that at the usual ambient temperature of 20-25°C in a draught-free environment laser Doppler flux is relatively stable. To prevent unwanted effects of ambient or local temperature changes on flux these authors state that an adaptation of 20-30 min of the subject to the conditions in the room is mandatory. This also includes the removal of heavy clothing to circumvent later adaptive vasoconstriction in response to lower ambient temperature.

From the above discussion it can be seen that there are a vast number of variables which may influence LDF measurements. In view of the questionable temporal and spatial reproducibility any comparison, between results obtained from different studies, with particular reference to the present study regarding the prediction of wound healing after amputation, must be very tentative. This approach is taken by Mars (1995), who suggests that in order for this diagnostic technique to be useful, each vascular laboratory should

establish its own resting LDF values. Bircher *et al.*, (1994) also encourage researchers or laboratories to establish their own standard operating procedure, including validation under their specific practical conditions. In addition Mars (1995) suggests that provocative testing comparing readings before and after a particular manoeuvre should be used. Bircher *et al.*, (1994) state that currently, measurement under controlled circumstances with a defined purpose, and recordings expressed in arbitrary units, are often the best that is achievable. The following section discusses various provocative tests which have been used to over come the problems of calibration and absence of absolute values. These are used in addition to resting laser Doppler flux.

2.6 Provocative Testing

2.6.1 Postocclusive Reactive Hyperaemia Test

Several investigators have concluded that the scatter of LDF resting values is too large to permit separation of patients with ischaemic peripheral vasculature from normal control subjects. Karanfilian *et al.*, (1984); Pabst *et al.*, (1985); Del Guercio *et al.*, (1986) and Kvernebo *et al.*, (1989), deem the Postocclusive Reactive Hyperaemia Test (PORH) (Figure 5) to be more clinically useful than absolute readings. These authors have demonstrated a standardised and highly reproducible postocclusive reactive hyperaemia response. The test has become a preferred method because it relies on measurement of relative changes and does not require calibration. The test involves monitoring of LDF before and after occlusion of arterial blood flow for 3 minutes with an arterial tourniquet (Bircher *et al.*, 1994). Typically one of three responses occurs: a normal increase in measured flux after release, delayed reactive hyperaemia following tourniquet release, or a blunted response, with no hyperaemia occurring (Mars, 1995).

The LDF response to PORH depends on the initial perfusion conditions among other factors. The extent to which the LDF response can be used to quantify the degree of arterial occlusive disease has not been clearly determined. Kvernebo *et al.*, (1989) analysed the PORH response in several groups of patients and control subjects. They found that the delay between tourniquet deflation and the first increase in recorded flux was the most sensitive index for separating normal subjects, claudicants, and patients with severe ischaemia (Fontaine III and IV classification).

In a previous study, Pabst *et al.*, (1985) used the peak PORH response and found significant differences between controls and severely ischaemic patients but less significant differences between those groups and claudicants. In an even earlier study, Karanfilian *et al.*, (1984) identified vascular beds with relatively high LDF output (finger, toe, forehead) and those with significantly lower signals (e.g. plantar and dorsal aspect of the foot). They also found significant differences in resting baseline values between control subjects and patients with peripheral arterial disease; however this latter group consisted primarily of patients with severe ischaemia. A better separation was obtained by using the PORH response, and the most sensitive index was found to be the time to maximal response (18 seconds in the control group versus 150 seconds in the patient group). The observations of Del Guercio *et al.*, (1986) were even more encouraging because they studied patients in Fontaine group II (claudicants) without signs or symptoms of severe ischaemia. From a qualitative viewpoint, they found a significant vasomotion response superimposed on the postocclusive reaction in about 80% of normal subjects, whereas only 35% of the patient group demonstrated this phenomenon. They also compared the PORH response induced by thigh cuff occlusion with that resulting from ankle cuff occlusion. The most pronounced differences was in the latency time between the end of occlusion and the subsequent resumption of cutaneous blood flow. Most encouraging was the difference in latency time when thigh compression was used: practically no overlap with the control group was seen, but there was some overlap of values

if ankle compression was applied. Differences in the site of the occlusive disease may have been responsible for this overlap.

A less reliable separation was obtained by Seifert *et al.*, (1988), who found a significant reduction of PORH response in patients with severe ischaemia but could not separate claudicants (Fontaine II classification) from the control group. The derivation of a reactive hyperaemic index in these groups showed a significant difference between the control and severe PVD groups at all sites on the limb. However, a distinction between mild and severe ischaemia could not be made at any site on the leg, an indication that the technique would probably be of little value in assessing amputation level. Fairs *et al.*, (1987) have concluded that in any event, producing reactive hyperaemia with severe ischaemia is questionable in terms of the pain it produces. In their experience they had found that pre-operative pain is not always well controlled. Their patients were often acute admissions in need of immediate amputation surgery, or their condition has deteriorated rapidly following other unsuccessful vascular procedures. Fairs *et al.*, (1987) argued that any technique which could painlessly obtain the same information would be preferable.

2.6.2 The Venoarteriolar Reflex

A second test involving the Venoarteriolar Response has also been used to improve the sensitivity of the LDF. This is the vasoconstrictive response of the skin microcirculation in the foot when moving from supine to a standing position, or when lowering the leg below heart height . On standing, LDF and vasomotion is normally reduced. Sundberg *et al.*, (1986) assessed the effect of leg position on LD flux. They examined the effect of increased venous pressure by occluding venous outflow with a 40 mmHg cuff. They found a decrease in LDF in both healthy subjects and patients. While leg elevation caused an increase in LDF in both groups, the response to leg dependency was more informative, with a decrease in LDF in control subjects and an increase in patients. However, these results require some

additional qualification because the significant increase occurred at 37°C, which may have influenced the venoarteriolar reflex, although the same temperature did not influence the response in the normal control group. These results are somewhat contradictory to those reported by Seifert *et al.*, (1988) who found a decrease in LDF values in sitting position in patients as well as control subjects. It is possible that a lower thermostat setting (32°C) may be the explanation. Belcaro and Nicolaides (1989), studied the effect of orthostatics on LDF in normal subjects, diabetics, claudicants, and patients with severe ischaemia without altering the skin temperature. They found a significant reduction of LDF on standing in normal volunteers, whereas there was no significant flux reduction in the patient group. These authors therefore concluded that in diabetics with microangiopathy, resting flow has been found to be greater than in controls and there is a reduction of the venoarteriolar response.

2.6.3 LDF Index

Thus far only two research articles have been found which have suggested the use of an LDF index in the prediction of amputation wound healing. Gebuhr *et al.*, (1989), measured LD flux at the sternum as well as three sites on the lower limb. They concluded that sternal recordings were unnecessary when evaluating wound healing potential in the lower limbs of PVD patients. Kram *et al.*, (1989) used a calf - brachial LDF index to predict below - knee amputation wound healing. They did not find however, that the index increased predictive accuracy compared to calf LDF measurements alone, although patients with wounds that failed to heal tended to have lower calf - brachial indexes.

2.7 Evaluating Wound Healing Potential using Thermal Testing

2.7.1 Controversy: Non-heating versus Heating

If LDF measurement is performed on unheated skin, readings can be obtained 3 minutes after application of the probe. Routine testing at the four sites would then take less

than 20 minutes. There is controversy as to whether skin heating is required. Some authors have developed criteria based upon the difference in the LD signal between non-heated and heated skin for prediction of wound outcome (Holloway *et al.*, 1983; Gebuhr *et al.*, 1989) and these authors, along with others, state that addition of cutaneous heating to LDF measurements improves the prediction of outcome for wounds in ischaemic skin (Holloway *et al.*, 1983; Matsen *et al.*, 1984; Allen *et al.*, 1987; Fairs *et al.*, 1987; Gebur *et al.*, 1989; Lantsberg *et al.*, 1991; Padberg *et al.*, 1992). However, cutaneous heating has not been used routinely with LDF measurements. With Karanfilian *et al.*, (1986); Kram *et al.*, (1989); Kvernebo *et al.*, (1989); Castronuovo *et al.*, (1987) reporting useful unheated values for evaluating wound healing potential pre-operatively.

$TcpO_2$ monitoring has been shown to be effective and reliable for evaluating wound healing potential (Oishi *et al.*, 1988; Wyss *et al.*, 1988; Mars *et al.*, 1993). A common problem with $TcpO_2$ monitoring is that low to zero readings are often found in wounds which healed (Franzeck *et al.*, 1982; Harward *et al.*, 1985; Allen *et al.*, 1987; Wyss *et al.*, 1988). In a study conducted by Padberg *et al.*, (1992) false negative results (wounds which healed when predicted to fail) occurred in 7% of the wounds studied (at the criteria with the best overall predictive accuracy for $TcpO_2$ (11mmHg). This meant that 2 of 11 wounds (1 above knee amputation, 1 toe amputation) healed with a $TcpO_2$ of zero. Wyss *et al.*, (1988) reported 4 of 11 amputations (3 below knee, 1 foot) which healed with a $TcpO_2$ of zero. Harward *et al.*, (1985) reported 3 of 7 toe amputations and 6 of 7 below knee amputations which healed with a $TcpO_2$ of zero.

This problem can be understood if the findings of Matsen *et al.*, (1984) are taken into account. The authors' results confirmed a previously hypothesised non-linear relationship between $TcpO_2$ and local cutaneous blood flow . They found that a $TcpO_2$ reading of zero may be obtained in the presence of significant local cutaneous blood flow. They stated however, that non-heated LDF measurements do not correlate with local skin perfusion

whereas $TcpO_2$ and LDF measurements reflect changes in arteriovenous gradient when made over areas of heated skin (44°C). They concluded that the addition of cutaneous heating to LDF measurements enhances the capability of LDF for stratifying ischaemic wounds between those with marginal and inadequate blood flow.

Kram *et al.*, (1989) however, employing a non-heated LDF, compared dual calf measurements to wound outcome in 29 below knee amputations. The results were promising, however, not all the limbs were ischaemic and there were only four wounds which failed to heal. The authors also did not describe how test results influenced their clinical decisions.

A study by Karanfilian *et al.*, (1986) compared non-heated LDF to $TcpO_2$ in a single group of forefoot wounds consisting of 20 amputations and 36 ulcerations. The authors concluded that $TcpO_2$ more accurately predicted outcome (95% vs 87%), and that LDF was less useful because of the high incidence (23%) of false negative predictions. They reported that the sensitivity of the LDF was 79% while the specificity 96% (see Chapter Five for a discussion of this studies results). They suggested however that both methods were significantly more sensitive ($p<0.05$) than Doppler ankle pressure measurements for predicting healing.

Neither Holloway *et al.*, (1983) or Fairs *et al.*, (1987) were able to differentiate amputation groups (healed or failed) from controls with nonheated measurements. Both Holloway *et al.*, (1983) and Fairs *et al.*, (1987) also calculated the relative increase in LDF flux produced by heating the skin (heated flux / baseline flux). Fairs *et al.*, (1987) found that a significant difference existed between the absolute heated flux of the control and both the BK and AK groups ($p<0.001$). The difference between the absolute heated flux of both amputee groups was also significant ($p<0.005$). A more significant difference between the BK and AK group was found when the relative increase in flux was considered ($p<0.001$).

The difference between the controls and BK or AK groups using this parameter was also significant ($p<0.001$). The correlation between $TcpO_2$ and the relative increase in LD flux was $r = 0.7$, $p<0.001$. Relative flux was plotted rather than absolute flux as the authors' experience had indicated that vascular reactivity is likely to be a better indicator of potential for healing than absolute flow level. The correlations were very similar though marginally poorer for the absolute heated flux/$TcpO_2$ characteristic.

Holloway *et al.*, (1983) reported findings from an uncontrolled pilot study using an LDF for amputation level selection. Primary wound healing for both above knee and below knee amputations was indicated when the flux achieved after heating was at least one third of that seen in controls. However, no exact values were given and the findings from Padberg *et als'.*, (1992) study differed substantially. The latter study found that all above knee amputations healed in situations where the heated flux was less than 30% of the control levels.

Pabst *et al.*, (1985) found, in a study of LDF in controls and patients with PVD, a significant difference in unheated, baseline flux in the great toe, between controls and severely ischaemic limbs. These authors did not however find differences at more proximal sites between controls and either mild or severe PVD. These latter findings confirm those of Holloway *et al.*, (1983); Fairs *et al.*, (1987) and Padberg *et al.*, (1992).

Gebuhr *et als'.*, (1989) study was similar to the present study. These authors studied 22 amputations. Each patient had recordings at four level: the dorsum of the foot, 10 cm below the knee, 10cm above the patella, and on the sternum. Both the unheated, resting flux and the heated flux (after two minutes of local heating 44°C) were recorded. The flux increase after local heating was always prompt, and after one to two minutes no further increase was obtainable. The sternal recordings always showed a higher basic flux than those from the leg, and also the greatest increase in flux after local heating. The unheated, baseline

flux, before heating, showed no correlation with the amputation level, and the sternal flux did not correlate to the extremity flows. The increase in flux after heating, however, was directly related to healing. Sternal recordings would appear to be unnecessary in the normal clinical situation. The authors reported that if an increase of five or more arbitrary units was found upon heating the skin, amputation at this level was expected to be successful. They therefore concluded that the most distal level with a positive local heat test should be the level of amputation and that a positive local heat test indicated the presence of a reserve capacity in the microcirculation which was needed to ensure healing. It is important to note in this study however that, although wound healing occurred in 18 out of 21 when cutaneous heating produced an increase of 5 or more arbitrary units over baseline non-heated values, out of the four amputations that failed to heal, 3 were incorrectly predicted to heal by the criteria selected.

The most recent study that reports on the use of absolute heated $TcpO_2$ and LDF values is that of Padberg *et al.*, (1992). The skin was heated to 45°C for both instruments. For predicting healing, criteria for both $TcpO_2$ and LDF could be established with 100% specificity and positive predictive value. They found however that more general use of their criteria resulted in an unacceptably low sensitivity, negative predictive value, and/or accuracy. Thus, it was concluded that while this criterion may be of value in the individual patient, it is not appropriate for more general application. For predicting failure, only criteria for the LDF could be established at 100% sensitivity and negative predictive value. Criteria could not be established for $TcpO_2$. False negatives occurred with $TcpO_2$ measurement at its lowest predictive value. Similarly to Franzeck *et al.*, (1982); Harward *et al.*, (1985) and Wyss *et al.*, (1988) and in support of the findings of Matsen *et al.*, (1984), Padberg *et al.*, (1992), found that patients with very low $TcpO_2$ values proved capable of healing with appropriate local wound care. This included two patients with $TcpO_2$ measurements of 0 mmHg. In contrast, no wounds healed at a LDF range <35mv. This criterion for LDF range provided a sensitivity and negative predictive value of 100%, while retaining a positive

predictive value of 83%, and an accuracy of 85%. Padberg *et al's.*, (1992) study therefore provided a direct comparison between absolute heated probe LDF and $TcpO_2$ measurements made at the same temperature and location in assessing the outcome of a single set of ischaemic wounds followed to definitive clinical outcomes. Both the instruments accurately assessed the outcome of ischaemic wounds. The authors concluded that $TcpO_2$ excels in prediction of wound healing, but is less precise at low values, while LDF excels in the prediction of wound failure, and can discriminate between marginal and inadequate skin blood flow.

2.8 Wound Healing Limitations

The ultimate objective of preoperative LDF and $TcpO_2$ testing is to derive a numerical value above which all amputations will heal and below which none will heal. It is crucial to be aware however, that wound healing is a complex process. It not only dependent on an adequate blood and oxygen perfusion in the cutaneous microvasculature, but on multiple factors. These factors are the reason why there is no universal non-invasive technique for predicting the outcome of an amputation.

Mars *et al.*, (1993) suggests that a major limitation relates to the fact that non-invasive tests are all specific, providing information on either perfusion or oxygenation of either the skin or the muscle. The major problem with this is that the perfusion to the skin and muscle at a particular site of amputation can be different. The reason being that the circulation to the tissues does not always originate at the same level of the axial arterial tree (Mars *et al.*, 1993). Therefore, information is gained about only one aspect of the healing process.

Burgess *et al.*, (1981) suggest that the following variables will influence wound healing even if a preoperative test indicates that the circulation is adequate for healing.

Firstly, that postoperative blood flow is different to preoperative bloodflow. The amputation removes distal vascular runoff as well as collateral pathways. Despite this fact wound healing is predicted on the preoperative circulatory status. Secondly, the tests do not specifically quantify the ability of the limb to heal although they do provide important information about the circulatory status. Thirdly, surgical technique (tissue handling, flap length, and shape and tension of the wound) varies from patient to patient. The result being that the demands placed on the healing potential of the skin and underlying tissue are different. Another factor relates to postoperative care where specifics such as rigid dressings may influence wound tension and local pressure compromising circulation to the healing skin. A final factor which may decrease potential for wound healing is poor patient health. Postoperative problems such as thrombosis of the arteries to the residual limb, wound infection, malnutrition, pneumonia, atelectasis, and pulmonary embolism, may all compromise the ability of the wound to respond to the metabolic challenge of tissue-healing. Due to the above limitations both Burgess *et al.*, (1981) and Mars *et al.*, (1993) state that it should be easier to predict wound failure than wound healing. In other words a preoperative test may indicate that there is adequate perfusion for wound healing. However, the result may still be jeopardised by poor surgical technique and postoperative care as well as intercurrent disease. On the other hand assuming that all the above variables are optimal, if a test indicates that circulatory status is poor, it is easier to predict that the wound will fail to heal.

