Perforating blood vessel selection in deep inferior epigastric artery perforator flaps

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Author’s Declaration

The work contained in this thesis has been completed and composed by the candidate and has not been accepted in any previous application for a degree. All sources of information have been specifically and accurately acknowledged.

Signed:

Date:

Definitions/Abbreviations

ANOVA	Analysis of Variance
CTA	Computed Tomography Angiography
DIEA	Deep Inferior Epigastric Artery
DIEV	Deep Inferior Epigastric Vessels
DIEP	Deep Inferior Epigastric Perforator
ICG	Indocyanine Green
IP	Ischaemic Preconditioning
IRI	Ischaemia Reperfusion Injury
LDF	Laser Doppler Flowmetry
LDI	Laser Doppler Imaging
MRA	Magnetic Resonance Imaging Angiography
ICG-NIR-VA	Indocyanine Green Near Infrared Video Angiography
PI	Pulsatility Index
PU	Perfusion Units
RI	Resistance Index
SIEA	Superficial Inferior Epigastric Artery
TRAM	Transverse Rectus Abdominis Muscle
VTE	Venous Thromboembolism

Abstract

Introduction: The DIEP flap is a popular choice for breast reconstruction, though selection of which perforating blood vessel(s) to supply the flap is still largely based on surgeon preference, with little evidence to support numbers or location of perforators. In addition, many surgeons routinely discard zone IV of the flap, limiting the size of transferrable tissue. The aim of this research was to investigate the effect of number and location of perforators within a DIEP flap, on the total pedicle flow and perfusion of zone IV fat and skin.

Methods: This research comprised of two studies; an animal model and a patient study:
1) 20 cranially-based abdominal epigastric perforator flaps were raised in Wistar rats on two perforators. The perforators were sequentially clamped and released in a randomised order and total pedicle flow (measured using microvascular flow-probes) and skin perfusion (measured using laser Doppler Flowmetry) was recorded on the following perforator combinations:

- P1 (superior perforator)
- P2 (inferior perforator)
- P1+2 (both perforators)

In addition, half of the animal flaps were randomised to receive a single (15 minute) period of pedicle-clamped ischaemic preconditioning after raising, with all measurements repeated to observe any effect.
2) 13 DIEP flaps were raised in post-mastectomy patients requiring breast reconstruction on two perforators. These were clamped and released as before to assess perfusion of fat and skin in zone IV using SPY Indocyanine-green-fluorescence-angiography scans on the same perforator combinations as in our animal study, listed above.

Results: All data were analysed using non-parametric analyses and revealed that in our animal model, total pedicle flow was significantly ($p<0.001$) greater on a single perforator compared to two but no significant differences were identified in the flap skin perfusion. In our clinical study a single superior perforator supplied zone IV significantly ($p=0.039$) better than both peroforators, though this was not observed with the single inferior perforator. No significant differences were seen in zone IV skin perfusion. A single period of ischaemic preconditioning significantly ($p<0.05$) increased the total pedicle flow, but not the skin perfusion in our rat model.

Conclusions: Possible reasons for these observed differences could be related to the flow dynamics and resistances specific to perforator flap anatomy and physiology and the possibility of vessel shunting in the subcutis.

Publications, Presentations, Prizes and Awards Arising from Thesis

Publications

- Douglas, H. Wilkinson, M. Mackay, I. 2014. The effect of perforator number and location on the total pedicle flow and perfusion of zone IV fat and skin of DIEP flaps. *Journal of Plastic Reconstructive and Aesthetic Surgery* 67(2),pp.212-218.

Presentations

- Effect of perforator number and location on the perfusion of fat and skin of zone IV of DIEP flaps. Douglas, H. Mackay, I. Scottish Plastic Surgery Meeting (BAPRAS), Dunkeld, 27th-28th October 2011.

- Effect of perforator number and location on the total pedicle flow and the perfusion of zone IV fat and skin of DIEP flaps. Douglas, H. Mackay, I. British Association of Plastic Reconstructive and Aesthetic Surgeons (BAPRAS). Newcastle, 11th-13th July 2012.

  and

- American Society of Plastic Surgeons, (ASPS). New Orleans, 26th- 30th October 2012.

Prizes

- 1st Prize for best research presentation: Effect of perforator number and location on the perfusion of fat and skin of zone IV of DIEP flaps. Douglas, H. Mackay, I. British Association of Plastic Reconstructive and Aesthetic Surgeons (BAPRAS). Newcastle, 11th-13th July 2012.

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Part I: Introduction

Chapter 1: Microvascular free tissue transfer

1.1 Background

Microvascular free tissue transfer (also know as 'free flap') has revolutionised the way in which reconstructive surgery is approached, allowing surgeons to reconstruct a defect (usually due to neoplasia or trauma) with tissue of a similar structure. This enables the surgeon to address the functional and cosmetic outcome of such procedures, as opposed to just healing alone. As early as the 1950's re-vascularised segments of the jejunum were used to repair oesophageal defects(Seidenberg et al. 1959), though the first free flap using cutaneous tissue was not performed until 1972(Harii et al. 1974b, Harii et al. 1974a, Rickard and Hudson. 2013).The ability to perform microvascular anastomosis to blood vessels under the operating microscope lent such a huge scope to the field of reconstructive surgery, that the next twenty years saw the development of a wealth of free flaps of varying composition (skin, fascia, muscle, bone) used for a wide range of conditions. However, early failure rates were very high(Percival et al. 1989), attributed to a steep learning curve of microvascular anastomotic skill and limitations regarding the understanding of vascular supply to tissues at that time(Suominen and Asko-Seljavaara. 1995).

Anatomical cadaveric studies by Taylor and Palmer in 1987 examined the relationship between specific blood vessels and the overlying tissue which these specific vessels supplied(Taylor and Palmer. 1987). This seminal work led to the introduction of the term angiosomes; describing a three-dimensional unit of tissue supplied by a particular source blood vessel and connected to neighbouring units of tissue by smaller and less significant vessels, termed 'choke' vessels(Taylor and Palmer. 1987, Inoue and Taylor. 1996). This has led to the construction of a virtual three-dimensional map of the body, allowing surgeons to choose which blood vessel to take depending on the tissues they wish to include in the flap, or vice versa. A flap based upon blood vessels which supplies one particular angiosome can usually include the adjacent angiosome within the flap, supplied through the choke vessels, but units further away than this are unreliably supplied by those vessels and the risk of necrosis is higher(Inoue and Taylor. 1996).

Building on the work by Taylor and Palmer, and with increased knowledge of the vascular supply of tissue flaps in the lower abdomen, the concept of perforator flaps was introduced and the first Deep Inferior Epigastric Perforator (DIEP) flap was performed in 1989 by Koshima et al(Koshima and Soeda. 1989). The DIEP flap takes only the lower abdominal skin and underlying fat supplied by perforating vessels (blood vessels which perforate the muscle to supply the overlying tissue) from the deep inferior epigastric artery (DIEA). The artery runs with a pair of small veins, called venae commitantes, which together form the Deep Inferior Epigastric Vessels (DIEV). These perforating blood vessels (called 'perforators') are dissected through the rectus sheath and rectus abdominis muscle to their origin of the DIEV that runs under the rectus

muscle, along the posterior rectus sheath. As the muscle and its supplying motor nerves are left relatively intact, this reduces the amount of donor site morbidity produced compared with a Transverse Rectus Abdominis Muscle (TRAM) flap, which removes the muscle through which the DIEV perforate as part of the flap, and leaves the abdomen more prone to herniation(Chevray. 2004, Taylor and Daniel. 1975).

1.1.1 TRAM/DIEP flap zones

The TRAM flap, and subsequently the DIEP flap, has been historically divided into different zones indicating differing levels of vascular supply. This was first performed by Scheflan and Dinner, based upon their clinical impression of better perfusion in the divided areas in 16 patients undergoing pedicled TRAM reconstruction with a deep superior epigastric artery supply(Scheflan and Dinner. 1983a, Scheflan and Dinner. 1983b). This was described before Taylor and Palmer's seminal work on angiosomes, but broadly based on a similar concept, with the researchers observing that the tissue either side of the pedicle being well-vascularised and noting that the tissue furthest from the pedicle was less well-vascularised. Figure 1.1 shows the accepted sites for zone I and zone IV of the flap (shown here as right-sided with the arrow indicating the supplying DIEA), with zone I directly above the supplying vessel, and therefore the primary angiosome, and zone IV the furthest away from the blood vessel, separated by another angiosome. The two remaining zones are labelled II and III, though which angiosome is preferentially supplied has been debated. Scheflan and Dinner originally described the medial adjacent angiosome as having better blood supply compared with the lateral, as mentioned, based upon initial clinical impressions(Scheflan and Dinner. 1983b, Scheflan and Dinner. 1983a).

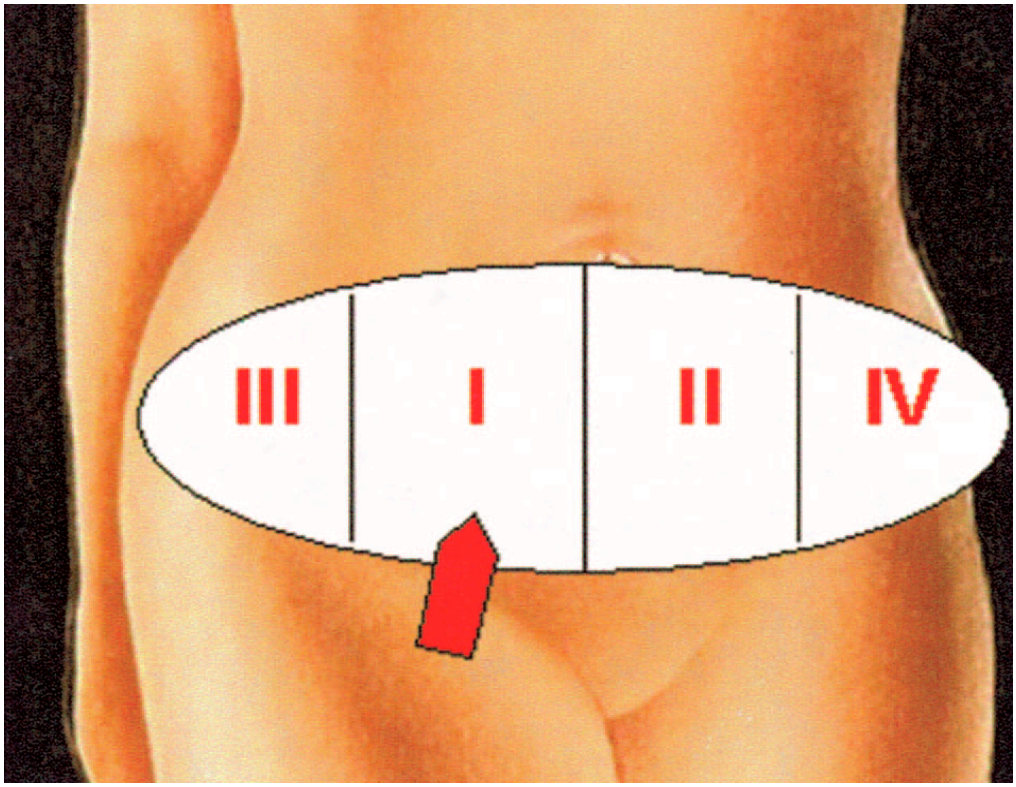


Figure 1.1: Original Scheflan TRAM/DIEP flap zones (Image permission granted by Elsevier Ltd. Holm et al. 2006)

However, later clinical studies by Dinner led him to reverse this decision, indicating that the lateral angiosome to zone I was superior(Dinner et al. 1983), as shown in Figure 1.2.

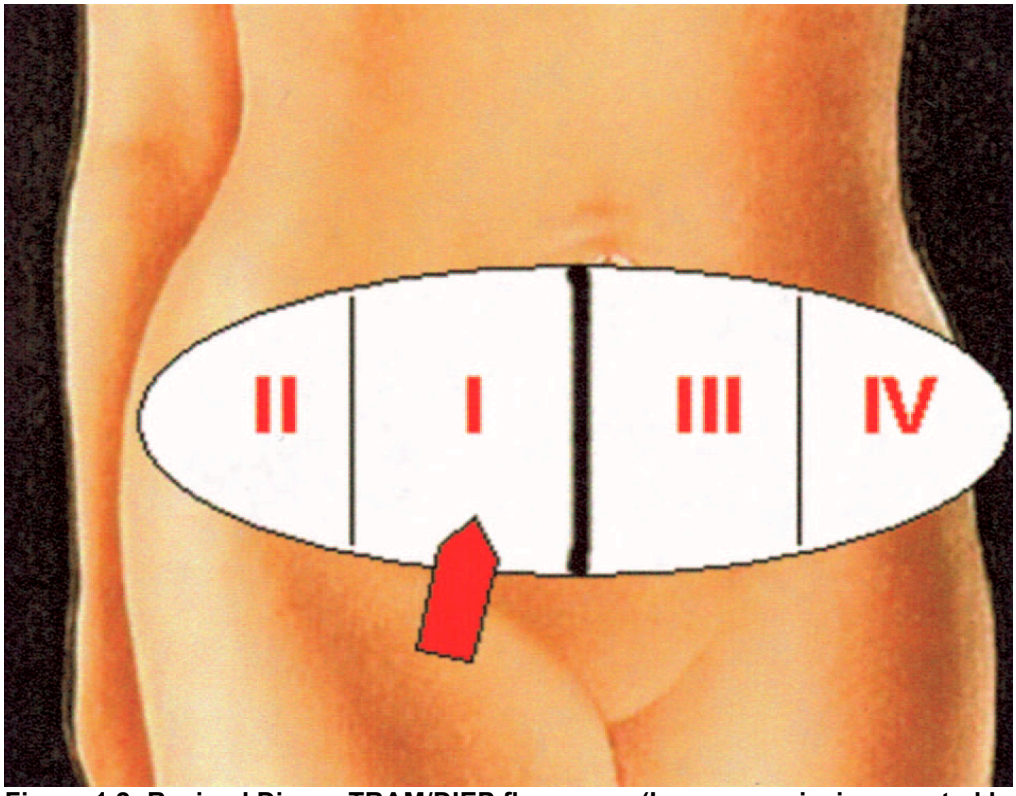


Figure 1.2: Revised Dinner TRAM/DIEP flap zones (Image permission granted by Elsevier Ltd. Holm et al. 2006)

More recent clinical studies assessing the perfusion of the DIEP flap zones quantitatively in-vivo using indocyanine green angiography agree with Dinner's later assessment and point out that the DIEA may have a different preferential vascular territory than the pedicled superiorly-based flap employed by Scheflan and Dinner(Holm et al. 2006). However, other authors have stated that the reality is in fact more complicated, and the further laterally a flap is taken, other angiosomes can be involved(Rickard. 2001). In addition, it has been suggested that grouping DIEP perforator flaps into four strictly defined zones may be an oversimplification, that perforator courses and orientations are unpredictable and perhaps perfusion may occur progressively rather than step-wise in fashion(Tregaskiss. 2006, Tregaskiss et al. 2007). More recently this debate has progressed to consider the concept that a perforator flap has a totally different vascular territory compared to that of a muscle flap angiosome, which has many perforators from a source vessel(Saint-Cyr et al. 2008, Saint-Cyr et al. 2009). More recent contrast cadaveric studies have shown results that agree with the initial zone mapping also(Bailey et al. 2010) though reported different perfusion patterns across the midline when different perforator rows were used(Saint-Cyr et al. 2008), which will be discussed further in Chapter 2. It is however, universally accepted that the unit of tissue directly above the supplying vessel (zone I) is the most reliably perfused, whereas the unit of tissue furthest away (zone IV) is the least reliably perfused.