2.9 CONCLUSION

There is therefore a lack of knowledge regarding the use of LDF in predicting amputation wound healing due to the instrumentational, methodological, physiological and morphological differences discussed above. It is hoped that this study will be able to fill this gap and in doing so establish the LDF as an acceptable diagnostic tool. The use of LDF is increasing in the fields of physiology and pharmacology and in the practical daily clinical evaluation of vascular disease. The monitoring of the effects of treatment on the

microcirculation appears to be one of the most promising fields for the application of LDF. Further technical development of the LDF combined with extensive clinical research application may make this method one of the most interesting non-invasive fields of investigation in vascular disease.

CHAPTER THREE

3.0 METHODS

3.1 Validation Study

The stabilisation time, biological zero, and dynamic range of the LDF was obtained in a validation study on 20 healthy subjects. This study was performed in the King Edward VIII Hospital laboratory where the ambient temperature ranged between 20 to 23°C. The study followed the guidelines provided by Bircher *et al.*, (1994), with emphasis on the repeatability and the reproducibility of the LDF.

Repeatability is defined by these authors as expressing "the situation under the same conditions, i.e., same operator, same apparatus, short time interval, identical sample". Reproducibility is defined as expressing "the situation under different conditions, i.e., different laboratories, samples, different operators, different days, different instruments."

A standard reactive hyperaemia experiment (Bircher *et al.*, 1994) (Figures 5 and 6) was performed to validate the LDF. Due to the fact that the amputee study assessed unheated and heated LDF probe results the validation study was performed using both probes.

3.1.2 Validation Procedures

3.1.2.1 Reproducibility Study

The reproducibility study was performed over five days on 10 subjects (Table II). Each subject was tested at the same time of day for five consecutive days. Each testing session lasted forty-five minutes. The subjects were all assessed in the supine position. At the beginning of a fifteen minute acclimatisation period a standard blood pressure cuff was placed around the lower calf. The mid-dorsum of the subject's right foot was shaved and cleaned with alcohol when necessary. An unheated LDF probe was placed onto the mid-dorsum of the foot via double-sided adhesive tape. The LDF averaging time (time constant) was set at 0.3 seconds for the entire duration of the present study. This setting gave a good dynamic response and enabled the operator to readily identify non-physiological spikes. These could then be taken into account in the final analysis. The LDF printing mode was set to continuous data. This meant that two new LDF flux value were printed by the on-line, dot matrix, serial printer every second. After 15 minutes the printer was switched on for 15 seconds. The printer printed 30 resting cutaneous LD flux readings. The printer was then switched off and the arterial supply to the foot was occluded by inflating the blood pressure cuff to between 200 - 250 mmHg. After 3 minutes the printer was switched on. Once the printer had printed 30 biological zero values the pressure in the cuff was released. The printer then recorded the first 15 seconds of the phase of reactive hyperaemia. For each subject per day, 90 unheated LDF measurements were obtained.

The phase of reactive hyperaemia lasted from 1- 3 minutes. The cutaneous blood flux then decreased to resting values. After 5 minutes from the release of the blood pressure cuff the LDF probe was heated to 45°C to achieve maximal vasodilation of the cutaneous vessels. After 3 to 5 minutes an equilibrium state was reached. The printer was then switched on for

15 seconds. The same protocol as for the unheated probe was followed. For each subject per day, 90 heated LDF measurements were obtained.

3.1.2.2 Repeatability Study

The repeatability study was performed on 10 subjects (Table II) during a single testing session which lasted two hours. The same protocol was used as in the reproducibility study except for the following changes. The unheated test was performed five times consecutively. In other words the probe was only heated after the fifth phase of unheated reactive hyperaemia. The heated test was then performed five times. For each subject, 450 unheated and 450 heated LDF measurements were obtained. The time interval between all tests (consecutive tests within the unheated and heated probe tests and between the two probe tests) was 5 minutes. This ensured that stable baseline values were reached after the period of hyperaemia.

3.2 Statistical techniques for assessing agreement of LDF measurements in validation study

In order to validate the LDF, the agreement between the LDF values test 1 - test 5 (repeatability), and (day 1 - day 5 (reproducibility), or in other words the measurement error for daily as well as day to day measurements, was assessed. The study addressed the problem of agreement between the measurements by considering how much they differed from each other. A modified method of that described by Bland and Altman (1986) was used.

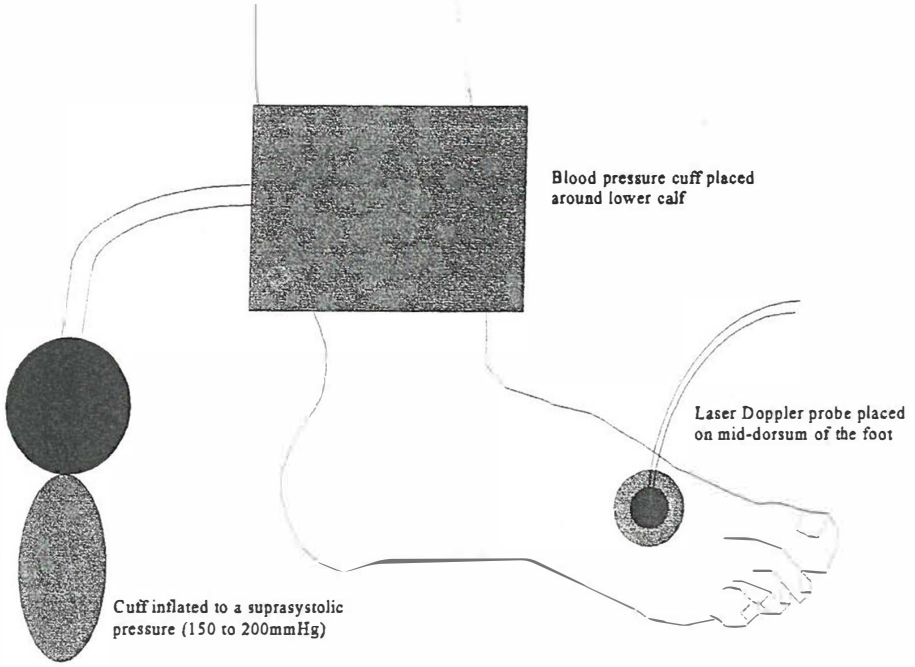

Figure 5: **Position of blood pressure cuff and LDF probe for standard reactive hyperemia experiment.**

Resting LD flux, Biological Zero, and peak flux (the peak value during the phase of reactive hyperemia) data, were reported as mean and standard deviation (SD) for each of the 5 sets of repeated measurements in the repeatability and the reproducibility study (Tables III and IV). The mean and SD were then calculated for the five tests combined and the five days combined. This mean and SD were used to calculate the coefficient of variation (Tables III and IV). Differences between measurements were calculated, and the average difference, i.e. the mean difference between measurements day 1 and day 2, day 2 and day 3, day 3 and day 4, and day 4 and day 5, (reproducibility); and test 1 and test 2, test 2 and test 3, test 3 and test 4, test 4 and test 5 (repeatability) were assessed (Tables III and IV). The subjects were then compared in a one-way analysis of variance and SD was found as the square root of the mean square error. The Repeatability Coefficient (RC), as adopted by the British Standards Institution was calculated by 1.96 (two standard deviations) multiplied by SD (Tables III and IV). If the RC = x (representing two standard deviations) then it is expected that 96% of the differences between measurements to be smaller than x.

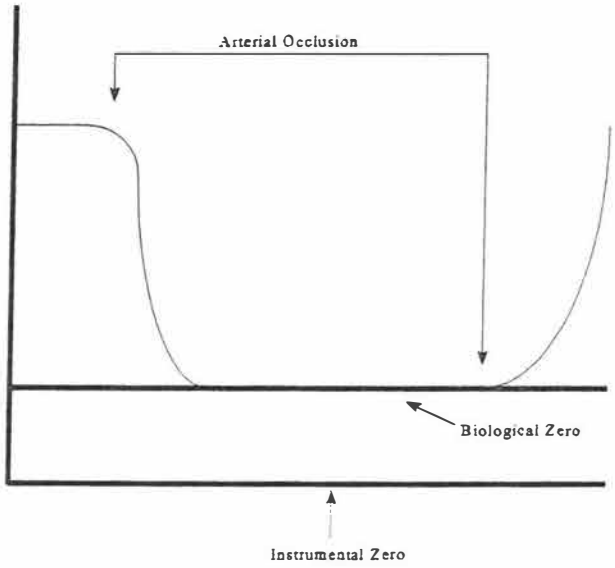

Figure 6: **Response of cutaneous blood flow during the POHR test as measured by LDF**

Adding or subtracting the RC from the average difference would indicate the RC range within which 96% of the differences between measurements would fall. If the difference lay outside the range this would suggest that the measurement was affected by some variable, other than biological or instrumental variables which account for the normal range of variation between repeated measurements.

Such information is critical when assessing whether a provocative test or pharmacological drug affects cutaneous blood flow. If, after applying a provocative test, for example the standard reactive hypereamic test, the resting cutaneous blood flow did not increase or decrease beyond the resting normal variation indicated by the RC, then it would suggest that the test had no effect on cutaneous blood flow. Any difference between the measurement

obtained during, or after the provocative test was applied, and the normal resting mean cutaneous blood flow, less than the RC, would be attributed to the normal daily or day to day variation and not as a consequence of the test. On the other hand, if the difference was greater than the range indicated by the repeatability coefficient then it suggest that the provocative test or vasodilator drug altered the cutaneous blood flow.

Bland (1987) reported that the measurement error may also be reported as the coefficient of variation, which is the SD divided by the mean, multiplied by 100 to give a percentage. This value has been used frequently in the literature to indicate the reproducibility or repeatability of the LDF and was therefore calculated in the present study in order to make comparisons. It is critical to note that this value depends on both the SD and the mean and therefore Bland (1987) recommended that it is not a good method for calculating measurement error, particularly if the range is great compared to the size of the smallest observations and the error does not depend on the value of the measurement.

3.3 Amputee Study

3.3.1 Subject Characteristics

Sixty patients with PVD, undergoing routine evaluation of wound healing potential, were studied at the Non Invasive Vascular Clinic, King Edward VIII Hospital. The unheated LDF probe was used on 60 patients and the heated probe on 35 of the 60 patients (Table II). The patients were not diabetic. General demographic data was obtained (Name, Sex, Age, Weight, Height) as well as a standard data proforma requiring information on the subject's presenting complaint, past medical history, medication, physical examination including presence of pulses, routine blood investigations, and the results of special vascular investigations. Each subject was given a verbal explanation of the procedures and reasons behind the testing. An interpreter was used in the case of Zulu speaking subjects. Informed

consent was obtained from each subject. The subjects had no prescriptions regarding food intake. They were asked to relax and to remain as still as possible during the investigation. Prior to testing, each patient lay supine for 20 minutes to acclimatise to ambient temperature of the laboratory, which ranged from 20 to 23°C. To avoid the possible confounding effect of previous heating of the skin, laser Doppler measurements were made before $TcpO_2$ measurement.

Table II: Demographic Data of Validation Study Subjects and PVD patients undergoing pre-operative assessment.

	Mean (years)	SD	Range	Male	Female
Reproducibility Study	32.8	12.18	23 - 53	4	6
Repeatability Study	36.1	14.04	21 - 59	4	6
Unheated Probe (n = 60)	57.68	15.36	30 - 87	34	26
Heated Probe (n = 35)	55.71	15.63	30 - 87	19	16

3.3.2 Instrumentation

3.3.2.1 LDF

LDF was performed with a Laserflo BPM^2 Blood Perfusion Monitor, Vasamedics, St Paul. This instrument has a low-power (2 milliwatt), solid state laser diode which is the source of a monochromatic infrared light at a frequency of 760 - 800 nm. The laser is conducted to the skin via an optic fibre which ends in a probe that is attached to the skin by means of a double sided adhesive ring. The fibre separation distance in the BPM^2 is approximately 0.5 millimetres. This yields a measurement depth of 1.0 millimetre. The measurement volume approximates a hemisphere of 1.0 to 1.5 millimetre radius. The BPM^2 has a Model TCO Temperature Control Option which is used for the local heating of skin

tissue. The TCO consists of a microprocessor based add-on hardware board that is mounted internal to the BPM[2]. A Model p-422 Heater Probe was used in the study in conjunction with a Model p-435 Softip Pencil Probe. The resulting signal was recorded as the laser Doppler flux in arbitrary units.

3.3.2.2 $TcpO_2$ Monitor

$TcpO_2$ measurement was made using a Hewlett Packard Transcutaneous Oxygen Monitor. The resulting signal was recorded as the $TcpO_2$ in mmHg. The $TcpO_2$ index, the ratio of limb to chest $TcpO_2$ was calculated for the amputation sites.

3.4 Unheated LDF Probe Procedures

LDF and $TcpO_2$ readings were taken at the three routine amputation sites (Figure 7), the mid dorsum of the foot (n = 33), 10cm below the tibial tuberosity over the anterior compartment (n = 61), 10 cm above the knee in the midline (n=24), and over the anterior chest wall, 5 cm below the clavicle in the mid-clavicular line (n =60). Due to the fact that this study was taking placing in a working environment it was not possible to obtain 60 complete sets of paired data (i.e. 60 subjects who had $TcpO_2$ and LDF measurements at all three lower limb sites). Measurement sites were shaved and cleaned with an alcohol solution when necessary. No zeroing procedure was undertaken. Readings were made after 3 minutes. The LDF averaging time was set at 0.3 seconds. After 3 minutes the printer was switched on and recorded the first 15 seconds of flux. Once again the LDF printing mode was set to continuous data. This meant that two new LDF flux value were printed by the on-line, dot matrix, serial printer every second. Thirty resting cutaneous LD flux readings were therefore recorded and the mean over the 15 seconds calculated for each subject. The sequence of sites tested was chest, above knee, below knee, foot. The unheated LDF index, the ratio of limb to chest LDF reading was calculated for the amputation sites.

3.5 Heated LDF Probe Procedures

LDF and $TcpO_2$ readings were taken at the three routine amputation sites (Figure 7), the mid dorsum of the foot (n =17), 10cm below the tibial tuberosity over the anterior compartment (n = 36), 10 cm above the knee in the midline (n=17), and over the anterior chest wall, 5 cm below the clavicle in the mid-clavicular line (n =35). Once again due to the fact that this study was taking placing in a working environment it was not possible to obtain 35 complete sets of paired data (i.e. 35 subjects who had $TcpO_2$ and LDF measurements at all three lower limb sites). Measurement sites were shaved and cleaned with an alcohol solution when necessary.

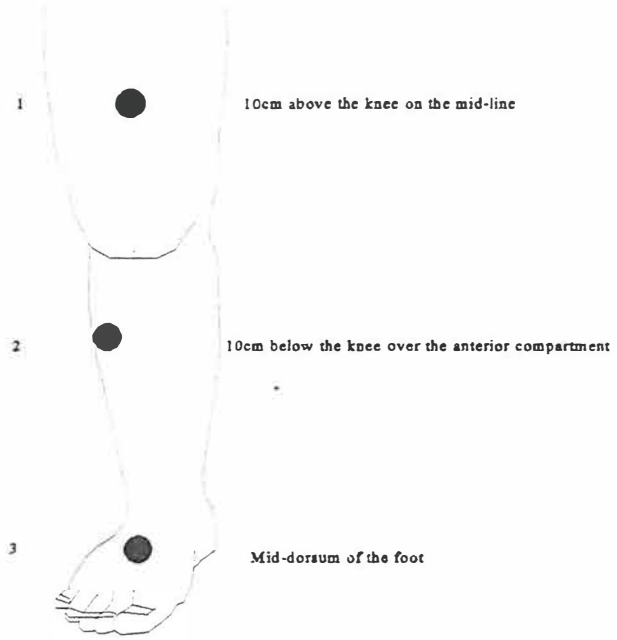

Figure 7: Probe placement sites on the lower limb

No zeroing procedure was undertaken. The LDF averaging time was set at 0.3 seconds. A resting unheated LD flux measurement was made after 3 minutes. After 3 minutes the

printer was switched on and recorded the first 15 seconds of flux. The LDF probe was then heated to 45°C. One 15 second recording was made after 5 minutes once the heated laser Doppler flux had stabilised. The printer configuration was identical to that used when recording unheated flux. Thirty heated flux values were therefore recorded and the mean over the 15 seconds was calculated for each subject. The sequence of sites tested was chest, above knee, below knee, foot. The heated LDF index, the ratio of limb to chest LDF reading, the Vascular Reserve (VR) or Heating Response (mean heated LD flux subtract the mean unheated LD flux) were calculated at each site.