1.1.2 Zone IV

Many surgeons routinely remove zone IV before transfer, believing it unreliable and prone to necrosis, which can limit the size of the flap(Granzow et al. 2006, Bailey et al. 2010). This can be clinically important if the defect to be reconstructed requires a large area of skin (e.g. burns or trauma reconstruction) or a large volume of tissue (e.g. fat to match a large contralateral breast post-mastectomy) for the reconstruction. A more common complication of DIEP flaps, indeed all autologous breast reconstructions, is that of fat necrosis; where although the overlying skin is healthy and viable, a variable amount of fat underneath is devitalised due to poor perfusion. This produces a hard, lumpy reconstruction with corresponding contour and size defects more likely. Fat necrosis rates in DIEP flaps are usually reported between 6-17%(Gill et al. 2004, Bodle et al. 2008, Chang et al. 2000), but have been reported as high as 35% in all autologous breast reconstructions(Nahabedian et al. 2002, Peeters et al. 2009) and can require debridement or ultrasound assisted liposuction to correct, which further reduces the size of the reconstructed breast(Hassa et al. 2010). Perhaps more importantly, areas of hard fat necrosis can be mistaken for recurrent cancers or disguise the feel of actual recurrences(Mandrekas et al. 1994). Many surgeons believe that the blood supply to the fat of zone IV is poorest, resulting in a much higher rate of fat necrosis in zone IV compared with other zones of better perfused tissue(Bailey et al. 2010) hence the often routine removal of this zone before transfer and limitation to the size of the flap. Some surgeons have advocated taking a very medial perforator to reduce the number of choke vessels required to traverse to zone IV, thinning the flap up to Scarpa's fascia or using delay procedures to enhance reliability(Vandevoort et al. 2002, Christiano and Rosson. 2010). Heitmann et al. examined the vascular staining of zone IV of DIEP flaps in their dye-injection study of 10 fresh cadaveric specimens and 5 intra-operative patient studies(Heitmann et al. 2000). The authors reported the majority of perforators located within 8cm of the umbilicus and that staining was poorest in zone IV for both cadaveric and patient studies, concluding that zone IV needed critical assessment in DIEP flaps. They did not however report on the location of the perforator injected in terms of row or position, only that the pargest perforator was selected to cannulate. The findings of Heitmann et al. were not supported by Cheng et al., whom reported their clinical outcome study of 74 DIEP flaps which included zone IV, finding low rates of partial flap failure (2.6%) and fat necrosis (13%) and concluding that zone IV was safe to take with the flap(Cheng et al. 2006b). The researchers did concede however, that the mean BMI of 22 in their patients was low and suggested that thicker flaps with more adipose tissue may have more pressure on the supplying vessels and thus render then more susceptible to necrosis. The method of identifying fat necrosis in their patient group was subjective, using either clinical examination or mammography, which is likely to reduce the accuracy of their fat necrosis data.

Chapter 2: Choice of perforator vessels in deep inferior epigastric artery perforator (DIEP) flap surgery

2.1 Perforator blood vessels

Perforator flaps are different to other kinds of flaps, both in anatomy and physiology. Essentially, the vascular branching pattern in an axial flap (a flap supplied by a named artery, reliably based on the angiosome concept) is similar to that of a normal circulation; the larger source artery which is taken branches into multiple vessels which all supply the overlying tissue, giving a very large cross-sectional vascular area, larger than the source vessel and the vascular resistance is multiple and in parallel. In perforator flaps, the perforator which supplies a particular area of skin and subcutaneous tissue is taken and traced to its supplying vessel, leaving all other vessels and structures behind. The perforator flap vascular tree is not just a smaller version of normal circulation, it is a specific long vessel segment, with all other branches sealed from the system. This produces a series of resistances, rather than the parallel type seen in systemic circulation and other axial flaps and a smaller total cross sectional area of vasculature(Rubino et al. 2006). This difference in resistance of perforator versus non-perforator systems and the subsequent effect upon flow in such a system will be further discussed in section 2.5.

The perforating blood vessels in a perforator flap will determine the vascular supply and drainage of the tissue to be transferred and as such is paramount to the survival of the flap. The selection of which perforators to choose is largely a clinical decision, made at the time of surgery depending upon which vessels appear to have the best calibre, a direct path through the muscle (and thus easier to dissect out without damaging the vessels, muscle or nerves) and location within the flap, thought to supply the largest area. The surgeon can tailor the flap to have different locations and number of perforating vessels depending upon the anatomy they find on preoperative imaging, during the operation or their own personal view regarding which perforating vessels supply or drain the flap more reliably.

In the case of the DIEP flap, the perforating blood vessels that supply the tissue emerge at different locations under the flap, depending on which units of tissue they will supply. These perforators can be separated into different anatomical groups, depending on their location emerging from the rectus sheath and entering the underside of the flap.

2.1.1 Pre-operative Imaging

Some surgeons routinely use radiological investigations to image the perforating blood vessels pre-operatively, and both computed tomography angiography (CTA) and magnetic resonance imaging angiography (MRA) have been used for this purpose. These imaging modalities can visualise the perforators in detail, including their size and course through the muscle, aiding the

surgeon in the decision of which vessel to select pre-operatively. CTA has been shown in several studies to decrease the duration of operating time, flap complications and donor site morbidity(Rozen et al. 2008a, Smit et al. 2009, Neil-Dwyer et al. 2009, Schaverien et al. 2011, Uppal et al. 2009, Ghattaura et al. 2010) and MRA to reduce the conversion of DIEP to TRAM(Neil-Dwyer et al. 2009) though debate regarding the best modality exists(Rozen et al. 2010a). Some facilities have the ability to map these perforators out on a 3D/4D image of the body to direct the surgeon to the desired vessel quickly(Saint-Cyr et al. 2008, Teoh et al. 2007). Casey et al (2009) retrospectively reviewed 287 DIEP flaps of which 101 received pre-operative CTA perforator imaging, whereas 186 had intra-operative perforator identification using a hand-held Doppler(Casey et al. 2009). They found a significantly decreased operating time and abdominal bulge in those patients who received pre-operative CTA, but no effect on clinical complications, failure rates or fat necrosis. Tillet et al. (2014) in their study of 142 consecutive DIEP flaps found that CTA was highly accurate in predicting the presence of perforators, but that intra-operative hand held Doppler was more accurate at accurately locating them through the abdominal wall, and suggested a combination of the modalities could be very useful(Tillett et al. 2014).

2.1.2 Perforasomes

As discussed in Chapter 1, the 3D vascular territory of a source vessel is termed an angiosome, a concept upon which many flap designs have been based. More recently the idea of the vascular territory of one or more perforators has been researched, advancing our knowledge of tissue vascularity and coining the phrase ‘perforasome’(Saint-Cyr et al. 2009). This is relevant to the perforator flap, as if only part of the branching system of the source vessel is to be utilised, then the overlying skin and subcutaneous tissue supplied by the perforator will be unique to that perforating vessel. Saint-Cyr at el. in an elegant cadaveric injection study mapped perforasomes in DIEP flaps and found that adjacent perforasomes can be recruited through direct linking vessels (supra-fascially) or indirect linking vessels (via the sub-dermal plexus)(Saint-Cyr et al. 2009). This can be seen more clearly in Figure 2.1.

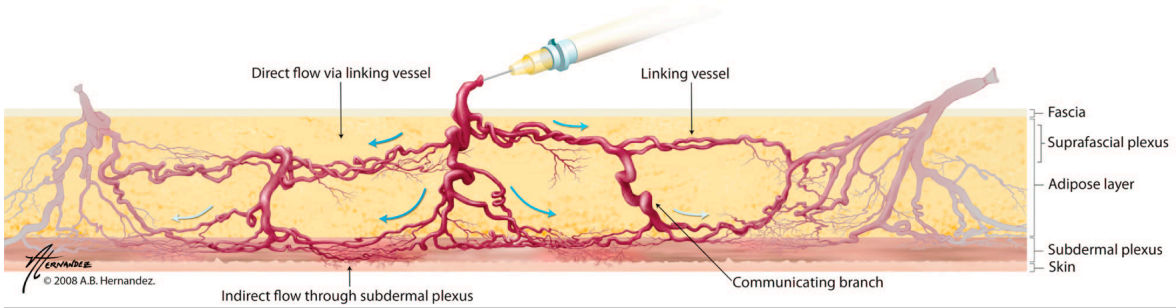


Figure 2.1: Perforasomes filling through direct and indirect linking vessels (Image permission granted by Lippincott, Williams & Wilkins Inc. Saint-Cyr et al. 2009)

The researchers concluded that the linking vessels between perforasomes can be opened by increasing the filling pressure of the perforator and that these linking vessels in DIEP flaps do cross the midline, but then travel from medial to lateral direction. This gives further evidence to

the furthest and thus worst perfused area being the named zone IV. However, they also observed that the perforasomes from perforators belonging to the same source artery were preferentially filled first, which would support the theory that ipsilateral perforasomes (i.e. those from the same DIEV) would be better supplied than contralateral perforasomes. The findings of Saint-Cyr et al. were supported by the cadaveric dye injection and patient CTA studies performed by Rozen et al.(Rozen and Ashton. 2010, Rozen et al. 2010b). This study, like the majority of those assessing the vascular anatomy and characteristics of flaps, is cadaveric and therefore cannot predict the effect of vessel tone and shunting which occurs in-vivo. However it thoroughly describes ways in which perforator flaps are different to other tissue flaps and explains the mechanisms by which the vessels are likely to fill and relate to each other, advancing our understanding of the perfusion of perforator flaps.

2.1.3 Anatomical side

When choosing the side of the body a DIEP flap should be based on, several factors may play a part. Sides of the abdomen with any scarring (e.g. appendix removal) are usually avoided, and any pre-operative imaging used can be reviewed to see if there is any difference between the deep inferior epigastric vessels on the left or right side of the patient. In actuality, particularly in the case of immediate reconstructions, the contra-lateral side to that of the mastectomy is often chosen to reduce operating time, as the mastectomy or vessel preparation can be undertaken at the same time as the abdominal flap is raised, reducing the time a patient is under anaesthetic and the increased risks of longer anaesthesia including venous thromboembolism (VTE)(Edmonds et al. 2004). If the vessels on a particular side of the flap do not look favourable, the other side can always be raised after the breast has been removed and the recipient vessels prepared.

2.2 Medial vs. Lateral row

2.2.1 Vascular territory

Having chosen a side of the abdomen on which to base the flap, the operating surgeon must then raise the flap on perforating vessels from the DIEA, which have very reliable branching patterns into two rows, termed “medial row” and “lateral row” perforators(Munhoz et al. 2004, Rozen et al. 2008b). These rows ramify into numerous perforating vessels which emerge from the rectus sheath in more medial or lateral anatomical positions over the muscle. Figure 2.2 shows a CT angiogram depicting the emergence of the different perforators from different rows.

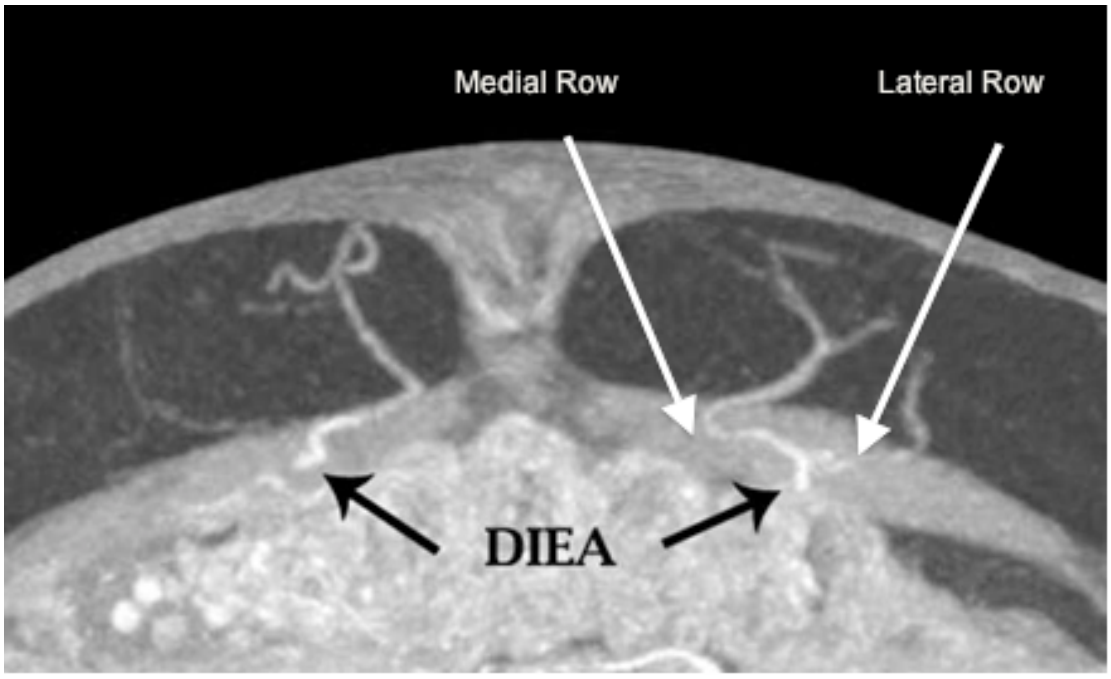


Figure 2.2: Medial and lateral rows of the Deep Inferior Epigastric Artery (Image permission granted by John Wiley and Sons. Rozen et al. 2011)

Despite differing numbers of DIEA main trunks, there are reliably two uniform rows, medial and lateral, of perforating vessels given from these trunks. Percentages and types of these differences in main trunks are shown in Figure 2.3, adapted by Rozen et al. from the original description by Moon and Taylor (Rozen et al. 2011, Moon and Taylor. 1988). This is explained both anatomically and embryologically by the two developmentally distinct heads of origin of each side of the hemi-rectus abdominis muscle(Rozen et al. 2011).

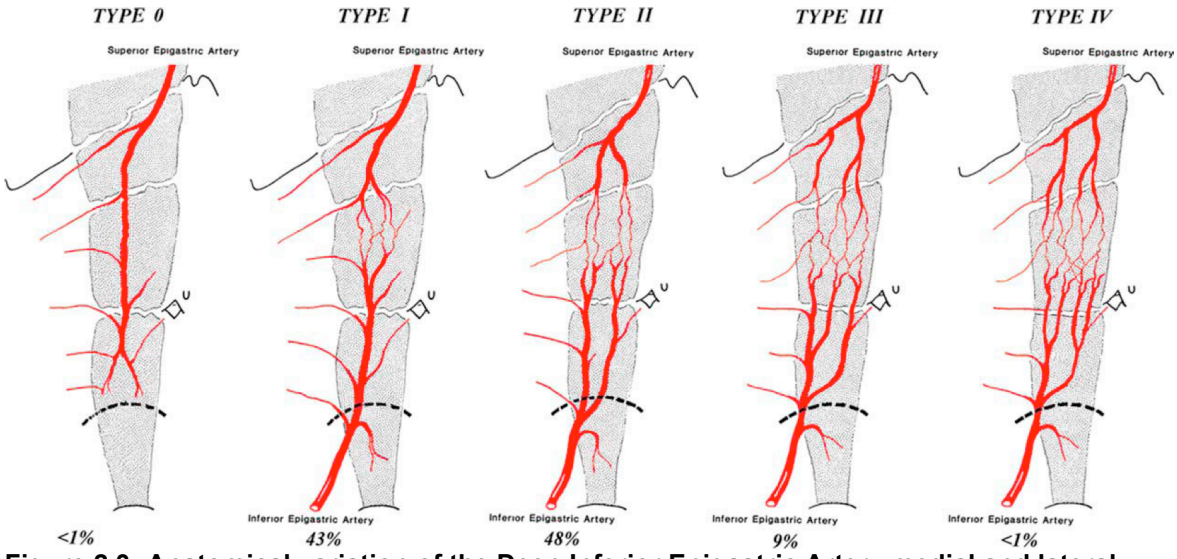


Figure 2.3: Anatomical variation of the Deep Inferior Epigastric Artery medial and lateral rows (Image permission granted by John Wiley and Sons. Rozen et al. 2011)