3.6 Statistical Analysis

Descriptive statistics were calculated at each site for both the unheated and heated, absolute and index LDF values. Comparisons were made of the respective values at each site. The data were also pooled and correlations between the unheated data and $TcpO_2$ (absolute and index) and the heated data and $TcpO_2$ (absolute and index) were calculated. For the 35 heated probe subjects the change from the unheated resting LD values to the heated LD values were compared at each site as well as overall. The difference was termed the laser Doppler fluxmeter vascular reserve (LDF VR) and was compared against the $TcpO_2$ index. A LDF VR index was also calculated and compared against the $TcpO_2$ index. The statistical analysis for comparison was performed using Spearman's rank correlation test.

Linear regression equations were calculated for both the unheated and the heated data for the following sets of data: pooled $TcpO_2$ index and absolute LDF, pooled $TcpO_2$ index and LDF index, pooled $TcpO_2$ index and LDF VR, pooled $TcpO_2$ index and LDF VR index.

The heated LDF absolute / $TcpO_2$ index data as well as LDF VR / $TcpO_2$ index data were also divided into those pairs classified as predicting wound healing by a $TcpO_2$ index of >0.55 (41 pairs) and those pairs classified as predicting wound failure by a $TcpO_2$ index of

<0.55 (29 pairs). A Spearman rank correlation value and linear regression equation was obtained for the predicted healing set and the predicted failure set to see whether the LDF and $TcpO_2$ values were correlated at both high and low perfusion states.

The unheated, heated LDF and LDF VR, as well as the $TcpO_2$ data underwent further analysis in order to determine whether there was a significant intragroup difference at the different amputation levels. This analyses was performed to determine whether the LDF and the $TcpO_2$ instruments were sensitive to the presence of PVD (indicated by a decrease in perfusion the more distal the measurement). The reason why the analyses was performed in the particular manner described above, was because for both the unheated and heated data only a few patients underwent evaluation at all three amputation levels. The majority were measured at two levels (either above-knee and below-knee, or below-knee and foot). Hence in order for the comparison of levels to be valid these combinations were separated from each other.

For statistical analysis a paired t-test was performed comparing the laser absolute values at the different levels, the $TcpO_2$ absolute and index values at the different levels, and the LDF VR values at the different levels. Overall for the unheated LDF data and the $TcpO_2$ there were 18 patients who were measured at above-knee and below-knee levels, there were 34 patients who were measured at below-knee and foot levels. For the absolute heated LDF, $TcpO_2$ as well as the LDF VR data, 12 patients had above-knee and below-knee measurements, while 17 had below-knee compared to foot measurements.

For all the analyses a level of probability of 0.05 was selected in order to reduce the chances of making a Type I error without increasing, too greatly, the chances of making a Type II error (Ferguson, 1981). The analysis was performed using the Microsoft Excel software package (with Astute, an additional statistics add-in for Excel).

3.6.1 Predictive Potential of the LDF

The unheated and heated LDF studies were run in parallel to the routine $TcpO_2$ test and were designed to determine how well the LDF could discriminate pre-operatively between levels of perfusion which indicated wound healing failure and levels which predicted wound healing.

For the LDF correlation results to be of clinical use they were analysed with respect to the present Gold Standard for amputation site selection, the $TcpO_2$ index of 0.55. In order to assess the influence that the Gold Standard had on determining the usefulness of the LDF, the correlation results were also analysed with respect to the following $TcpO_2$ indices: 0.5; 0.53; 0.57; 0.6. The decision was made that the absolute heated LDF / TcpO2 index (0.55) (Figure 12) correlation and the LDF VR / TcpO2 index (0.55) (Figure 15) correlation would be used to assess the usefulness of the heated LDF values for pre-operatively predicting wound healing. This was due to the fact that these comparisons had the highest correlation.

When comparing the results from two diagnostic tests like LDF and $TcpO_2$, using the $TcpO_2$ index as the Gold Standard for predicting healing, four situations are possible: a) a true-positive (TP) result: the LDF value pre-operatively predicted wound healing failure and the wound will fail to heal according to the Gold Standard (this value is interpreted as the Sensitivity of the Test); b) a false-positive (FP) result: the LDF pre-operatively predicts failure but the wound heals; c) a false negative (FN) result: the LDF pre-operatively predicts healing but the wound fails to heal; and d) a true-negative (TN) result: the LDF pre-operatively predicts healing and the wound heals (this value is interpreted as the Specificity of the test). The best diagnostic test is one with a small percentage of false-positives and false-negatives in other words the test which has the highest accuracy (Altman *et al.*, 1994)

The LDF was therefore evaluated through calculating the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy for several absolute heated LDF and LDF VR cut-off points in relation to various $TcpO_2$ indices (0.5, 0.53, 0.55, 0.57 and 0.6). Sensitivity was defined in this study as the ability of the heated LDF values to predict wound healing failure (how good the test was at identifying inadequate perfusion, i.e. identifying the diseased) (Hulley *et al.*, 1988). Specificity was identified as the ability of the LDF to predict primary healing (how good the test was at identifying adequate perfusion, i.e. identifying the non-diseased (Hulley *et al.*, 1988). The PPV and NPV give an indication of the probability that the test would give the correct diagnosis. The PPV was the proportion of patients with LDF test results that predicted failure who were correctly diagnosed (according to the selected $TcpO_2$ cut-off index) . The negative predictive value (NPV) was the proportion of patients with LDF test results that predicted healing who were correctly diagnosed (according to the selected $TcpO_2$ cut-off index). Wagner *et al.*, (1988) and Altman *et al.*, (1994) state that the positive predictive value is a more clinically useful figure than sensitivity or specificity. Finally the accuracy of the LDF was calculated to determine the overall LDF measurement error taking all four of the situations TP, FP, TN, and FN into account.

Sensitivity, specificity, PPV, NPV and accuracy of the LDF were calculated through the use of the binary table equations (Figure 8) (Dwars *et al.*, 1992) (which manipulated values from the absolute heated LDF / $TcpO_2$ index and the LDF VR / $TcpO_2$ index correlation graphs (Figures 12 and 15). For both graphs the $TcpO_2$ index was placed on the X-axis. The cut-off index was set at the five values mentioned above and the vertical line (shown in each graph) through the data, from the $TcpO_2$ index was used to separate healing from non healing wounds. On the right hand side of this line the $TcpO_2$ data predicted that wounds would heal while on the left hand side of the line the data predicted that the wounds would fail. For each $TcpO_2$ cut-off index, out of the total number of measurements (70 for each graph), the data that predicted wound healing failure (values below the cut-off) and the

number that predicted wound healing (values at or above the cut-off) differed which played a role in altering the usefulness of a particular LDF cut-off point in relation to the selected $TcpO_2$ index.

For each $TcpO_2$ index used as a cut-off, several absolute heated LDF values (ranging from 4 a.u. to 6 a.u.) and LDF VR values (ranging from 2.8 a.u. to 4.7 a.u.) were selected on the Y-axis to represent heated LDF values or cutaneous blood flow cut-offs which may be useful for pre-operative assessment of wound healing. The horizontal line through the data represents the absolute, heated LDF or LDF VR value which showed the division between healing and non healing wounds. Data below this line predicted that wounds would fail to heal while data above this line predicted that wounds would heal.

These two lines divide the graphs into four sections. These four sections represent the four classifications shown in the binary table (Figure 8). For example, from Figure 12 it can be seen using a selected cut-off absolute LDF value of 4.9 that the top left hand section represents those LDF measurements which predicted healing according to the LDF but were predicted to fail according to the $TcpO_2$ index. These values are called the false negative (FN) results because the LDF suggests that there is no indication of PVD or no compromise of perfusion, however according to the $TcpO_2$ index the wound will fail to heal postoperatively. The top right hand section shows those measurements which predicted healing according to the LDF and the $TcpO_2$ index. These measurements are called true negatives (TN) because the LDF showed that there was no indication of PVD or compromise of perfusion and the $TcpO_2$ index predicted healing. The bottom left hand corner shows those LDF measurements which predicted failure and the $TcpO_2$ index predicted failure. These values are called true positives (TP) because the LDF values indicated the presence of PVD and blood flow compromise and the $TcpO_2$ index predicted wound healing failure. The bottom right hand corner shows those values where the LDF predicted failure and the $TcpO_2$ index predicted healing. These values are called false positives (FP). The LDF values

indicated the presence of PVD and blood flow compromise, while the $TcpO_2$ index predicted that wounds would heal.

The FN, TN, TP, FP, together with the total LDF predicted failure result (TP + FP) and LDF predicted heal results (FN + TN) and the total failed wounds (TP + FN) and healed wounds (FP + TN) predicted by the $TcpO_2$ index were counted for each absolute heated LDF and LDF VR selected cut-off value in relation to the various $TcpO_2$ indices. These values were used to calculate the sensitivity, specificity, PPV, NPV and accuracy of the absolute heated LDF and LDF VR selected cut-off values.

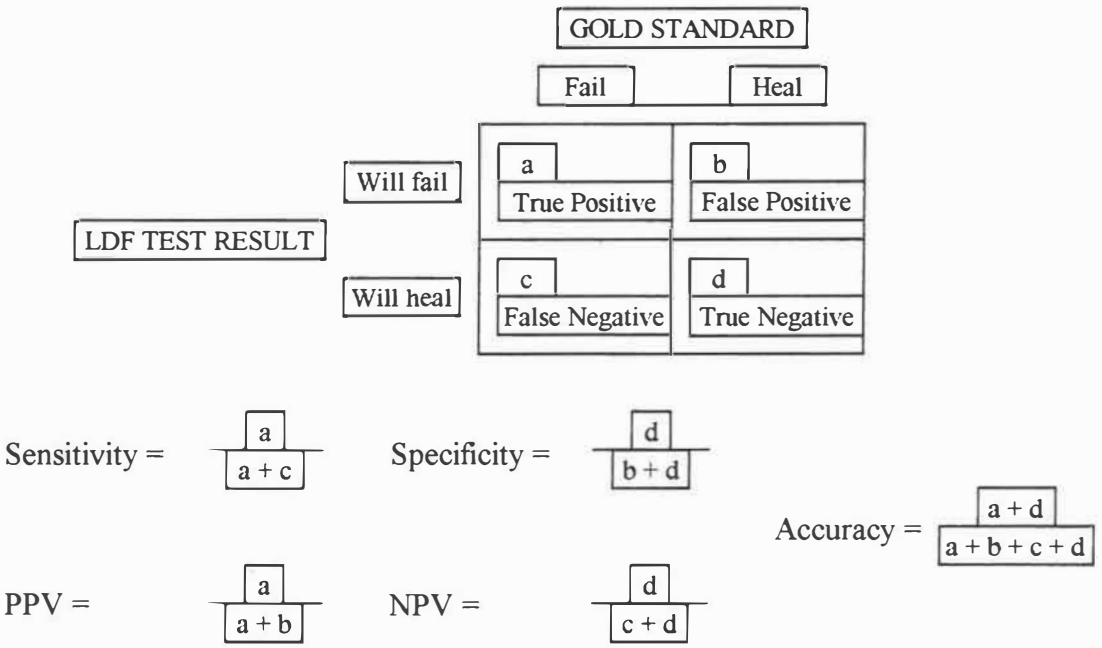

Figure 8: **Binary table evaluates sensitivity, specificity, PPV, NPV and accuracy of LDF for pre-operatively predicting wound healing.**

3.6.2 Predictive Potential Statistical Procedure

The aim of the present study was to calculate an absolute LDF value or LDF VR value that would be the most useful for pre-operatively evaluating wound healing potential. This present study used the **Receiver Operator Characteristic** (ROC) curve (Altman *et al.*, 1994) to make a more sensitive selection for the cut-off. This is a graphic way of portraying the trade-offs involved between improving either the LDF's sensitivity or its specificity. As was stated earlier sensitivity and specificity were calculated for several absolute, heated LDF and LDF VR measurements in relation to various $TcpO_2$ indices. For each absolute, heated LDF or LDF VR value in relation to a particular $TcpO_2$ index , sensitivity was plotted against 1-specificity.

According to Altman *et al.*, (1994), the ideal test is one that yields a curve that coincides with the upper left corner of the graph (100% sensitivity and 100% specificity). A worthless test would give a straight line from the bottom left corner to the top right corner: with each incremental gain in sensitivity being matched by an equal loss in specificity. A global assessment of the performance of the test [called the diagnostic accuracy (Zweig *et al.*, 1993)] is given by the area under the ROC curve and is calculated through the use of the following equation: Area under Curve = $\frac{1}{2}\{(a_1 + a_2) * (b_2 - b_1)\}$ (Zweig *et al.*, 1993).

Most ROC curves have a very steep section, in which the sensitivity increases a great deal while the false-positive rate hardly changes. It makes little sense to choose the cut-off point in this section, since moving up the curve will increase sensitivity without substantially reducing specificity. Similarly, selecting the cut-off point in the flat region, in which the sensitivity stays about the same while the false-positive rate increases, is unwise. The best cut-off point is where the ROC curve "turns the corner" (Hulley *et al.*, 1988).

An important advantage of ROC curves is that the curves for different diagnostic tests can be compared; the better a test, the closer its curve is to the upper left corner and consequently the closer the area under the curve is to one (Altman *et al.*, 1994). Figures 17 to 226 show the ROC curves for the absolute, heated LDF and LDF VR calculations in relation to the TcpO2 indices of 0.5; 0.53; 0.55; 0.57; 0.6. The absolute heated LDF value or LDF VR which is closest to the top left hand corner for each graph was chosen as the most useful value for that set of data. The sensitivity, specificity, PPV, NPV, and accuracy for each of these most useful points is displayed in Table X.

The linear regression equations obtained from the LDF / $TcpO_2$ index correlation and the LDF VR / index correlation were also used to calculate LDF and LDF VR values that corresponded with the $TcpO_2$ index of 0.55. The sensitivity and specificity as well as the positive and negative predictive percentages and the accuracy for these values were calculated and are also displayed in Table X. The site specific absolute, heated LDF data and LDF VR data was also compared to the Gold Standard $TcpO_2$ index at each lower limb site (Table IX).

The absolute, heated LDF or VR value (with respect to the various $TcpO_2$ indices) that was closest to the top left hand corner of the ROC curve and was part of a curve which had an area closest to one, was interpreted as being the most useful and accurate cut-off point for preoperatively evaluating wound healing potential in patients with PVD.

CHAPTER FOUR

4.0 RESULTS

4.1 LDF Validation

4.1.1 Repeatability

Repeatability was defined as expressing the LDF measurements over a short time period with the same operator, and an identical sample under the same conditions. Table III shows the mean and standard deviation for the heated and unheated resting cutaneous blood flow value, biological zero, peak flux value, as well as the temporal variation over the 2 hour period for the 10 subjects tested. The results show the significantly different ($p<0.0001$) magnitude in the mean signal between the unheated and heated LDF probe for both resting flux and peak flux. There was no significant difference between the biological zero values. The mean heated resting LDF value was 88.9% higher than the unheated flux value while the heated peak flux was 69.8% higher than the unheated peak flux. The heated peak flux was 19.4% higher than the heated resting flux while the unheated peak flux was 70.38% higher than the unheated peak flux.

The repeatability coefficient (RC) indicates the value within which 96% (two standard deviations) of the differences between repeated measurements over a two hour period will fall. The closer the RC to zero the smaller the difference between repeated measurements. In other words the RC gives an indication of the normal temporal variation in cutaneous blood flow over a two hour measurement period.

Subtracting and adding the RC to the average difference indicates the range within which 96% of the differences (representing normal variation due to biological and instrumental variables) over a two hour measurement period are expected to fall and within which 96% of normal LDF flux values will fall. The RC range for the heated resting absolute LDF values was - 3.37 to + 2.51, while for the unheated absolute resting values it was - 0.67 to + 0.69. The biological zero ranges were low being - 0.17 to + 0.16 and - 0.22 to + 0.22 for heated and unheated measurements respectively. The peak flux RC range for the heated LDF

Table III: Heated and unheated LDF data from Repeatability Study using standard POHR test

REPEATABILITY [Data are reported as mean (SD)]						
	HEATED (a.u.)			**UNHEATED (a.u.)**		
	Resting	**B-Zero**	**P-Flux**	**Resting**	**B-Zero**	**P-Flux**
Test 1	16.52 (7.93)	0.49 (0.099)	21.64 (11.59)	1.945 (0.87)	0.474 (0.16)	6.32 (3.50)
Test 2	16.82 (9.18)	0.44 (0.066)	21.08 (11.33)	1.93 (0.63)	0.501 (0.143)	6.56 (3.56)
Test 3	17.67 (9.32)	0.54 (0.14)	21.12 (11.32)	1.94 (0.7)	0.465 (0.081)	6.27 (3.360
Test 4	18.18 (10.04)	0.48 (0.10)	22.31 (11.45)	2.01 (0.70)	0.49 (0.17)	6.42 (4.03)
Test 5	18.25 (9.89)	0.51 (0.10)	22.41 (10.61)	1.89 (0.53)	0.46 (0.13)	7.18 (4.08)
Average	17.49 (0.79)	0.49 (0.037)	21.71 (0.63)	1.94 (0.04)	0.48 (0.017)	6.55 (0.37)
T1-T2	-0.296 (1.9)	0.053 (0.094)	0.558 (5.43)	0.014 (0.69)	-0.027 (0.216)	-0.25 (1.59)
T2-T3	-0.85 (1.36)	-0.106 (0.091)	-0.039 (1.74)	-0.01 (0.475)	0.036 (0.141)	0.298 (1.22)
T3-T4	-0.51 (1.41)	0.063 (0.09)	-1.19 (2.15)	-0.068 (0.44)	-0.023 (0.17)	-0.153 (0.79)
T4-T5	-0.067 (1.68)	-0.028 (0.09)	-0.092 (2.22)	0.118 (0.474)	0.027 (0.18)	-0.76 (1.163)
Ave Difference	-0.432 (0.33)	-0.0045 (0.079)	-0.19 (0.73)	0.0135 (0.078)	0.0033 (0.033)	-0.216 (0.43)
Repeatability Coefficient (RC)	1.96 *1.501 = 2.94	1.96 * 0.083 = 0.163	1.96 * 2.618 = 5.131	1.96 * 0.349 = 0.684	1.96 * 0.112 = 0.2195	1.96 * 1.024 = 2.007
Coefficient of Variation %	4.5	7.5	2.9	1.7	3.5	5.6

(- 5.32 to + 4.94) was higher compared to the unheated LDF (- 2.22 to + 1.79) peak flux range. From Table III it can be seen that the coefficient of variation was low ranging from 1.7% for the resting unheated flux to 7.5% for the heated biological zero.