In 1993 Itoh and Arai performed an anatomical study of 34 DIEP cadaveric specimens, describing the difference in these vessel rows, measuring vessel caliber and concluding that the lateral row

appeared dominant in more than 80% of specimens(Itoh and Arai. 1993). The authors followed this with a case series of DIEP flaps preferentially taken on lateral row perforators and reporting 100% flap survival, though information regarding hernia rates or abdominal bulge was not presented. These findings were not supported by Schaverien et al.(2008) whom looked at 10 cadaveric and 2 abdominoplasty DIEP flaps, performing injection studies into the largest medial and lateral perforators and found that medial row perforators supplied a more central territory, with supply of zone IV, whereas lateral row perforators supplied an ipsilateral area, with no cross over or perfusion of zone IV(Schaverien et al. 2008b). The numbers of flaps examined in this study was small and the data observational, but patterns of filling were elegantly displayed using high quality imaging. Bailey et al. (2010) supported the findings of Schaverien et al. in their cadaveric contrast injection study of 11 medial row single perforator DIEP flaps, reporting that whilst medial perforators reliably perfused across the midline, lateral perforators did not, suggesting medial dominance of a larger vascular territory(Bailey et al. 2010). The researchers also noted that flow was seen through indirect linking vessels at the subdermal plexus level, and direct linking vessel flow was observed between perforators in the same (medial) row and between medial and lateral rows. This further supports the theory of perforasomes and the methods of vascular communication between them.

Rozen et al (2010) examined 10 fresh cadaveric abdominal walls and 145 CT angiograms of patients abdominal wall vasculature and reported that medial perforators were larger and more likely to ramify towards the contralateral side of the abdomen in the subcutaneous tissue, giving a larger and more reliable contralateral territory than lateral perforators(Rozen et al. 2010b). The researchers agreed with the perforasome concept and suggested that original zoning of either classification was oversimplistic and the areas either side of the perforator were ‘zone II’ with areas further away being less reliable, with positional differences for medial and lateral row perforators. Figure 2.4 illustrates their concept.

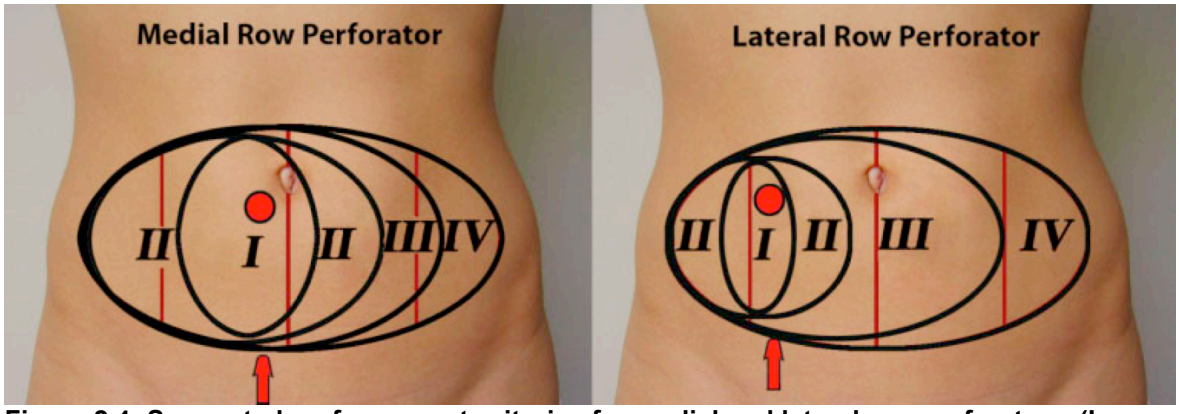


Figure 2.4: Suggested perforasome territories for medial and lateral row perforators. (Image permission granted by John Wiley and Sons. Rozen et al. 2010)

Wong et al (2010) injected 36 cadaveric flaps (22 medial and 14 lateral perforators) with contrast dye and measured the area of tissue perfused by each using 3-dimensional CT angiography(Wong et al. 2010). They found that medial perforators supplied a significantly larger vascular territory than lateral row perforators, and supported the findings of Schaverien et al

(2008) regarding the lack of perfusion across the midline for lateral perforators, suggesting that for a medial perforator the traditional Scheflan classification of the DIEP zones applies, whereas for a lateral row perforator the revised Dinner zone classification is correct. The authors concluded therefore that the in-vivo study by Holm. et al which supported the Dinner zone classification (Holm et al. 2006), was an oversimplification, as the different row perforators have different vascular territories and upon which the zones of the flap differ, though it is worth noting that the study by Holm et al (2006) did not split their group into medial and lateral perforators for analysis. The studies regarding vascular territory of different perforator rows have been largely based upon cadaveric or ex-vivo tissue, which as previously mentioned cannot automatically be taken as in-vivo evidence due to the lack of blood flow, neural and humoral effects on the vessels, the effects of which in relation to these anatomical studies are unknown.

2.2.2 Vessel tortuosity/Ease of dissection

The time taken for perforator dissection is significantly affected by the type of course a perforator takes through the rectus muscle or fascia and a more tortuous course can increase the likelihood of vessel, muscle and nerve damage(Vandevoort et al. 2002).

Blondeel et al. (1999) in their reported experience of 100 free DIEP flaps, reported simpler intra-muscular courses in lateral row perforators, and felt that they were the larger calibre and dominant vessels though as this was reported clinical experience, the authors had no measurements or quantitative data to support this opinion(Blondeel. 1999). Munhoz et al. (2004) in their fresh cadaveric study of 30 DIEP flaps reported that the intramuscular course of lateral perforator vessels was significantly less tortuous than medial row perforators(Munhoz et al. 2004), and concluded that concluded that the calibre of the vessels, and not the row, should be the clinical deciding factor for raising of the flap, but that lateral perforators are likely to provide easier dissection with less muscle and nerve damage. These findings were supported by Tansatit et al. (2006) with similar results in their cadaveric study of 31 DIEP flaps, reporting lateral row perforators as larger in calibre and with more direct intra-muscular courses(Tansatit et al. 2006). This group however used formalin-preserved cadavers instead of fresh, which is known to affect the size and laxity of the tissues, and may affect the comparability of this study with other groups using fresh cadaveric material.

Rozen et al (2008) in a cadaveric study of 20 cadaveric hemiabdomens (18 fresh and 2 embalmed with alcohol/glycerol) examined the relationship of the perforator rows to motor nerves supplying the infra-umbilical part of the rectus abdominis muscle(Rozen et al. 2008b). The authors reported that the intercostal nerves supplying the muscle entered its postero-lateral surface and ran medially, often traversing or travelling with the lateral row perforators, but not near to the medial row perforators. They concluded that medial row perforators are separated from the main motor nerves more than lateral perforators and proposed that dissection of medial row perforators was less likely to cause nerve damage and thus herniation and bulging.

These cadaveric and clinical studies would suggest that perhaps medial row perforators are more reliable at supplying the central portion of the DIEP flap, particularly when centrally based whilst the lateral row perforators are larger and have a more direct route from the main vessel to the rectus sheath, though may not provide as much perfusion to the opposite side of the flap. However, the majority of all of these studies have been performed as injection contrast studies in cadaveric or devitalised tissues, with only one cadaveric study looking specifically at zone IV(Schaverien et al. 2008b). The findings reported are observational, in small numbers of flaps with little quantitative data to report. In the few clinical studies performed, zone IV was discarded(Holm et al. 2006, Bozikov et al. 2009). Therefore the perfusion of zone IV on the medial and lateral perforator rows was an area chosen to examine in-vivo in this research.

2.3 Number and location of perforators

Within an individual perforator row, medial or lateral, the surgeon must decide the location of the perforator(s) and how many perforators to base the flap upon when raising the flap. This decision is often based upon the size of the perforators; if one large perforator is dominant, many surgeons take this alone, whereas if several smaller ones exist then two or three may be taken(Granzow et al. 2006). Some surgeons advocate taking one perforator, believing that this will reliably perfuse the flap whilst minimising damage to muscles and nerves with less dissection. Other surgeons routinely take two perforators, believing that the flap supply and perfusion territory will be superior with less chance of flap failure or fat necrosis. Investigators report that in their experience 25% of flaps are taken on one, 50% on two and 25% on three or more perforators(Granzow et al. 2006, Strauch. 2009).

Blondeel et al (1999) reporting clinical experience with 100 prespectively database-examined free DIEP flaps used a single perforator to perfuse the flap in over 50% of cases and stated the belief that it is not the number of perforators used, but their distance from the midline which affects flap perfusion, especially in zone IV, which they had included in 55% of their flaps safely with the use of a medial row perforator(Blondeel. 1999). Vandevoot et al. in their retrospective clinical study of perforator topography in 100 DIEP flaps reported no difference in complications when flaps were taken on one versus two or three perforators, though the raw data was not available for the researchers to review (Vandevoort et al. 2002). The authors also presented data on time spent dissecting the different numbers of perforators, reporting that dissection of one, two and three perforator flaps took an average of 110min, 138min and 180mins respectively, concluding that perforator dissection was the main determinant of the length of the operation. Gill et al (2004) retrospectively reviewed 758 free DIEP flaps and found that the overall complication rate was significantly ($p<0.02$) increased if more than one perforator was used, and as the number of perforators included in the flap increased, so did rates of fat necrosis, though this failed to reach statistical significance. Gill et al. suggested that larger size and centrality of a single perforator could give more reliable perfusion than a wider distribution of small calibre vessels(Gill et al. 2004).

Hallock et al. (2004) using a rat model comparing LDF flow and flap survival on a TRAM flap, a multiple perforator DIEP flap and a single perforator DIEP flap(Hallock and Rice. 2004). The researchers reported higher zone I perfusion in the TRAM flaps versus both DIEP flaps, low flow in all flap zone IV but no significant difference in flap survival in any group. Not only did this study add weight to the DIEP and single-perforator DIEP evidence, but also described a robust animal model upon which to study these flaps.

Figus et al (2006) prospectively studied 16 DIEP flaps all raised on either 1 or 2 perforators (perforator row not reported) and found no correlation between perforator number and complication rates(Figus et al. 2006), findings that were supported by Cheng et al. who in their study of 74 DIEP flaps over 2 years found no significant correlation between perforator number, or percentage of flap weight used and complication rate(Cheng et al. 2006a).

Bozikov et al (2009), retrospectively reviewed 100 DIEP flaps (zone IV discarded in all cases) and found no correlation between perforator row and incidence of fat necrosis but reported a significantly ($p=0.038$) increased risk of fat necrosis (assessed clinically and by USS) when a single perforator was used compared with two or more perforators. It is worth noting however, that only 5 flaps were harvested on a single perforator, as opposed to 75 flaps harvested on 2 or 3 perforators, and 14 on 4 perforators. This small group comparison to a much larger one may have biased the statistical analysis and also introduces the possibility of selection bias favouring more perforators. The authors also reported higher fat necrosis rates (identified through examination and confirmed using USS and mammography) in obese patients and those who needed revisional procedures(Bozikov et al. 2009). These findings were supported by Baumann et al (2010) who prospectively assessed 248 free flaps, combinations of TRAM, DIEP and superficial inferior epigastric artery (SIEA) flaps(Baumann et al. 2010). The authors reported significantly ($p=0.007$) lower rates of fat necrosis when using more numbers of perforators, advising that 3-5 perforators produced the lowest risk of fat necrosis, especially in muscle-sparing TRAM. However this study was heavily criticised for the grouping of DIEP flaps with non-perforator based SIEA and muscle-sparing TRAM flaps with different vascular territories and their method of recording perforator numbers(Rozen et al. 2010c). In addition, Baumann et al. did not use USS correlation of the clinically identified and subjectively-measured fat necrosis, which was measured at a relatively early time of 6 weeks post-operatively when swelling and evolving changes could affect both detection and later outcome.

Lee et al (2010) retrospectively compared the outcomes of different compositions of perforators within DIEP flaps, destailed in Figures 2.5-2.7.

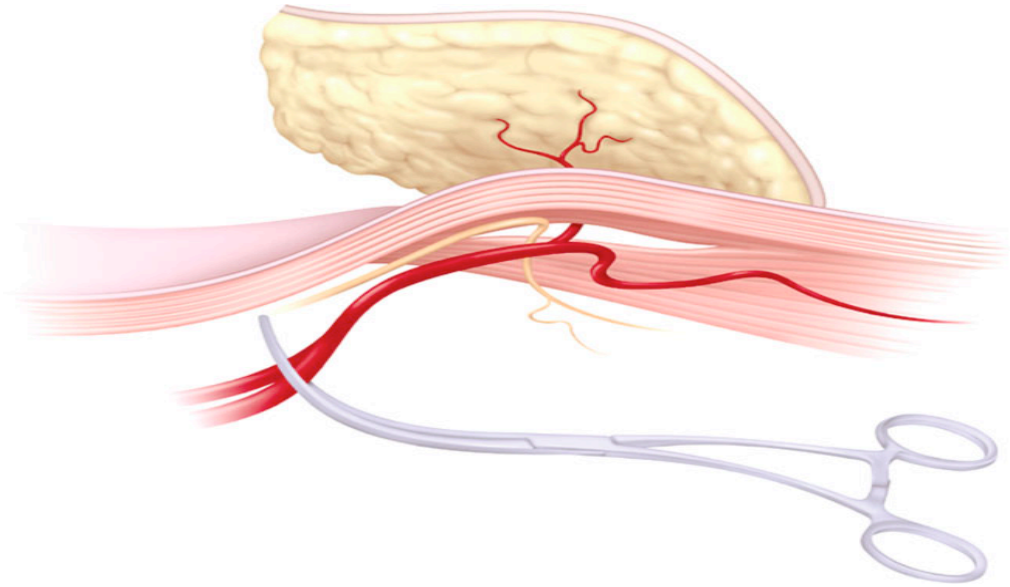


Figure 2.5: Flap based on one perforator from a single row – nerve preserved (Image permission granted by John Wiley and Sons. Lee et al. 2010)

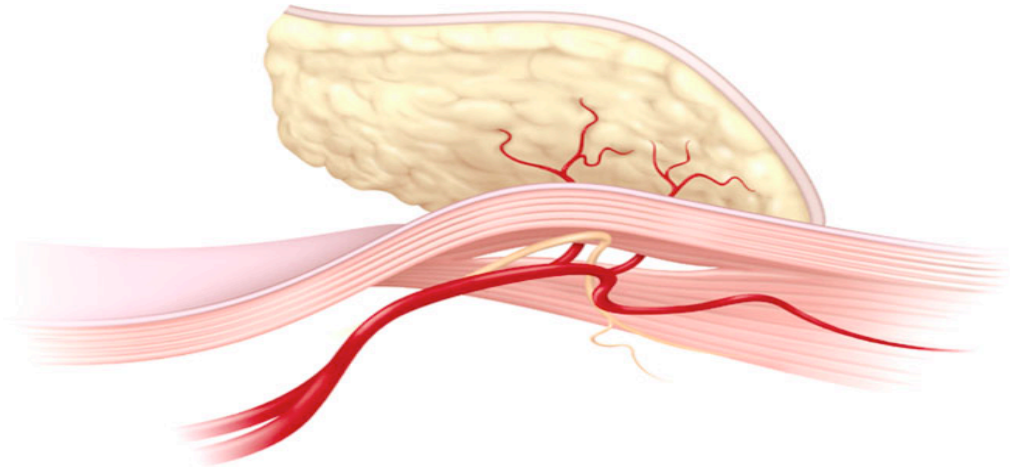


Figure 2.6: Flap raised on two perforators from a single row - minimal nerve sacrifice (Image permission granted by John Wiley and Sons. Lee et al. 2010)