4.1.2 Reproducibility

Reproducibility was defined as expressing the LDF measurements under different conditions, specifically in the present study, over different days. Table IV shows the mean and SD for the heated and unheated resting cutaneous blood flow value, biological zero, and the peak flux value for the test conducted once daily over five consecutive days for the ten subjects tested. The results show the significantly different ($p<0.0001$) magnitude in the signal between the unheated and heated flux for both resting and peak flux. There was no significant difference between the biological zero values. The mean heated resting flux value was 88.4% higher than the unheated LDF value while the heated peak flux was 67.9% higher than the unheated peak flux value. The heated peak flux was 35.8% higher than the heated resting flux while the unheated peak flux was 76.85% higher than the unheated resting value.

The RC indicates the value below which 96% of the differences between repeated measurements over five day period will fall. The closer the RC to zero the smaller the difference between repeated measurements. In other words the RC gives an indication of the normal temporal variation in cutaneous blood flow over a 5 day measurement period.

Subtracting and adding the RC to the average difference indicates the range within which 96% of the differences (representing normal variation due to biological and instrumental variables) over a 5 day period are expected to fall and within which 96% (two standard deviations) of normal LDF flux values will fall. The RC range for the heated resting LDF values was - 9.72 to + 8.04, while for the unheated resting values it was - 1.65 to + 1.51. The biological zero ranges were low being - 0.24 to + 0.27 and - 0.198 to + 0.184 for

heated and unheated measurements respectively. The peak flux RC range for the heated LDF (- 14.05 to + 11.19) was higher than the unheated LDF (- 4.82 to + 5.46) peak flux range.

Table IV: Heated and unheated LDF data from Reproducibility Study using standard POHR test

REPRODUCIBILITY [Data are reported as mean (SD)]						
	HEATED (a.u.)			**UNHEATED (a.u.)**		
	Resting	**B-Zero**	**P-Flux**	**Resting**	**B-Zero**	**P-Flux**
Day 1	12.32 (6.05)	0.47 (0.196)	17.09 (7.04)	1.44 (0.54)	0.44 (0.15)	7.87 (3.01)
Day 2	17.09 (7.04)	0.53 (0.15)	26.51 (10.56)	1.56 (1.19)	0.41 (0.14)	8.15 (3.31)
Day 3	16.45 (9.26)	0.59 (0.17)	26.59 (8.93)	2.05 (1.39)	0.46 (0.11)	7.67 (3.11)
Day 4	12.95 (4.12)	0.48 (0.085)	22.99 (6.81)	1.81 (0.84)	0.47 (0.15)	6.85 (2.25)
Day 5	15.66 (7.65)	0.41 (0.15)	22.81 (7.72)	1.74 (0.88)	0.47 (0.16)	6.59 (1.67)
Average	14.89 (2.14)	0.49 (0.068)	23.2 (3.87)	1.72 (0..24)	0.448 (0.025)	7.43 (0.67)
D1-D2	-4.78 (4.44)	-0.06 (0.21)	-9.42 (6.92)	-0.12 (1.32)	0.025 (0.173)	-0.29 (5.63)
D2-D3	0.65 (5.23)	-0.058 (0.195)	-0.077 (6.05)	-0.49 (1.46)	-0.047 (0.12)	0.47 (2.66)
D3-D4	3.51 (8.21)	0.11 (0.129)	3.59 (7.43)	0.24 (0.97)	-0.005 (0.15)	0.83 (3.34)
D4-D5	-2.72 (5.35)	0.065 (0.14)	0.185 (5.73)	0.07 (0.39)	-0.001 (0.14)	0.26 (2.92)
Ave Difference	-0.84 (3.66)	0.015 (0.087)	-1.43 (5.58)	-0.76 (0.31)	-0.007 (0.03)	0.32 (0.41)
Repeatability Coefficient (RC)	1.96 * 4.53 =8.88	1.96 * 0.1278 =0.250	1.96 * 6.44 =12.62	1.96 * 0.809 =1.586	1.96 * 0.0976 =0.191	1.96 * 2.62 =5.135
Coefficient of Variation %	14.4	13.9	16. 7	14	5.6	9.0

The repeatability coefficients for the resting and peak flux data for the reproducibility study, using the heated and the unheated LDF probe, were considerably higher than the respective values in the repeatability study. The RC for the resting heated flux in the repeatability study was 2.94 while for the reproducibility study it was 8.88 (Tables III and

IV). The repeatability coefficients for the peak flux data were 5.131 and 12.62 respectively. The RC for the resting unheated flux in the repeatability study was 0.684 while for the reproducibility study it was 1.586. The unheated peak flux in the repeatability study was 2.007 while for the reproducibility study it was 5.135.

The coefficient of variation was high ranging from 5.6% in the unheated biological zero to 16.7% in the heated peak flux. The coefficient of variation results, together with the RC results indicate large temporal variations between day-to-day measurements of cutaneous blood flux compared to repeated measurements on the same day.

4.2 LDF Amputee Results

4.2.1 Unheated LDF Compared to $TcpO_2$

The absolute LDF and $TcpO_2$ measurements, and the LDF index and the $TcpO_2$ index at the different sites are expressed as means and one standard deviation and are shown in Table V . The ranges of the readings were, LDF 0 - 12.0 arbitrary units, $TcpO_2$ 0 - 77 mmHg, the LDF index 0 - 3.0 and the $TcpO_2$ index 0 - 1.43. The highest mean readings for LDF and $TcpO_2$ were measured at the chest. On the leg the highest values were obtained at the foot with the LDF while the $TcpO_2$ values were lowest at the foot. Significant correlation was found between LDF and $TcpO_2$ at the foot, and between the $TcpO_2$ and LDF index at the below knee site and at the foot.

Pooling the data for the various sites there was a significant correlation between LDF and $TcpO_2$, ($n= 178$), $r = 0.34$, ($p<0.0001$), and between the LDF index and the $TcpO_2$ index, ($n= 118$), $r = 0.17$, ($p = 0.062$). There was poor correlation between LDF and the $TcpO_2$ index ($n = 118$), $r = 0.13$, ($p = 0.17$).

Table V: Unheated Laser Doppler fluxmetry, $TcpO_2$, laser Doppler index and the $TcpO_2$ index expressed as means and one standard deviation are shown for the different measurement sites. The indices are the limb to chest ratios. Correlation of the results at each site was by Spearman's rank correlation which was considered significant when $p < 0.05$.

	Laser (au)	$TcpO_2$(mmHg)	p =	Laser Index	$TcpO_2$ Index	p =
Chest (n=60)	3.94 ± 2.07	51.12 ±12.09	0.46			
AK (n=24)	1.74 ± 1.11	36.08 ±18.15	0.66	0.61 ± 0.41	0.72 ± 0.22	0.35
BK (n=61)	1.6 ± 0.9	33.57 ±18.04	0.39	0.52 ± 0.41	0.63 ± 0.28	0.09
Foot (n=33)	1.93 ± 1.33	15.24 ±12.81	0.0009	0.53 ± 0.63	0.29 ± 0.23	0.0014

For the correlation to be of clinical use it should be analysed with respect to the present criteria for amputation site selection. In the Durban Metropolitan Vascular Service this is based on the $TcpO_2$ index of 0.55. Sixty - five pairs of readings were above 0.55 and 53 pairs of readings below 0.55. The breakdown at each level was the following: Above - knee level, 21 pairs above 0.55, 3 pairs below 0.55; below - knee level, 41 pairs above 0.55, 20 pairs below 0.55; foot, 3 pairs above 0.55, 30 pairs below 0.55. The LDF values relative to the $TcpO_2$ index are shown in Figure 9 and the LDF index values relative to the $TcpO_2$ index are shown in Figure 10. The range of the scatter of LDF readings is such that there is no absolute LDF value or LDF index which has a high predictive power when compared to the $TcpO_2$ index of 0.55.

Various absolute $TcpO_2$ values have been suggested as predictive of amputation wound healing. These range from 0 to 20 and 40 mmHg (Malone *et al.*, 1987; Wagner *et al.*, 1988; Sarin *et al.*, 1991). Despite a significant correlation between absolute LDF and $TcpO_2$ readings there is no LDF level which has a high predictive power. This is shown in Figures 9 and 10 which shows the laser Doppler absolute flux with respect to the TcpO2 index of 0.55 (indicated by the solid line), and in Figure 11 by the random scatter of the data.

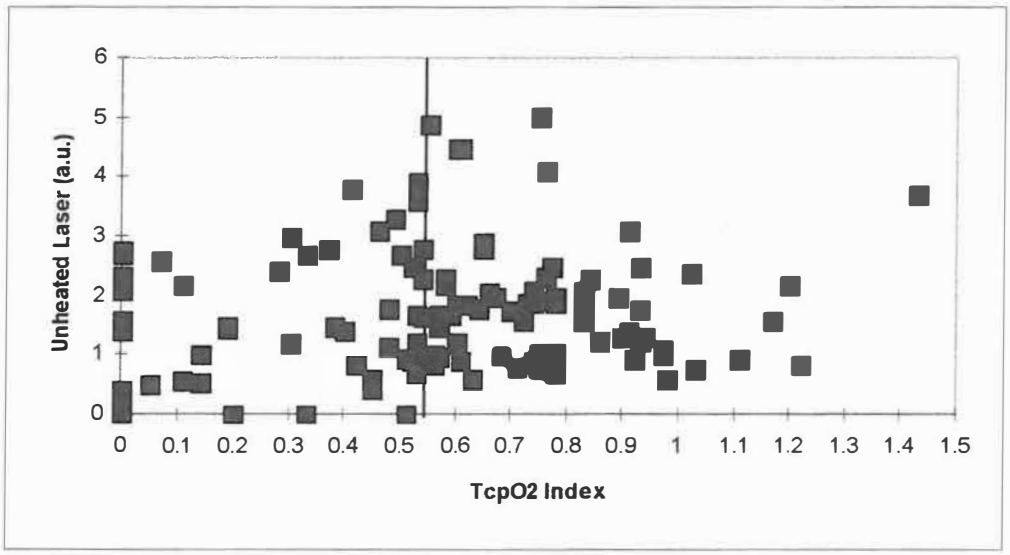

Figure 9: **Scattergram showing the relationship between the $TcpO_2$ index and unheated LDF (n = 118). The Spearman rank correlation coefficient is r = -0.001, (p = 0.99). No amputation site with a $TcpO_2$ index of less than 0.55 would be expected to heal. The linear regression equation is y = 1.42 + 0.55x**

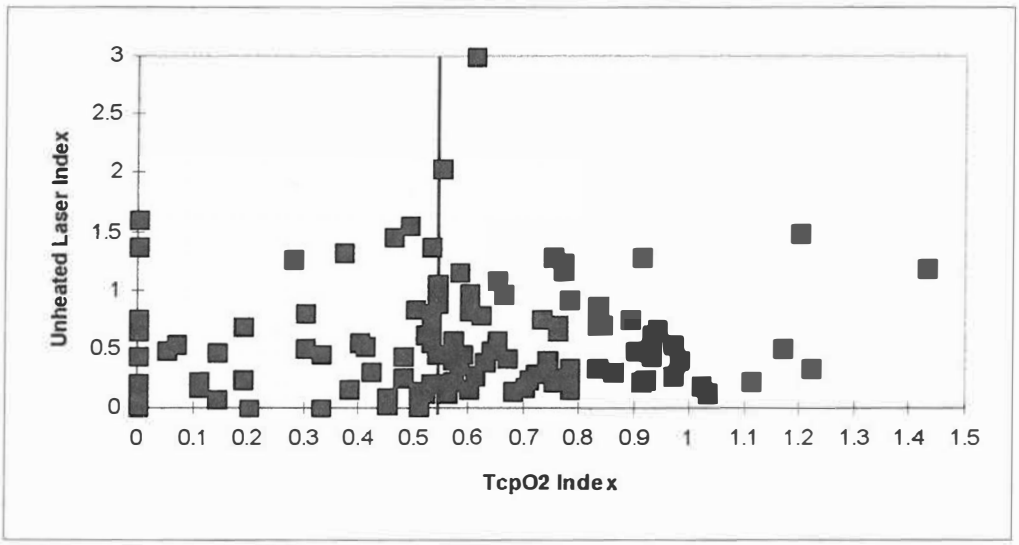

Figure 10: **Scattergram showing the relationship between the $TcpO_2$ index and unheated LDF index (n = 118). The Spearman rank correlation coefficient is r = 0.233, (p = 0.025). No amputation site with a $TcpO_2$ index of less than 0.55 would be expected to heal. The linear regression equation is y = 0.46 + 0.18x**

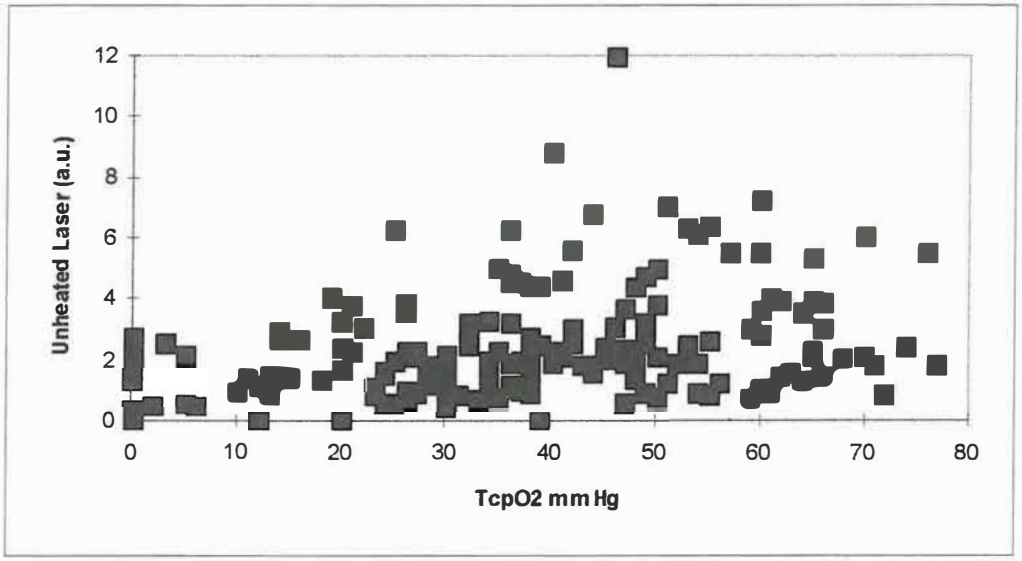

Figure 11: **Scattergram showing the relationship between $TcpO_2$ (mmHg) and unheated LDF (au) (n = 178). The Spearman rank correlation coefficient is r = 0.409, ($p < 0.0001$).**

4.2.2 Unheated LDF's ability to identify the presence and characteristics of PVD

From Table VI it can be seen that the absolute unheated LDF found significant differences between flux at the three different levels. For the above-knee / below-knee analysis the below-knee flux was significantly ($p<0.05$) lower than the above-knee flux. The table shows an interesting result at the foot. There was a difference between the flux of the below-knee and the foot in the below-knee / foot combination, however the foot flux was significantly ($p<0.05$) higher than the below-knee value. This finding will be discussed in detail in Chapter Five. The table shows that while unheated LDF was unable to display clearly the presence of PVD both the absolute $TcpO_2$ values and the $TcpO_2$ index were able to do so, measuring significantly ($p<0.05$) lower flux values at each lower amputation level.

Table VI: Intra-group difference at the different levels (above-knee compared to below-knee, below-knee compared to foot). Unheated Laser Doppler, $TcpO_2$ and the $TcpO_2$ index expressed as means and one standard deviation are shown. Statistical analysis by a Paired t-test, which was considered significant when $p < 0.05$.