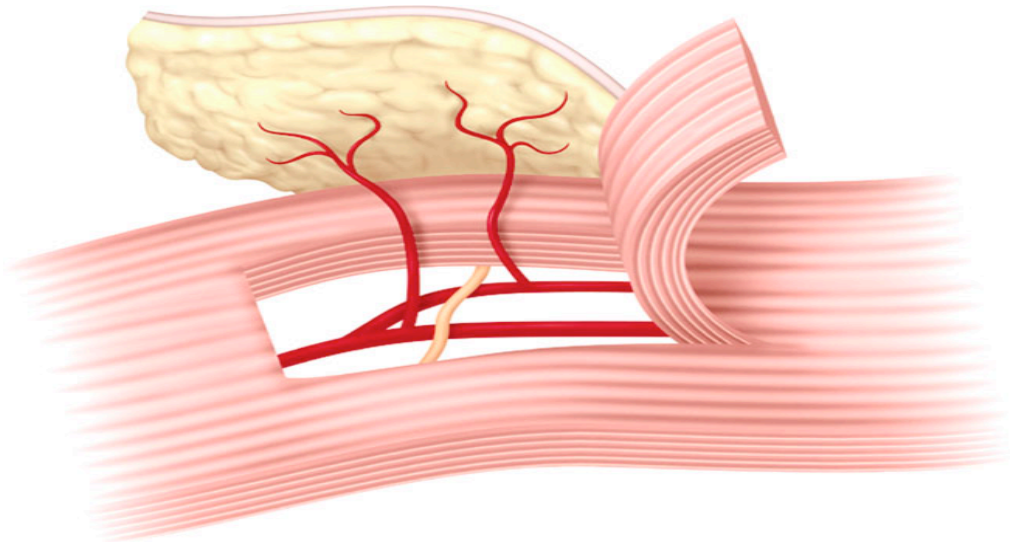


Figure 2.7: Flap raised on two perforators from both medial and lateral rows - significant nerve and muscle sacrifice (Image permission granted by John Wiley and Sons. Lee et al. 2010)

Lee et al. reported that taking two perforators from the different rows in a flap produced a reduction in fat necrosis which just reached statistical significance (p=0.049) compared to a single perforator flap, but more abdominal bulging, though this failed to reach statistical significance. The authors reported no significant difference observed in clinical outcomes between the one and two perforator supplied flaps from a single row(Lee et al. 2010). Taking perforators from both medial and lateral rows is not commonly performed, due to the significant muscle and nerve sacrifice which Lee et al. describes, and this would probably fall into the category of a muscle sparing TRAM flap rather than a DIEP flap for this reason.

A cadaveric contrast injection study by Bailey et al. (2010) suggested that the largest medial perforators are found within 3cm of the umbilicus, and these should be seen as preferential to take to supply the flap as a single perforator(Bailey et al. 2010). The researchers followed this cadaveric study with 16 clinical cases of DIEP flaps raised on a single large medial perforator, selected in the majority of cases by pre-operative CT angiography. The perfusion was not assessed quantitatively intra-operatively but by clinical impression and areas which appeared underperfused excised. Flaps consisted of either zone I/III, zone I/II/III or zone I/II/III/IV. Though they had no flap failures, they reported a fat necrosis rate of 25%, mainly in zone III of the original zone classification and concluded that when using a single medial row perforator the lateral half of zone III and all of zone IV should be excised. This study was interesting, however though it expanded upon perfusion of the DIEP flap relating to perforator row, the subjective measurement of flap perfusion intra-operatively and the lack of any detail as to the method of detecting fat necrosis limit its findings in terms of clinical application.

Few researchers have commented on the position of the perforator within the flap, as regards to the vertical element (superior vs. inferior) rather than the zones based on the horizontal axis of the flap. Keller (2006) reported much lower tissue oxygenation readings in the inferior portion of zone I in a flap with a superiorly placed perforator(Keller. 2006). He proposed a mathematical model suggesting that flow, vessel radius and resistance, and distance from perforators may be more significant than angiosome allocations of the flap, though Holm. et al criticised this model as over simplistic, assuming constant resistance in vessels which alter tone and thus radius and resistance(Keller. 2006). Hallock et al (2010) agreed with Keller, suggesting that the sequential perforasome concept would logically apply vertically as well as horizontally, and highlighted that this may necessitate individual approach to flap design(Hallock. 2010).

2.4 DIEP flap problems

2.4.1 Fat necrosis

As mentioned in Chapter 1, fat necrosis is a commonly reported problem in DIEP flaps, and indeed some surgeons advocate the use of surgical delay procedures to reduce the incidence of fat necrosis, particularly if a large volume flap is required encompassing zone IV(Christiano and Rosson. 2010). Fat necrosis in DIEP flaps has been reported as much higher than in free TRAM

flaps. Kroll et al (2000) in a retrospective review of 310 breast reconstructions reported higher fat necrosis in DIEP versus TRAM flaps (62% vs. 13%) though these findings were not significant when examined across all flap groups (p=0.101), the identification of fat necrosis was clinical and subjective and significant sub-group selection bias existed (i.e. DIEP flaps only used when small flaps were required) between the TRAM and DIEP group, reducing the impact of this stud significantly(Kroll. 2000). Scheer et al (2006) reported similar findings in theira retrospective review of 130 unilateral and bilateral TRAM and DIEP flaps, reporting significantly ($p<0.001$) higher fat necrosis in unilateral DIEP flaps, proposing that taking a larger than hemi-zone of the flap could increase the fat necrosis rates. Unfortunately, data regarding methods of fat necrosis detection, follow up times, sizes or zones of flaps or numbers of perforators used was not presented, again, limiting the findings(Scheer et al. 2006).

Gill et al (2004) looked at fat necrosis in a 10-year retrospective review of 758 DIEP flaps, reporting significantly higher rates of fat necrosis when post-operative radiotherapy was used and also trends of increasing fat necrosis the more perforators were included in the flap, though this latter association failed to reach statistical significance and zone IV was discarded in all flaps(Gill et al. 2004). The authors retrospectively identified patients with fat necrosis by using documented examination findings in case notes, and was therefore less accurate than a prospective study with radiological support. Figus et al (2006) in a prospective study of 16 DIEP patients reported significantly increased fat necrosis, identified using USS, in patients with higher BMI and with longer operating and ischaemia times, concluding that ischaemia or inadvertent vessel damage due to more difficult dissections may have contributed(Figus et al. 2006).

Peeters et al (2009) examined fat necrosis in 202 DIEP flaps using physical examination and USS identification. The researchers found a much higher rate (35%) of fat necrosis detected by USS than clinically (14%) but that radiotherapy, smoking, BMI and bilateral reconstructions were not significantly associated with higher fat necrosis rates(Peeters et al. 2009). Bozikov et al (2009) as mentioned previously, reported single perforator use, high BMI and revisional procedures all significantly increased fat necrosis rates found on examination and confirmed with USS(Bozikov et al. 2009). Baumann et al (2010) reported higher rates of fat necrosis with smaller numbers of perforators, smoking, inclusion of zone III of the flap and higher in DIEP/SIEA flaps than muscle-sparing TRAM, though as discussed this came under criticism for the heterogeneous group of flaps studied and the subjective methods used to identify fat necrosis(Baumann et al. 2010).

2.4.2 Venous congestion

Venous congestion in DIEP flaps is a recognised complication which occurs more commonly than with TRAM flaps, often observed in zone IV but sometimes in a large portion of the flap. Carramenha e Costa et al. (1987) performed 12 cadaveric (2 fresh, 10 formalin fixed) injection studies of TRAM flaps and noted that the superficial epigastric venous system of the abdominal integument appeared to be larger and more dominant than the deep system(Carramenha e Costa

et al. 1987). Blondeel et al. (2000) reviewed their experience with 240 DIEP's and 271 free TRAM's and noted that in cases where near-total flap venous congestion was observed, there was a very large superficial inferior epigastric vein (SIEV), suggesting the flap was reliant on the superficial system for its venous drainage and required a second venous anastamosis using the SIEV to improve the congestion(Blondeel et al. 2000). The authors also examined 15 fresh cadaveric and 2 fresh abdominoplasty DIEP specimens and though branches between the superficial and deep venous systems were found in all specimens, they reported a variable and unreliable pattern of superficial vein branches crossing the midline. Blondeel et al. linked these findings with the clinical observation of blue mottled colour and dark venous bleeding occasionally seen in zone IV of DIEP's intra-operatively, concluding that it is likely that poor venous outflow rather than unsustainable arterial inflow makes zone IV unreliable. Though largely based on opinion rather than data, the clinical observations of such an experienced group supported with fresh cadaveric anatomical findings are worth noting.