Combination	Laser (au)	p =	$TcpO_2$ (mmHg)	p =	$TcpO_2$ Index	p =
A-K / B-K (n=18)	1.91 (1.17)		38.88 (15.95)		0.75 (0.22)	
	1.17 (0.98)	0.027	16.5 (12.49)	<0.0001	0.31 (0.22)	<0.0001
B-K / Foot (n=34)	1.56 (0.75)		39.32 (14.44)		0.74 (0.28)	
	2.03 (1.32)	0.023	16.0 (12.48)	<0.0001	0.33 (0.25)	<0.0001

4.2.3 Heated LDF Compared to $TcpO_2$

The absolute LDF and $TcpO_2$ measurements, and the LDF index compared to $TcpO_2$ index, VR compared to $TcpO_2$ index, and the VR index compared to $TcpO_2$ index at the different sites are expressed as means and one standard deviation and are shown in Tables VII and VIII. The ranges of the readings were, LDF 0 - 27.8 arbitrary units, $TcpO_2$ 0 - 77 mmHg, LDF index 0 - 1.4, $TcpO_2$ index 0 - 1.43, VR 0 - 18.38 and VR index 0 - 1.81. The highest mean readings for absolute LDF, LDF VR and $TcpO_2$ were measured at the chest. On the leg the highest values were obtained at the above knee site, while the lowest values were at the foot for absolute resting laser Doppler flux, LDF VR, $TcpO_2$ absolute and index values. Significant correlations were found between LDF and $TcpO_2$ absolute and index values, and LDF VR (absolute and index) and $TcpO_2$ (index) at the foot and below knee.

Pooling the data for the various sites there was a significant correlation between LDF and $TcpO_2$, ($n= 105$), $r = 0.63$, ($p<0.0001$), and between the LDF index and the $TcpO_2$ index, ($n= 70$), $r = 0.68$, ($p<0.0001$). There was a significant correlation between LDF and the $TcpO_2$ index, ($n=70$), $r = 0.72$, ($p<0.0001$). There was a significant correlation between

LDF VR index and the $TcpO_2$ index (n= 70), r = 0.64, ($p<0.0001$). The highest correlation was between the LDF VR and $TcpO_2$ index, (n=70), r=0.74, ($p<0.0001$).

Table VII: Heated Laser Doppler fluxmetry, $TcpO_2$, laser Doppler index and the $TcpO_2$ index expressed as means and one standard deviation are shown for the different measurement sites. The indices are the limb to chest ratios. Correlation of the results at each site was by Spearman's rank correlation which was considered significant when $p < 0.05$.

	Laser (au)	$TcpO_2$(mmHg)	p =	Laser Index	$TcpO_2$ Index	p =
Chest (n=35)	18.06 ± 4.13	55.71 ± 11.23	0.12			
AK (n=17)	9.98 ± 4.84	39.17 ± 18.98	0.71	0.58 ±0.33	0.73 ± 0.37	0.71
BK (n=36)	7.9 ± 4.96	36.25 ± 20.25	<0.0001	0.46 ± 0.33	0.62 ± 0.31	<0.0001
Foot (n=17)	5.19 ± 5.16	17.29 ± 13.32	0.0001	0.29 ± 0.30	0.31 ± 0.23	0.0004

Table VIII: Laser Doppler VR, $TcpO_2$ index and VR index expressed as means and one standard deviation are shown for the different measurement sites. Correlation of the results at each site was by Spearman's rank correlation which was considered significant when $p < 0.05$.

	VR	$TcpO_2$ Index	p =	VR Index / $TcpO_2$ Index	p =
Chest (n=35)	14.2 (4.54)				
AK (n=17)	7.24 (4.48)	0.73 (0.37)	0.34	0.51 (0.32)	0.32
BK (n=36)	6.45 (4.7)	0.62 (0.31)	<0.0001	0.53 (0.42)	0.0003
Foot (n=17)	3.47 (4.3)	0.31 (0.23)	0.0029	0.29 (0.4)	0.028

The LDF values relative to the $TcpO_2$ index are shown in Figure 12, the LDF index values relative to the $TcpO_2$ index are shown in Figure 13, and the LDF absolute values relative to the $TcpO_2$ absolute values are shown in Figure 14. The LDF VR absolute values relative to the $TcpO_2$ index are shown in Figure 15 while the LDF VR index values relative to the $TcpO_2$ index values are shown in Figure 16.

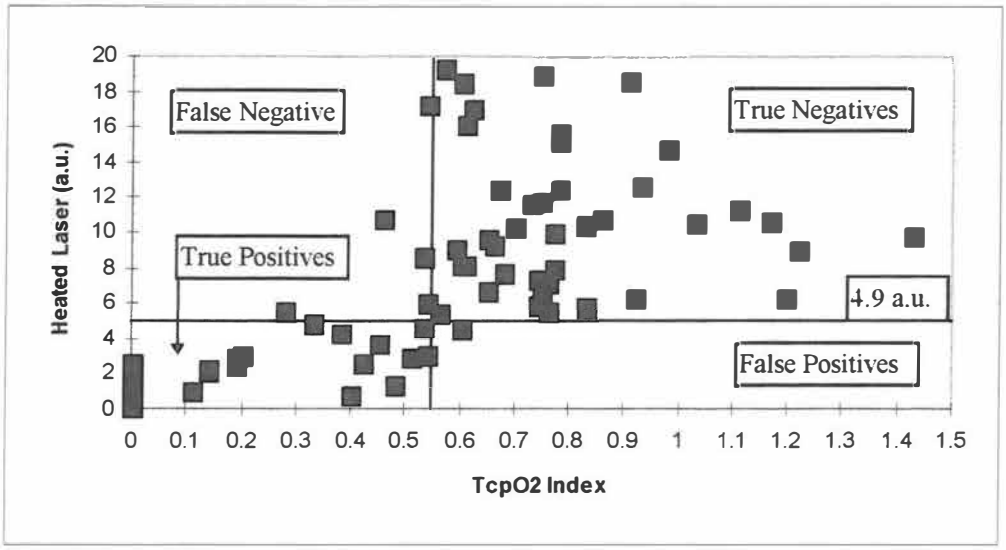

Figure 12: **Scattergram showing the relationship between the $TcpO_2$ index and heated LDF (n = 70). The Spearman rank correlation coefficient is r = 0.72, ($p<0.0001$). No amputation site with a $TcpO_2$ index of less than 0.55 would be expected to heal. A heated LDF of 4.9 au is shown as a potential predictive value. The linear regression equation is y = 2.38 + 9.4x**

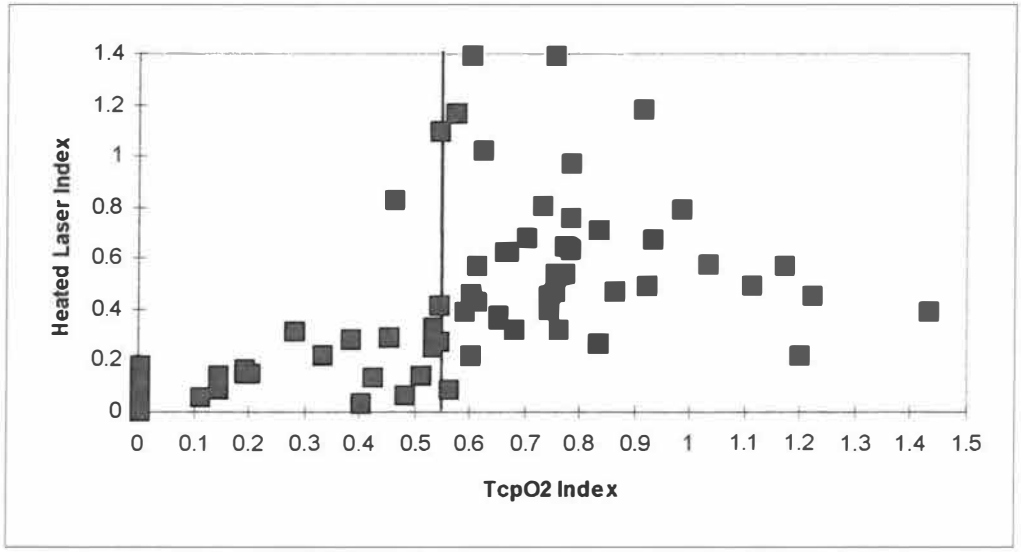

Figure 13: **Scattergram showing the relationship between the $TcpO_2$ index and heated LDF index (n = 70). The Spearman rank correlation coefficient is r = 0.68, ($p<0.0001$). No amputation site with a $TcpO_2$ index of less than 0.55 would be expected to heal. The linear regression equation is y = 0.16 + 0.5x**

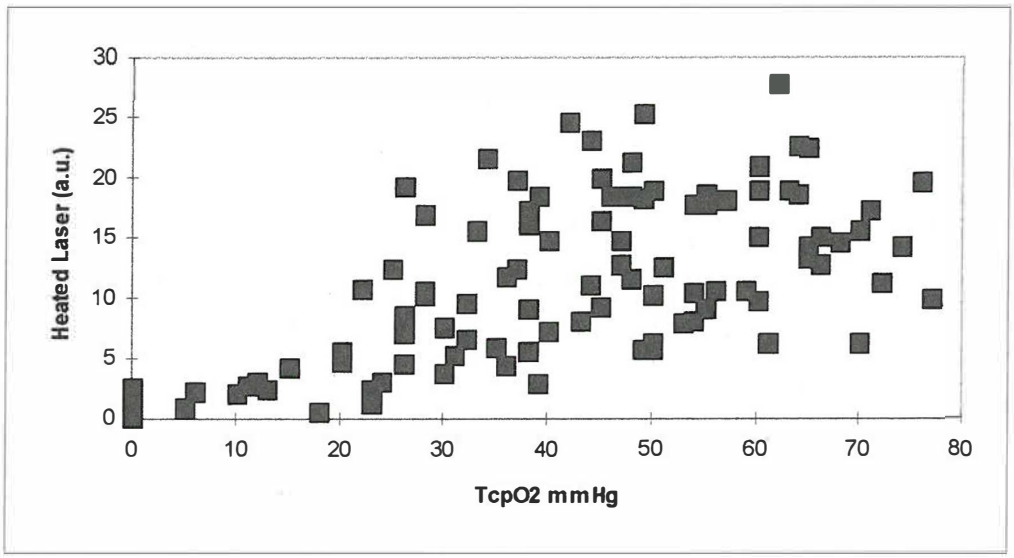

Figure 14: **Scattergram showing the relationship between $TcpO_2$ (mmHg) and heated LDF (au), (n=105) . The Spearman rank correlation coefficient is r = 0.63, (p<0.0001)**

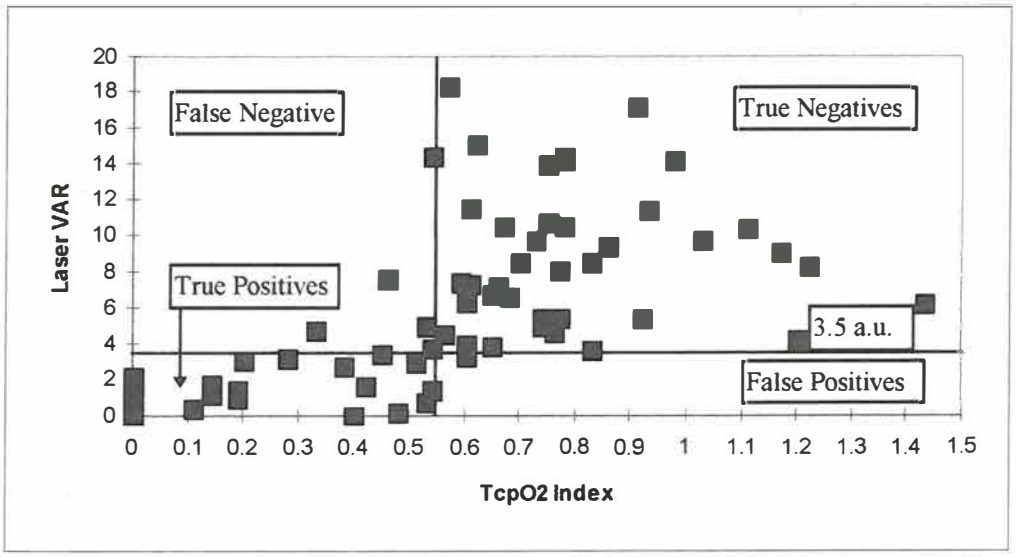

Figure 15: **Scattergram showing the relationship between the $TcpO_2$ index and LDF VR (n = 70). The Spearman rank correlation coefficient is r = 0.74, (p<0.0001). No amputation site with a $TcpO_2$ index of less than 0.55 would be expected to heal. An LDF VR of 3.5 is shown as a potential predictive value. The linear regression equation is y = 1.1 + 8.57x**

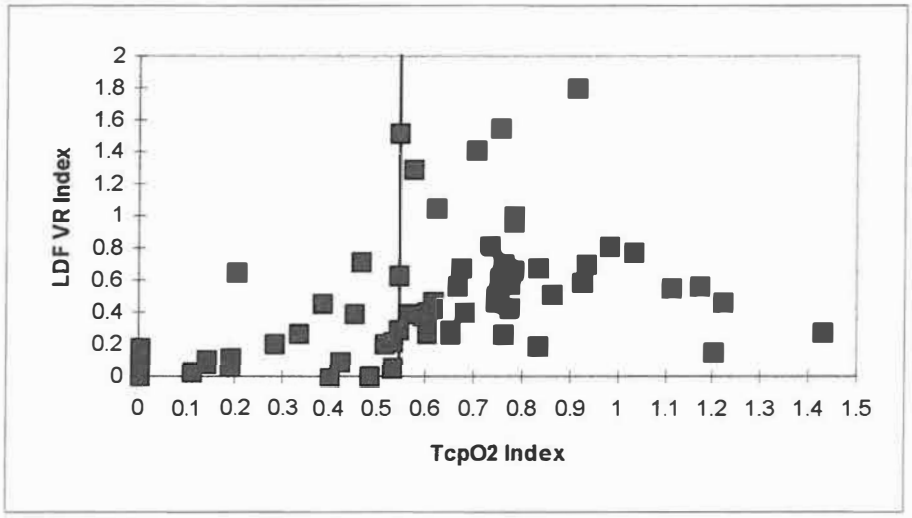

Figure 16: **Scattergram showing the relationship between the $TcpO_2$ index and LDF VR index (n = 70). The Spearman rank correlation coefficient is r = 0.64, ($p<0.0001$). No amputation site with a $TcpO_2$ index of less than 0.55 would be expected to heal. The linear regression equation is y = 0.15 + 0.56x**

4.2.4 Heated LDF's Ability to Distinguish Severity of PVD

From Table IX it can be seen that when using a heated LDF probe the results reflect the existence of PVD. The table shows that there were significant differences for the absolute heated LDF and LDF VR values between the different amputations sites, with the values decreasing the more distal the measurement.

Table IX: Intra-group difference at the different levels (above-knee compared to below-knee, below-knee compared to foot). Heated Laser Doppler, LDF VR, $TcpO_2$ and the $TcpO_2$ index expressed as means and one standard deviation are shown. Statistical analysis by a Paired t-test, which was considered significant when $p < 0.05$.

Combination	Laser (au)	p =	LDF VR (au)	p =	$TcpO_2$(mmHg)	Pp =	$TcpO_2$ Index	p =
A-K / B-K (n=12)	10.9 (4.4)		7.85 (4.23)		44.8 (13.2)		0.8 (0.3)	
	4.9 (5.5)	0.003	3.95 (4.99)	0.01	16.4 (13.8)	<0.0001	0.3 (0.3)	<0.0001
B-K / Foot (n=17)	9.3 (4.4)		8.01 (4.26)		43.8 (13)		0.8 (0.2)	
	5.2 (5.2)	0.007	3.47 (4.29)	0.002	17.3 (13.3)	<0.0001	0.4 (0.3)	0.0009*

4.3 Receiver Operator Characteristic Curves

Figures 17 to 26 show the ROC curves which resulted from determining the sensitivity and specificity of the absolute heated LDF and LDF VR values with respect to the five $TcpO_2$ indices. The points on each curve represent an absolute heated LDF or LDF VR value. Each figure has two text boxes. The left hand box indicates the absolute, heated LDF or LDF VR value plotted that was nearest to the top left hand corner of the graph. The right hand box indicates the area under the curve.

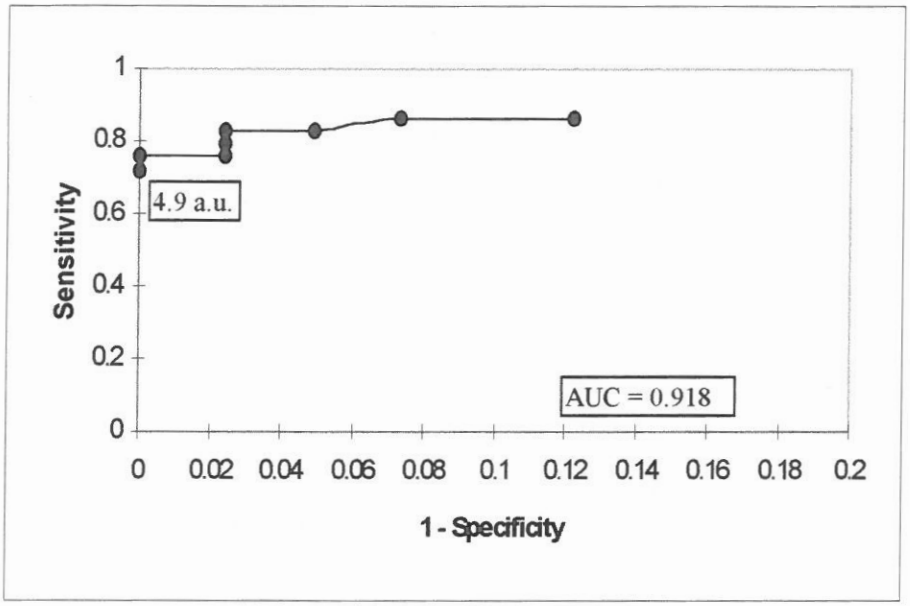

Figure 17: **ROC curve displaying sensitivity and specificity of absolute LDF values using a TcpO2 index of 0.55.**

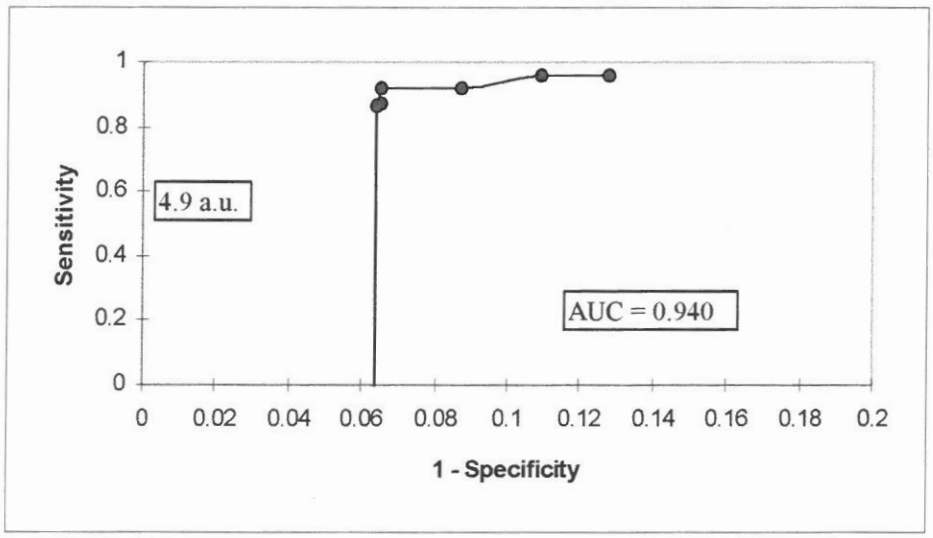

Figure 18: **ROC curve displaying sensitivity and specificity of absolute LDF values using a TcpO2 index of 0.53.**

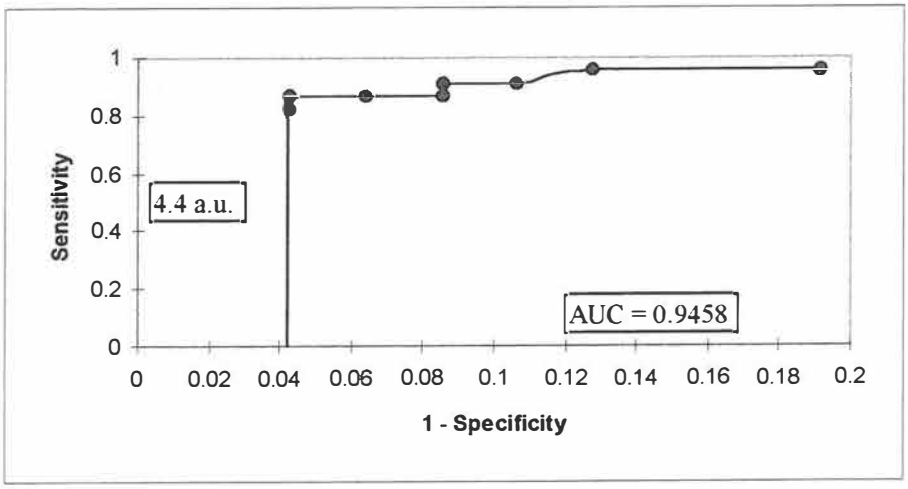

Figure 19: **ROC curve displaying sensitivity and specificity of absolute LDF values using a TcpO2 index of 0.50.**

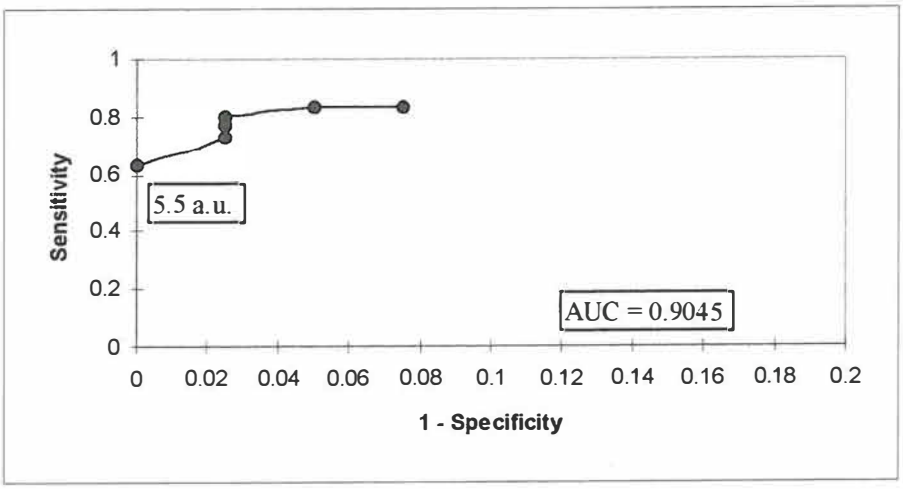

Figure 20: **ROC curve displaying sensitivity and specificity of absolute LDF values using a $TcpO_2$ index of 0.57.**

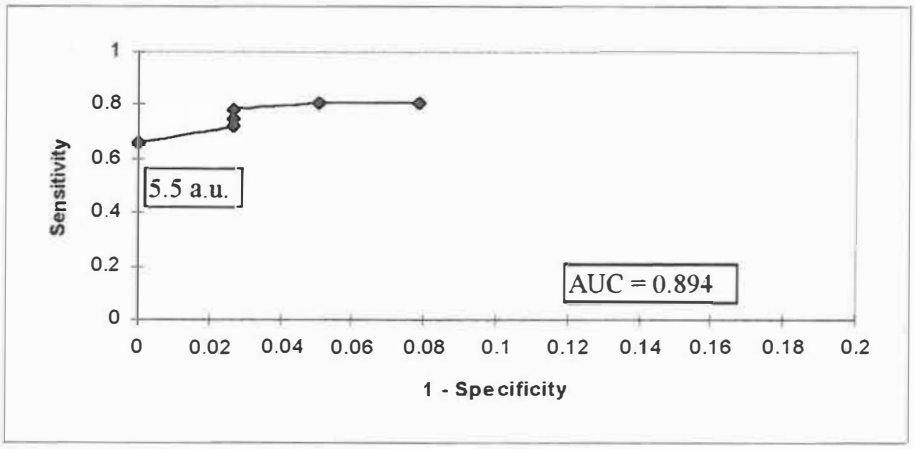

Figure 21: **ROC curve displaying sensitivity and specificity of absolute LDF values using a TcpO2 index of 0.60.**

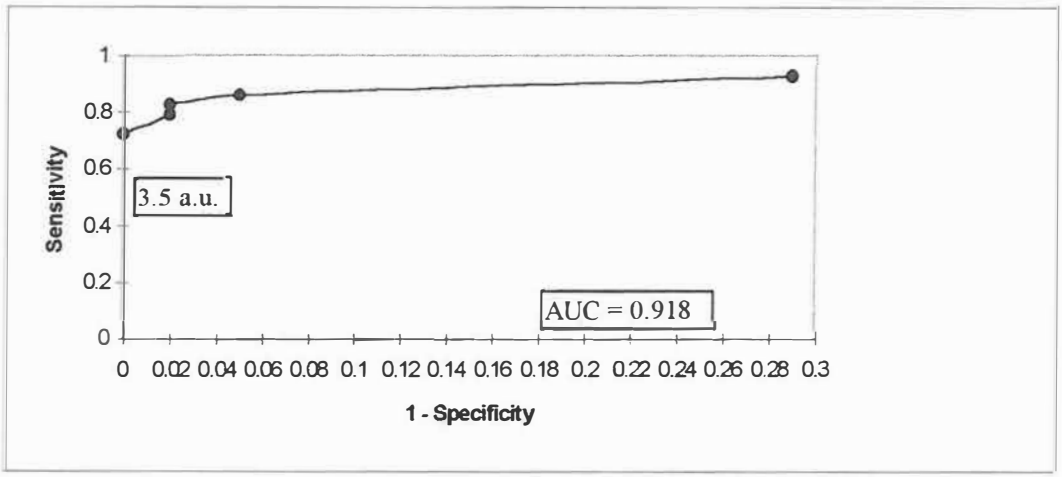

Figure 22: **ROC curve displaying sensitivity and specificity of LDF VR values using a TcpO2 index of 0.55.**

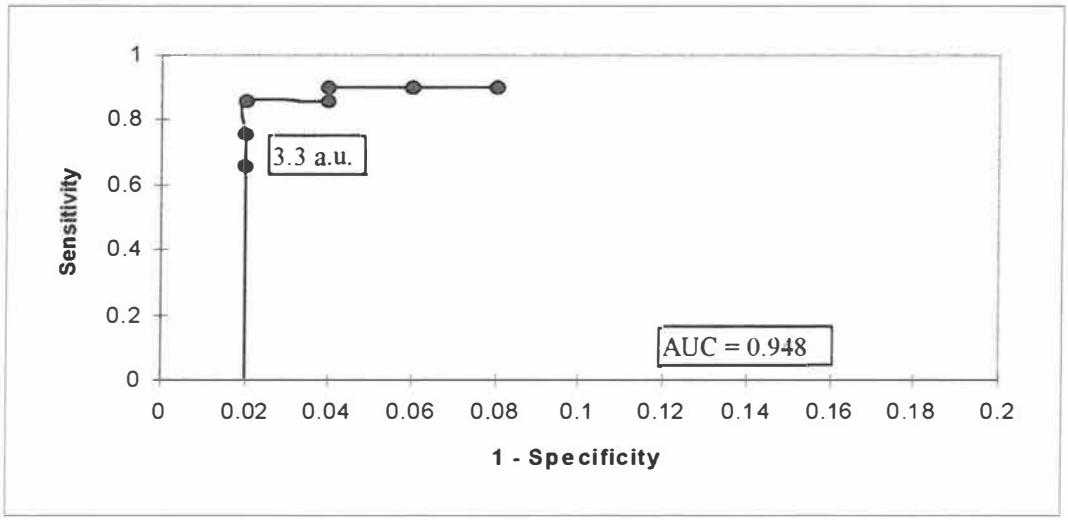

Figure 23: **ROC curve displaying sensitivity and specificity of LDF VR values using a TcpO2 index of 0.53.**

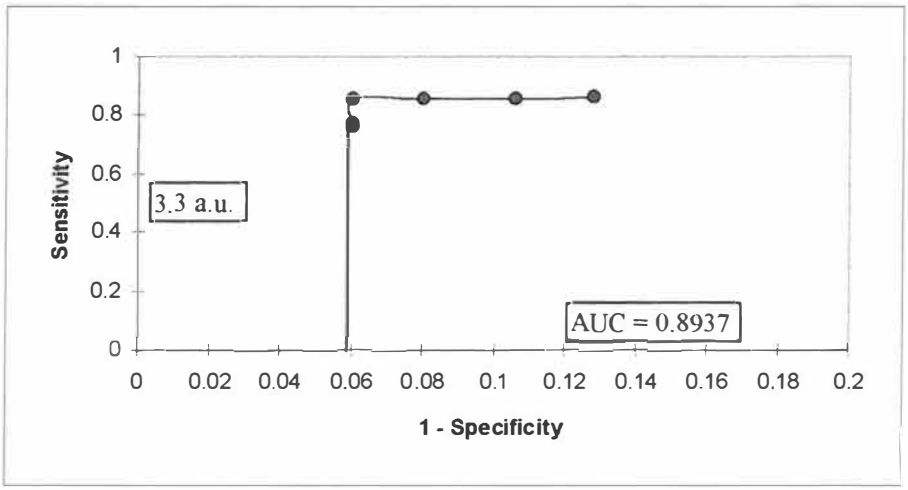

Figure 24: **ROC curve displaying sensitivity and specificity of LDF VR values using a TcpO2 index of 0.5.**

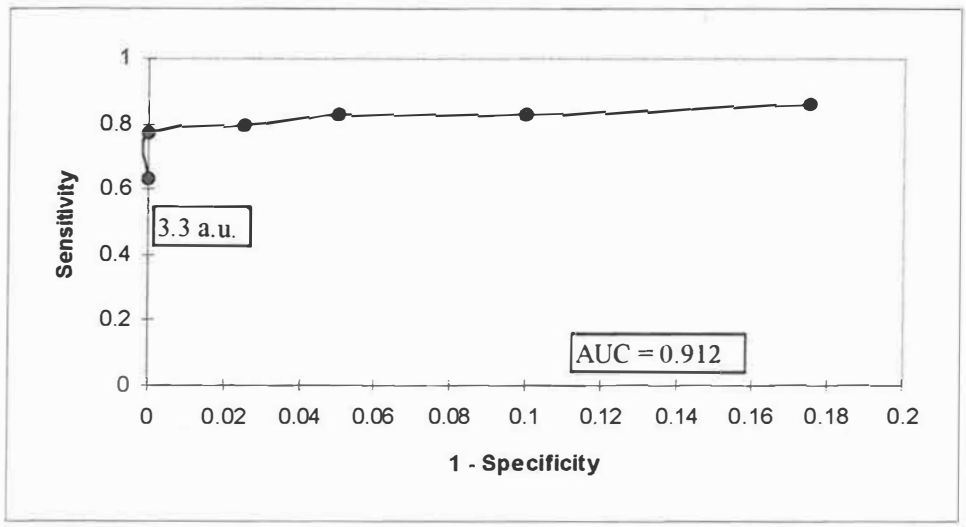

Figure 25: **ROC curve displaying sensitivity and specificity of LDF VR values using a TcpO2 index of 0.57.**

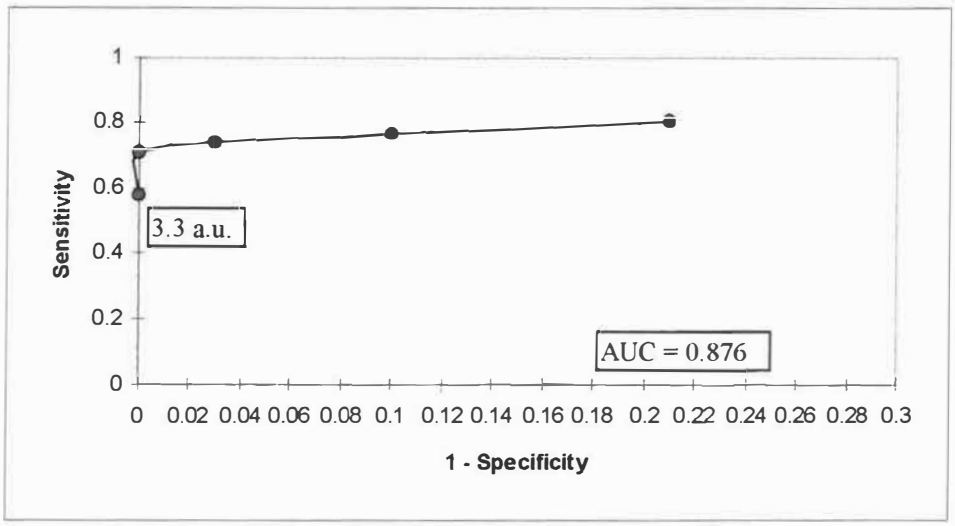

Figure 26: **ROC curve displaying sensitivity and specificity of LDF VR values using a TcpO2 index of 0.60.**

4.4 The most useful LDF value

Table X shows the sensitivity, specificity, positive predictive value, negative predictive value and accuracy of the most useful absolute heated LDF or LDF VR cut-off point as determined by the ROC curves.

Table X: Area under the curve (AUC), absolute LDF or LDF VR value (LDF), Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), and accuracy of the most useful absolute LDF or LDF VR cut-off point as determined by the ROC curve.

ROC Curve	AUC	LDF	Sen %	Spe %	PPV %	NPV %	Acc %
LDF / 0.5	0.9458	4.4	86.96	95.74	90.9	93.75	92.85
LDF / 0.53	0.940	4.9	91.6	93.48	88	95.55	92.85
LDF / 0.55	0.918	4.9	82.76	97.56	88.88	96	91.43
LDF / 0.57	0.9045	5.5	80	97.5	96	86.66	90
LDF 0.6	0.894	5.5	78	97.4	96	84	88.57
LDF / 0.55	-	7.55	89.66	68.29	90.32	66.66	77.14
LDF VR / 0.5	0.8937	3.3	86	94	86.36	93.75	91.43
LDF VR / 0.53	0.948	3.3	86	98	94.74	94.1	94.29
LDF VR / 0.55	0.918	3.5	82.76	97.56	88.88	96	91.43
LDF VR / 0.57	0.912	3.3	77	100	100	85.1	90
LDF VR / 0.6	0.876	3.3	71	100	100	81.25	87.14
LDF VR / 0.55	-	5.88	93	71	94	69	80

4.4.1 Absolute Heated LDF

The results from Table X indicate that for the absolute heated LDF data a value of 4.4 a.u. would be the most useful value for pre-operatively predicting wound healing potential if the $TcpO_2$ index was 0.5 This is due to the area under the ROC curve from which this value was obtained being closest to one (0.9458) compared to the other absolute heated LDF ROC curves.

For the $TcpO_2$ index of 0.55 (The Gold Standard) an absolute heated LDF value of 4.9 a.u. is shown as a possible predictive level (Figure 12 and Table X). Below this level, one amputation would be expected to heal. Twenty - four readings fall below LDF = 4.9 a.u. and all have a $TcpO_2$ index less than 0.55. Of the remaining 45 readings above LDF = 4.9 a.u., 40 are above $TcpO_2$ index = 0.55 and would be expected to heal. Five readings are above LDF = 4.9 a.u., but below $TcpO_2$ index = 0.55 and would therefore be expected to fail. The predictive value of a positive test (LDF < 4.9 a.u.) is 88.88% and of a negative test (LDF > 4.9 a.u.) is 96%. From Table X it can be seen that this value produces a sensitivity of 82.76%, specificity of 97.56% and an overall accuracy for pre-operatively predicting wound healing or failure of 91.43%.

Based on the linear regression equation for the data in Figure 12, the $TcpO_2$ index of 0.55 corresponds to a heated LDF value of 7.55 a.u.. At this level the predictive value of a positive test (LDF < 7.55 a.u.) is 90.32% and of a negative test (LDF > 7.55 a.u.) is 66.66%. From Table X it can be seen that this value produces a sensitivity of 89.66%, specificity of 68.29% and an overall accuracy for pre-operatively predicting wound healing or failure of 77.14%.

4.4.2 LDF VR

Table X shows that for the LDF VR, a value of 3.3 a.u. would be the most useful value for pre-operatively predicting wound healing potential if the $TcpO_2$ index was 0.53. The area under the ROC curve from which this value was obtained was closest to one (0.948) when compared to the other absolute LDF VR ROC curves.

For the $TcpO_2$ index of 0.55 (The Gold Standard) a LDF VR value of 3.5 a.u. is shown as a possible predictive level(Figure 15 and Table X). Below this level, one amputation would be expected to heal. Twenty - four readings fall below LDF VR = 3.5 a.u.

and all have a $TcpO_2$ index less than 0.55. Of the remaining 45 readings above LDF VR = 3.5 a.u., 40 are above $TcpO_2$ index = 0.55 and would be expected to heal. Five readings are above LDF = 3.5 a.u. but below $TcpO_2$ index = 0.55 and would therefore be expected to fail. The predictive value of a positive test (LDF VR< 3.5 a.u.) is 88.88% and of a negative test (LDF VR > 3. a.u.) is 96%. From Table X it can be seen that this value produces a sensitivity of 82.76%, a specificity of 97.56% and an overall accuracy for pre-operatively predicting wound healing or failure of 91.43%.

Based on the linear regression equation, the $TcpO_2$ index of 0.55 corresponds to a LDF VR value of 5.8 a.u.. At this level the predictive value of a positive test (LDF VR< 5.8 a.u.) is 94% and of a negative test (LDF VR > 5.8 a.u.) is 69%. From Table X it can be seen that this value produces a sensitivity of 93% and a specificity of 71% and an overall accuracy for pre-operatively predicting wound healing or failure of 80%.

4.4.3 Low Perfusion compared to High Perfusion

The heated absolute LDF values and the LDF VR values were divided into healing and non-healing groups according to the $TcpO_2$ index value of 0.55. The two groups were correlated independently of each other with the $TcpO_2$ index. From Table XI it can be seen that there were significant ($p<0.05$) positive correlations, for both the LDF values ($r = 0.7$) and the LDF VR values ($r = 0.59$), in the non-healing sub group, while in the healing group there were poor correlations ($r = 0.07$ and 0.16 respectively) The reason for this may be due to the fact that the LDF is sensitive to low perfusion states. This factor will be discussed further in Chapter 5.

Table XI: **Laser Doppler fluxmetry compared to $TcpO_2$ index, and LDF VR compared to $TcpO_2$ index divided into healing and non-healing groups by $TcpO_2$ cut-off index of 0.55. Correlation of the results at each site was by Spearman's rank correlation which was considered significant when $p < 0.05$.**

LDF / $TcpO_2$ Index	r =	p =	Linear Regression Equation
Heal (n=41)	0.07	0.66	y = 11.6 + -1.2x
Non-heal (n=29)	0.7	<0.0001	y = 1.34 + 9.1x
LDF VAR / $TcpO_2$ Index			
Heal (n=41)	0.16	0.33	y = 8.23 + 0.46x
Non-heal (n=29)	0.59	0.0007	y = 0.54 + 7.02x

CHAPTER FIVE

5.0 DISCUSSION

Fagrell (1994) has stated that the LDF technique has been used extensively in clinical practice for evaluating physiological and pathophysiological conditions in the cutaneous microcirculation of humans. Inadequate blood flow of the skin microcirculation, not meeting the requirements for tissue nutrition is the final cause of the failure of wounds to heal. Hence, the LDF technique has been proposed as being useful for pre-operative evaluation of wound healing potential (Holloway *et al.*, 1983, Lantsberg *et al.*, 1991; Padberg *et al.*, 1992).

Fagrell, (1994) and Hoffmann *et al.*, (1994) have argued that at times the interpretations of the LDF results have been uncritical and that for meaningful interpretations, there are a number of inherent methodological problems that have to be taken into account. This applies especially when measurements are performed on the skin (Fagrell, 1994).

5.1 Validation Study

If a method for blood flow monitoring has any place in the laboratory or in clinical practice, it is necessary to ensure that the results are reproducible (Petersen *et al.*, 1994) and repeatable (Bircher *et al.*, 1994). The methodological error of a LDF was reported by Tenland *et al.*, (1983) to be lower than 6%. This value represented the coefficient of variation for repeated measurements of the Brownian mobility of a stable emulsion, and indicated the instrumental reproducibility.

The skin has a complex vascular geometry and furthermore, blood flow is not a static phenomenon, but represents both spatial and temporal variations. Consequently the validity of the LDF method for clinical use depends on *in vivo* studies within the specific environment, and under the same conditions, in which clinical application will be taking place. The validation process in the present study therefore took place in the King Edward VIII Vascular Laboratory.

The repeatability of a LDF (using an unheated probe) when measuring cutaneous blood flow, was analysed by Tenland *et al.*, (1983). These authors measured resting cutaneous blood flow continuously on the forehead and forearm for 20 minutes. They reported that temporal coefficients of variation (standard deviation divided by the mean) varied from 4% to 11% in low blood flow areas and between 8% and 19% in high blood flow areas respectively (Tenland *et al.*, 1983). The coefficient of variation for resting cutaneous unheated laser Doppler flux in the present study was considerably lower at 1.7%. The coefficient of variation for resting cutaneous flux using the heated probe, in the present study, was only slightly higher at 4.5%. The reason for the reduced variation when using the unheated probe, and the low variation for the heated probe, compared to the findings of Tenland *et al.*, (1983), may be due to the fact that the present LDF is made by a different manufacturer and is also a more technologically advanced model. It must noted however, that the measurements for the present study were obtained from the mid-dorsum of the foot, while Tenland *et al.*, (1983) used the forearm and forehead. The vascular geometry beneath the probe at each of these sites is different and may have played a role in influencing the results.

Tenland *et al.*, (1983) found that the reproducibility of their unheated LDF was poor. They found tenfold differences, during four days of repeated measurements, in all skin areas, including locations with A-V shunts. Similarly, in another study which assessed the reproducibility of a LDF, Tuominen *et al.*, (1992) found day to day LDF level coefficients of

variation of between 20-30%. Compared to the above findings, the LDF in the present study showed lower coefficients of variation for resting heated (14.4%) and unheated (14%) cutaneous laser Doppler flux, indicating slightly better reproducibility. These two values are also within the repeatability range reported by Tenland *et al.*, (1983). The reason for the reduced percentage may again be due to difference between the manufacturer as well as instrumental technological advances and the different methods used in the two studies (for instance positioning of the probe.)

Interpretation and comparison of the Repeatability Coefficient (RC), used to assess the repeatability and reproducibility of the LDF in the present study, is difficult. The reason being that no LDF studies, to the best of the author's knowledge, have used this method to evaluate the repeatability and reproducibility of LDF for measuring cutaneous blood flow. Chapter Three discussed the reason why this method is preferred to the coefficient of variation which has been used by other researchers. The data from the present study shows firstly, that the repeatability of the LDF (comparison of continuous measurements over a time period on a single day) is better than the reproducibility (comparison of day to day measurements). The results indicated that differences between continuous measurements on a single day (repeatability study) were small (Table III), with the RC being 2.94 and 0.684 for the resting heated and unheated values respectively. The RC's for the reproducibility study (day to day measurements) (Table IV) were comparatively higher, being 8.88 and 1.56 respectively. The interpretation of these results has been given in Chapters Three and Four and will not be discussed in this chapter. The results from the present validation study therefore suggest that the repeatability of the LDF cutaneous blood flux signal is better than the reproducibility. In other words the flux variation over two hour time period is small while over day to day measurements it is large.

Various validation studies have contradicted the findings of Tenland *et al*, (1983), and to some extent the reproducibility findings of the present study. Sundberg *et al.*, (1984) found a very modest hour-to-hour and day to day variation. An excellent temporal correlation was also reproduced by other groups (Ninet and Fronek, 1985; Muller *et al*, 1987). These two groups found that baseline reproducibility as well as responses to various skin vasomotor reflexes and relative blood flow changes during cooling and heating were satisfying. It is important to realise however, that the present validation study was performed in a working environment in order to ascertain whether the LDF gave readings which were reproducible in a clinical setting. Previous validation research was performed in rigorously controlled settings where confounding variables were kept at a minimum. Various environmental variables within the King Edward VIII Vascular Laboratory may have affected the validation study results (in particular the day-to-day results).

Spatial variation may also have influenced the results in the present validation study. Tenland *et al.*, (1983) found that repeated measurements at the same probe position yielded a coefficient of variation of 25 %. Petersen *et al.*, (1994) suggested that regional changes in the vascular arrangement may influence variation. Therefore, in relation to spatial variation, LDF probe placement may have played an important role in influencing the results of the validation study and may have been responsible for the high variation in the reproducibility study in comparison to the results from the repeatability study. This may have been overcome through marking the exact area with a permanent marker so that the probe was placed in the identical position each day.

Petersen *et al.*, (1994) discusses a further issue which may explain the results found in the present validation study. They state that blood flow not only differs in closely located skin areas, but that inter-individual variation is very pronounced. Tenland *et al.*, (1983)

demonstrated up to 10-fold differences in normal subjects, while Kvernebo *et al.*, (1988) concluded that reproducibility was good in a given population, but not in individual subjects. The fact that blood flow readings can be so variable at the same anatomical site in a particular individual and that only 10 subjects were used in the present validation study may explain the high day-to-day variation in the reproducibility study.

The rather great variations in blood flow value recorded might be a result of inhomogeneities in the microvasculature within the measuring volumes. More specifically the spatial variation in the LDF values may be explained by the different number of capillary loops per defined surface area or changes in venule-capillary density under the probe (Ryan, 1973). Landis, (1938) reported that estimations of the number of capillaries show more than a six-fold variation within an area of $1mm^2$.

Lukkari-Rautiarinen *et al.*, (1989) sum up the issues which may have influenced the present validation study. They suggest that the intra-individual changes detected during continuous monitoring over a day without detachment of measuring devices indicated physiological fluctuations in peripheral microcirculation. The day-to-day variation was wider because of problems related to the re-attachment of the measuring devices and also problems related to biological variations. These were most probably the variables which influenced the present study, despite using provocative testing (Postocclusive Reactive Hyperaemia Test and LDF heating), controlling the room temperature, as well as relaxing the patient as much as possible in order to minimise the cutaneous blood flow variation caused by internal and external factors. The large fluctuations in cutaneous blood flow over the five day test indicated the great difficulties in performing microcirculatory blood flow studies where effects over days or months are of interest.

5.2 Laser Doppler Comparison With Non-Invasive Methods

Chapter Two has discussed numerous methods which have been developed to measure lower limb perfusion. The washout of $Xenon^{133}$ reflects blood flow, vital capillaroscopy monitors blood flow velocity in the outer capillaries, and venous occlusion plethysmography estimates total blood volume flow to the extremity (Petersen *et al.*, 1994). It is common practice to evaluate a new technique, such as LDF, by comparing it with established methods. Petersen *et al.*, (1994) points out however, that the researcher must keep in mind that different methods may measure different parameters. For example, Fagrell (1986) found high LDF values in skin, which was emptied of erythrocytes, as observed by vital capillaroscopy. Petersen *et al.*, (1994) states that LDF is the only technique that estimates the flux of moving particles in the range of laser light penetration.

Therefore it is critical to understand that there is no "gold standard" against which to compare the LDF skin blood flow measurements. Direct correlation to venous outflow of the skin is impossible, and the $Xenon^{133}$ washout technique, although displaying a good correlation with LDF measurements in a few studies (Stern *et al.*, 1977; Bisgaard and Kritensen, 1984), has subsequently been shown to display a poor correlation with LDF blood flow measurements, during steady state conditions and a standardised vasodilatation. (Klemp and Staberg,1985). Although the $TcpO_2$ index has been used as the "Gold Standard" in the present study, the fact that it has been used, was a major limitation to the study especially when using the unheated LDF probe. This issue is discussed in the following section.

5.3 Unheated LDF Comparison with $TcpO_2$

The microvascular bed of the skin is composed of the nutritional capillaries and the thermoregulatory vessels. The nutritional capillaries are the most superficial ones (depth of 10-50 um) and normally contains a very low volume of blood (approximately 5-10% of the cutaneous bloodflow). In the subpapillary, thermoregulatory, vascular bed, which is located 0.05-2.0mm from the skin surface, the dominating vessels are venules, and only a small portion are arterioles. The volume of blood in this vascular compartment has been estimated to be at least 95% of all blood in the skin (Ostergren, 1984) and consequently the blood flow in the nutritional capillaries will only be a small percentage of the total skin blood flow.

The nature of the $TcpO_2$ and the measuring depth is defined by Beinder *et al.*, (1994) as, the measurement of the reduction current produced by surplus oxygen molecules, which are available under the condition of maximal hyperaemia, and which diffuse from the cutaneous nutritional capillary loops to the skin surface. On the other hand the measuring depth of the LDF in the skin is at least 1-2mm using a standard probe (Vasamedics, 1991). Hence the predominating part of the signal will be coming from the subpapillary vessels, and only a minute part from the nutritional capillaries. It is for this reason that the LDF technique cannot be used for evaluating skin capillary nutritional circulation, but only the total skin microcirculation in humans.

From the above discussion it follows that a comparison between unheated LDF cutaneous blood flow and $TcpO_2$ measurements is technically an evaluation between two different instruments measuring different variables, at different depths and under different conditions in the skin microcirculation. The LDF measures red blood cell concentration essentially in the thermoregulatory layer, while the skin is in a baseline state, whereas oxygen pressure is

monitored under the condition of maximal hyperaemia in the nutritional capillary loops. To add to the dilemma, both instruments have various methodological limitations which have already been discussed in Chapter 2.

The findings from the present study showed a significant but poor correlation between unheated LDF and $TcpO_2$ ($r = 0.34$); and LDF Index and $TcpO_2$ index ($r = 0.17$) Belcaro *et al.*, (1988c) reported similar results with the correlation of $TcpO_2$ to resting LDF being $r = 0.4$. These findings contradict those of Matsen *et al.*, (1984) who found that non-heated LDF measurements did not correlate with local skin perfusion. However, it must be noted that the present study sample size was much larger than the Matsen *et al.*, (1984) study. How sample size is related to correlation outcomes is discussed in the following paragraph.

Bland *et al.*, (1986) state that sample size is a critical factor when calculating the correlation between two variables. These authors state that the correlation coefficient measures the strength of a relation between two variables and not the agreement between them. They argue that two tests will have perfect agreement only if the points lie along the line of equality, while they will have perfect correlation if the points lie along any straight line. They state that correlation depends on the range of the true quantity in the sample, and since researchers usually try to compare two methods over the whole range of values typically encountered (as in the present study), a high correlation is almost guaranteed. These points are critical when interpreting the results from the unheated LDF probe study as well from the heated LDF probe study.

For the unheated study, although a significant correlation was found, there was no agreement between the two variables. This was indicated by the fact that the range of scatter of the unheated LDF readings was such that there was no absolute LDF value or LDF index which

had a high predictive power for evaluating wound healing potential, when compared to the $TcpO_2$ index of 0.55. The results from the present study therefore contradict those of Karanfilian *et al.*, (1986); Castronuovo *et al.*, (1987) Kram *et al.*, (1989); and Kvernebo *et al.*, (1989) who reported useful unheated values for evaluating wound healing potential.

5.4 Unheated and heated LDF Ability to Distinguish Severity of PVD

The symptoms of PVD, intermittent claudication, rest pain, ulceration and finally gangrene are all manifestations of insufficient oxygen and nutrient delivery at a cellular level, secondary to relative degrees of arterial occlusion. It is therefore expected that tests of perfusion of both the macro and microcirculation should reflect a fall in perfusion as the tests are performed more distally in the atherosclerotic patient. The magnitude of the segmental fall in perfusion parameters is dependent on the site and severity of the disease process. With respect to this characteristic of PVD, Wyss *et al.*, (1988) have argued that because there is a continuously increasing probability of failure as the degree of ischaemia at the site of amputation increases, it is impossible to calculate a predictive threshold. They see the level of ischaemia, however it is measured, as a risk factor rather than the sole cause of failure of healing of an amputation. Their argument is valid, however the objective of any predictive or diagnostic study is to determine a threshold value which is the ***most useful*** in clinical practice. The characteristic of lower limb perfusion in the PVD patients in the present study will now be discussed in relation to the unheated and heated LDF results.

Tables V, VI, and VII, VIII show that for the unheated and heated LDF study, while the absolute, heated LDF, heated LDF index, LDF VR, absolute $TcpO_2$ measurements and the $TcpO_2$ index reflected a fall in mean values as the site of measurement moves distally, the highest mean unheated absolute LDF value on the lower limb occurred at the foot. The unheated LDF

probe does not seem useful for distinguishing PVD characteristics. However, this may not be due to instrumental limitations as shown by the following discussion.

Belcaro *et al.*, (1989) have shown that unheated resting cutaneous LDF readings may be high around the margin of diabetic ulcers. They have attributed the rise in LDF, in these patients with microangiopathy, to shunting of blood to the thermoregulatory plexus and the dermal capillary loops. The very wide range of unheated LDF readings obtained at the foot, 0 - 4.9 a.u. may be due to a similar phenomenon. Similarly, ischaemia is associated with increasing peripheral vasodilatation in an attempt to improve oxygen and nutrient supply to the tissues. In the severely ischaemic foot the LDF value may be very high when compensation is successful - maximal vasodilatation in the presence of an adequate inflow, or it may very low if the inflow is insufficient despite maximal vasodilatory compensation.

5.5 Heated LDF Comparison with $TcpO_2$

Human skin contains many thermoreceptors and is thus very sensitive to changes in temperature. Cochrane (1986) argued that since the major function of the skin is to maintain body temperature, temperature variation should be used to evaluate its response. The author concluded that there are several reasons why evaluating the response of skin blood flow to local heating is the most clinically useful. Firstly, it is very simple to perform and only requires the subject to remain still during the 5 to 10 min needed to complete the test. Secondly, reproducible patterns of behaviour are observed in normal controls. The reason being that heating causes a maximal *constant* flow (hyperaemic stabilisation) in venules and arteriovenous shunts in the sampled area (Wahlberg *et al.*, 1994). Thirdly, human skin contains many thermoreceptors and is thus very sensitive to changes in temperature.

The microvascular structure was discussed briefly when evaluating the Validation Study results. Further detail is required in this section to provide an understanding of the effect that skin heating has on the microcirculatory blood flow. Tseng *et al.*, (1995) stated that the dermal arteries branch off to form metarterioles and then precapillary sphincters before giving rise to the true capillaries that form the nutritive vessels. Direct communication between the arterioles and venules, the arteriovenous anastosmoses, can be found at the fingertips, palms of the hands, toes, soles of the feet, ears, nose and lips where they function in thermoregulation. Smooth muscle fibres are found surrounding the vessel walls of the arteriovenous anastosmosis, the metarterioles and the precapillary sphincters. These smooth muscle fibres are controlled mainly by the sympathetic nervous system, which causes vasoconstriction (Witzleb, 1989). There are no known vasodilatory nerve fibres to the cutaneous vessels, and vasodilation is brought about by a decrease in constrictor tone as well as by local production of bradykinin in sweat glands and by vasodilator metabolites (Ganong, 1991). It is the large venous plexus that is involved in temperature regulation. Blood flow in response to thermoregulatory stimuli can vary from 1 to as much as 150ml/100g of skin per minute by shunting blood flow through the anastomoses (Ganong, 1991). Local heating suppresses the sympathetic tone and induces increased blood flow by opening up many of the arteriovenous anastomoses (Witzleb, 1989). Heating of the skin "arterializes" the capillary bed by local vasodilation (Karanfilian *et al.*, 1986) causing maximal hyperaemia. Guyton, (1986) states that with excessive heating, the increased activity of the sweat glands causes them to release the enzyme kallikinin, which in turn splits the polypeptide bradykinin from globulin in the interstitial fluids. Bradykinin in turn is a powerful vasodilator, that could account for the greatly increased blood flow when sweating begins to occur.

Cutaneous blood flow is determined by one of the following factors: vascular tone, vascular lumen size and vascular number (Guyton, 1986). The vascular tone is controlled by a complex neurohumoral system (West, 1990). Taylor *et al.*, (1984) proposed that the maximal

flow obtained after a thermal stress test by raising the skin temperature to 45°C is due to the abolition of the cutaneous vascular smooth muscle tone.

Various researchers (Holloway *et al.*, 1983; Matsen *et al.*, 1984; Allen *et al.*, 1987; Fairs *et al.*, 1987; Gebur *et al.*, 1989; Lantsberg *et al.*, 1991; Padberg *et al.*, 1992).) support the notion that the addition of cutaneous heating to LDF measurements improves the prediction potential when evaluating wound healing. In particular Matsen *et al.*, (1984) stated that LDF and $TcpO_2$ measurements reflect changes in arteriovenous gradient when made over areas of heated skin (44°C).

The results from the present study support these past studies, indicating that using a heated LDF probe (45°C) improves the pre-operative prediction potential for evaluating wound healing in PVD patients. Significant correlations were found between LDF and $TcpO_2$; LDF index and $TcpO_2$ index; LDF and $TcpO_2$ index ($r = 0.72$) and LDF VR index and $TcpO_2$ index. The strongest correlation was found between LDF VR and $TcpO_2$ index ($r = 0.74$). All of these correlations except for LDF VR Index / $TcpO_2$ index had `p' values of $p<0.0001$. The reason for this strong correlation was discussed earlier with the unheated data. Simply put though, the strong significance was probably due to the comparison being made over the whole range of heated LDF values obtained.

The LDF VR / $TcpO_2$ correlation is slightly higher than that of Fairs *et al.*, (1987), who found that the correlation between $TcpO_2$ and the relative increase in LDF flux (LDF VR) was $r = 0.7$ ($p<0.001$). These authors also found that the correlations were very similar although marginally poorer for the absolute heated flux / $TcpO_2$ characteristic. Cheatle *et al.*, (1991) also found a significant correlation between $TcpO_2$ Index and LDF VR of $r = 0.524$, $p <0.001$).

Holloway *et al.*, (1983); Fairs *et al.*, (1987); and Gebuhr *et al.*, (1989) have all indicated that vascular reactivity is likely to be a better indicator of potential for healing than absolute flux level. Gebuhr *et al.*, (1989) reported very similar findings to the present study. They found that their sternal LDF measurements always showed a higher basic flux than those of the leg and the unheated, baseline flux, before heating, showed no correlation with the amputation level. They suggested that sternal recordings would appear to be unnecessary in the normal clinical situation. The fact that the LDF index values and the LDF VR Index values, from the present study, did not show a stronger correlation to $TcpO_2$ than the absolute LDF values, support this argument.

5.6 Possible LDF Predictive Levels for pre-operatively Evaluating Wound Healing Potential

In the present study, both the heated LDF value of 4.9 a.u. and the LDF VR value of 3.5 relative units were found to be possible predictive levels. It is at this point that the limitation of this study must be noted. In studies which have reported the sensitivity, specificity etc. of LDF cut-off points they have calculated these values in relation to the actual outcome of wound healing post-operatively (Karanfilian *et al.*, 1986; Padberg et al., 1992). The present study used the $TcpO_2$ index of 0.55 as the Gold Standard with the assumption that the index is 100% accurate. It is a fact that no diagnostic test is 100% accurate and hence in reality the predictive ability of the LDF has been calculated according to a "Gold Standard" which has its own measurement error. The result is that the predictive percentages of the LDF a.u. and the LDF VR and hence the predictive accuracy of the LDF are elevated.

Karanfilian *et al.*, (1986) reported that the accuracy of LDF for predicting wound healing was 87%, the sensitivity was 79% and the specificity was 96%. Their definition of sensitivity is

opposite to the definition of sensitivity in the present study and that given by Hulley *et al.*, (1988). Karanfilian *et al.*, (1986) define sensitivity as the ability of the LDF to reflect adequate blood flow when it is present. In other words the ability of the LDF to predict healing. Their results show that out of 29 patients whose wounds healed the LDF predicted that 23 would heal. Accepting that there has been a mistake when defining the parameter in the text these authors have shown that their LDF had a specificity of 79% (according to the present studies definition of specificity), and a sensitivity of 96%. This sensitivity value is considerably better than that of the present study, whilst the ability to predict healing (specificity) is low compared to the present study.

The accuracy of the LDF and LDF VR in the present study was 91.43%. Karanfilian *et al.*, (1986) concluded that LDF was less useful than $TcpO_2$ for evaluating wound healing due to the high incidence of false negative predictions (23%). False negatives are values which predict healing however the wound fails to heal. The false negative (FN) percentage for the present study was 17.24%. This value explains why the accuracy of the LDF in the present study was higher than in the study by Karanfilian *et al.*, (1986). This value was calculated by subtracting the sensitivity (True negatives) value of 82.76% from 100% (which represents the total number of wounds that the $TcpO_2$ index of 0.55 predicted to fail).

The positive predictive value in the present study for both criteria was higher than that of Padberg *et al.*, (1992) who found a positive predictive value of 83% and the overall accuracy of their most useful LDF cut-off value to be 85%. The outcome of the study by Padberg *et al.*, (1992) is more relevant to the results from the present study. This is due to the fact that these authors also used an LDF probe that was heated to 45°C while Karanfilian *et al.*, (1986).

5.7 Reasons for Low Sensitivity

Various researchers have suggested factors which may have caused the low sensitivity of the threshold criteria calculated for the LDF. Franzeck *et al.*, 1982; Harward *et al.*, 1985; Allen *et al.*, 1987; and Wyss *et al.*, (1988), found that the $TcpO_2$ measurement may be zero when some nutritive flow of the blood to the skin is still present. These authors found that wounds healed even with low $TcpO_2$ values. The blood flow is compromised and as a result the oxygen delivered to the cutaneous microvasculature is reduced. There comes a time when the bloodflow is so compromised (but still present) that there is only enough oxygen being delivered for the metabolic needs of the local skin. The result is that no extra oxygen is available to diffuse to the surface of the skin (Wyss *et al.*, 1981). Therefore no oxygen diffuses across into the $TcpO_2$ probe, and consequently a reading of zero is obtained. This is the reason why laser Doppler flux reading value may be obtained even when $TcpO_2$ is zero.

Such readings as those discussed above have been reported in past research (Harward *et al.*, 1985; Ratliff *et al.*, 1984). These authors have argued that a $TcpO_2$ measurement of zero at the site of an amputation does not always indicate a degree of ischaemia that precludes healing after an amputation. According to Matsen *et al.*, (1984) these results may be due to the fact that a non-linear relationship exists between $TcpO_2$ and local cutaneous blood flow. Padberg *et al.*, (1992) clarifies this by stating that $TcpO_2$ excels in the prediction of wound healing, but is less precise at low values while LDF excels in the prediction of wound failure. The results of the study by Karanfilian *et al.*, (1986) also support this LDF argument.

In the present study the two prediction criteria that were calculated were obtained from the sets of data which displayed the strongest correlation, absolute heated LDF / $TcpO_2$ index and LDF VR and $TcpO_2$ index. The calculations were based on the theory that a linear relationship

existed between the two variables. This non-linear relationship may therefore explain why the correlations for each set of data were not as strong as they could have been if a linear relationship existed. This relationship may also explain why the sensitivity of the absolute heated LDF and LDF VR was low.

Table XI however, shows results which may provide data in opposition to the non-linear relationship argument at low perfusion states. Through dividing the data (absolute heated LDF, LDF VR and $TcpO_2$) into healing and non-healing groups (according to the $TcpO_2$ index of 0.55), there was only a significant correlation between the non-healing data. This result suggests seems to indicate that the $TcpO_2$ data was still following a linear relationship with laser Doppler flux at low perfusion states. However, this result requires further research.

5.8 Effect of the Clinical Setting on Research

Due to the need to use the $TcpO_2$ monitors, which were already positioned in the Vascular Laboratory at King Edward VIII Hospital, data collection took place during consulting hours in the Vascular Laboratory. This meant that the data collection may have been influenced by numerous environmental variables which were impossible to control for in a working environment.

Due to the fact that there are only two vascular technicians in the Vascular Laboratory, and that there is usually a backlog of patients, obtained from a number of hospitals around KwaZulu Natal (King Edward VIII, Addington, Wentworth, R.K. Khan), it was crucial that the LDF measurement protocol used did not affect the normal timing and flow of activity within the laboratory. Although the LDF provides rapid almost immediate data and is easy to use in a controlled measurement setting, the rushed atmosphere of the vascular laboratory was not

conducive to rigorous research. Cutaneous blood flow is extremely sensitive to environmental conditions and the LDF is extremely sensitive to blood flow changes. This meant that in many cases measurements could not be used due to abnormal blood flow values caused by environmental influences in the laboratory.

Although variables such as ambient temperature and excessive movement of the patient's limbs were monitored, the atmosphere of the laboratory was on occasions far from relaxed, with many patients, nurses, and doctors, as well as the researcher, being in the laboratory at one time. This meant that there was continuous noise and mental stimulation for the patient. This situation often caused patients to move and fidget and any such mental or physical activity may have influenced the blood flow readings.

5.9 The Effect of Biological Zero

The effect that physiological, anatomical, instrumental, methodological, environmental, temporal, and spatial variables may have had on the LDF blood flow measurements in this present study have already been discussed in detail. The effect of biological zero has also been mentioned. What is important to note is that the interpretation of LDF readings may have been improved in the present study by subtraction of the biological zero from the unheated or heated LDF value, or perhaps by the evaluation of biological zero itself. However, this was not done in the study due to the following reason. In a pilot study, attempts at measuring biological zero in patients with severe peripheral vascular disease resulted in pain and the movement artefacts which affected the readings obtained in most patients, prolonging measurement duration. Due to the difficulty already present in trying to maintain a relaxed, motionless patient such measurements were not feasible.

6.0 CONCLUSION

The primary amputation revision rate (excluding guillotine amputations) for lower limb amputations, at King Edward VIII Hospital, is around 23% (Chapter Two). This percentage indicates the effect that the $TcpO_2$ index had over seven years (1989 - 1995). This was a considerable reduction in the primary revision rate of 35% which existed before the $TcpO_2$ index was introduced by Mars *et al.*, (1993). The failure rate in those patients undergoing $TcpO_2$ assessment is less than 5% (Mars *et al.*, 1993). Not all patients are assessed because of the time required to perform the $TcpO_2$ test.

The present study found that the LDF had an overall accuracy of 91.43% when pre-operatively predicting the outcome of amputations in PVD patients. The measurement error was therefore around 10% which suggests that by using the LDF on all patients, the primary revision rate may be reduced further. However, it must be remembered that this LDF value is based on the $TcpO_2$ Gold Standard, which has its own measurement error. Despite this limitation, the result from this present study suggests that through the use of provocative testing (heating and by measuring LDF VR), the widespread implementation of LDF is just as useful as limited use of $TcpO_2$ measurement for pre-operatively evaluating wound healing potential in PVD patients.

high incidence of diabetes; air, water and food contamination; physical inactivity; poor diet; and tobacco-smoking.

Figure 1: **Total number of patients undergoing lower limb amputation for PVD and Diabetes combined, trauma and other (1984 - 1995).**

In 1993, Mars *et al.*, addressed the problem of amputation revision surgery at King Edward VIII Hospital and outlined a programme involving pre-operative assessment of amputation wound healing potential that would save the hospital R1,07 million annually. In this study the authors reviewed the Natal Provincial Administration's centralised computer records of all patients admitted to King Edward VIII Hospital between 1984 and 1988. They found that during the 5-year period, 965 patients required 1563 lower limb amputations for PVD, 222 of these patients died in hospital. The primary revision rate, in other words, the number of first-time amputations that required revision, was 51%. The in-hospital mortality rate was 23,1%

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PRESENTATIONS

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