Figus et al. examined 16 DIEP flaps, measuring perfusion with Laser Doppler Flowmetry (LDF) near to and distant from the perforator preoperatively and at regular intervals postoperatively, to give a measure of arterial perfusion(Figus et al. 2006). They also used Lightguide Reflectance Spectrometry (LRS), which measures oxygenated haemoglobin (and thus a measure of how well the venous drainage of de-oxygenated haemoglobin was). Patient results were divided into 3 outcome groups; those with no complications, those with fat necrosis and those which required re-exploration in theatre. Figus et al. demonstrated that following free flap inset the skin perfusion near to the perforator increased compared to pre-operative measurements in successful flaps and those with fat necrosis, whilst in those which required re-exploration they did not reach pre-operative levels for around 12 hours. They suggest that the initial increase in perfusion is due to the increased flow through a smaller vessel, though why this would not be observed in the revised flaps was not explained, as they stated that no arterial problems were present in any flap. Perfusion of the skin away from the perforator decreased compared to pre-operative levels in all flaps, but improved more rapidly in flaps with no complications than those with fat necrosis or which required re-exploration. LDS values decreased in all groups post-operatively, but increased more rapidly in the flaps with no complications and fat necrosis than the group which required exploration. Figus et al reported that they use the SIEV as an additional venous anastamosis in 3 flaps – once intra-operatively when the flap appeared congested before division with a turgid superficial vein and in two other cases which required return to theatre due to venous congestion noted post-operatively and concluded that this may be necessary in certain cases when the superficial venous system appears dominant. This research is one of the few physiological studies which attempted to quantatively assess both perfusion and congestion in DIEP flaps and correlate their findings with clinical outcomes.

In their injection study of venous anatomy in 10 fresh cadaveric and 2 fresh abdominoplasty DIEP specimens, Schaverien et al (2008) reported a variable morphology of venous anatomy, with connections between the superficial and deep system seen, but no midline crossover at all in one flap and deep and superficial crossover in another(Schaverien et al. 2008a). Rozen et al (2009)

examined the venous system of DIEP flaps using venography in 8 fresh cadaveric specimens and CTA examination of the DIEP venous system in 100 pre-operative patients(Rozen et al. 2009). They found a variable pattern of venous perforator size, a good degree of midline branch crossover (94% in cadavers and 86% in CTA's) and identified superficial to deep communication in most cases, though admitted that some of these communicating branches were too small to be identified by CTA. Based on their findings the authors proposed that a DIEP venous perforator of <1mm diameter, SIEV >1.5mm, small communicating veins or midline branches could potentially predict the need for additional venous anastamosis using the SIEV, though these suggestions were not correlated with any clinical data.

Building on their earlier findings, Schaverien et al. (2010) retrospectively reviewed 54 DIEP flaps and their MRA scans, and found that the flaps which had suffered from venous congestion did not have venae commitantes with direct connections to branches of the SIEV(Schaverien et al. 2010). Ninety-eight percent of the flaps which did not have congestion problems had these direct venous connections demonstrated on MRA. These findings strongly suggest that perforators with direct connections to the SIEV branches are predictive of adequate flap drainage and the authors suggest that pre-operative imaging of these perforators for selection can assist in reducing this complication.

Hallock et al (2010) supported the perforasome work of Saint-Cyr et al.(2009) and reiterated the individual nature of such perforators and possible territories of venosomes by describing their experience of a large right medial perforator which produced left hemi-flap venous congestion, the majority of which resolved quite rapidly on the abdomen(Hallock. 2010). They underlined the unpredictable nature of some flap drainage and that it is likely to be the main cause of vascular compromise in a flap rather than arterial supply.

Figus et al (2012) looked at the venous perforator anatomy of DIEP flaps, using duplex Doppler scanning to identify and measure perforating arteries and veins and compare them with the source vessels(Figus et al. 2012). The researchers concluded that though correlation existed between the size of the perforator veins and their corresponding DIEV, this differed on different sides of the abdomen and there was no correlation with the SIEV. The concept of a ‘perforator complex’ was discussed, linked with their findings, that where a perforating vessel could have any combination of artery, vein and nerve. They highlight that this could explain the congestion see in flaps with an adequately sized perforator, if there was no vein accompanying the artery and conclude that a large perforating vein should be identified when raising the flap to ensure adequate drainage, as this supercedes the need for a larger artery.

2.5 Flap flow and perfusion

When considering the vascular supply to any flap the key mechanisms of arterial inflow, perfusion of the flap tissue constituents and venous outflow must be considered. The relationships between these physiological components has been examined and using laws of

physics regarding electrical and hydraulic flow and models based upon such laws, experiments designed and variables tested.

Gosling et al. (1971) described the use of Doppler ultrasound measurement of arterial waveforms as a reliable method of detecting arterial flow and the effect of any occlusion(Gosling et al. 1971). Measuring the size of the flow pulse and the transit time of the flow the authors could reliably detect vascular occlusions caused by peripheral vascular disease. The measurement of pulsatility index (PI) was described in this paper, which is the maximum flow velocity minus the minimum flow velocity, divided by the mean flow velocity, shown in the formula below.

$PI = (Flow_{MAX} - Flow_{MIN}) / Flow_{MEAN}$

This was measured in different areas distal to stenotic vascular lesions and Gosling et al. describe the 'damping' of the pulse and the smaller value of PI with reduced arterial flow and abnormal waveforms when occlusions were proximal to the measurement. Evans et al. (1980) in canine experiments with arterial flow and stenoses found that whilst PI reduced dramatically with tight stenotic proximal lesions, milder stenoses produced a variety of values(Evans et al. 1980). This indicated some compensatory decrease in peripheral resistance, similar to that seen post-exercise, affected PI in these milder occlusions. They concluded that PI was not solely a measure of arterial inflow and was influenced by peripheral resistance also. Later studies confirmed that PI was a reliable measure of downstream vascular resistance(Petersen et al. 1997, Radermacher et al. 2003).

Sasmor et al (1992) describe blood flow velocity in a flap as imperative to the patency of anastamosis and illustrate that flow is inversely proportional to the downstream microcirculatory resistance(Sasmor et al. 1992). Proposing that different tissues on different parts of the body possess different microvascular resistances, they collected data on flap vascular resistance using pressure transducers in 30 patient free flaps, 16 cadaveric flaps and 17 canine flaps. The authors reported lower resistances in muscle flaps than soft tissue flaps or fascial flaps and lower resistances on flaps found on the trunk when compared to those from limbs. In an attempt to correlate these results with clinical outcomes, Sasmor et al. retrospectively reviewed 175 of their free flaps and reported significantly ($p<0.04$) higher complications in fascial/fasciocutaneous flaps compared with muscle trunks flaps. They concluded that increased vascular resistance in these flaps causes clinical problems, by reducing arterial inflow, reducing venous outflow and causing vascular stasis near anastamoses increasing the likelihood of thrombus. This study has several methodological flaws and did not separate the cadaveric and animal flaps from the patient free flaps for the reader to assess the potential differences between ex-vivo and animal tissues to free flaps. In addition there were no perforator flaps to assess or sizes of vessels used to correlate findings, However, the concept is interesting and raises important questions regarding flow rates and resistance in different flaps and clinical outcomes.

Salmi et al (1995) revisit the measurements of PI and describe the resistance index (RI) of a blood vessel, likening it to the resistance in an electrical circuit and measured using the following formula:

RI = (Peak systolic velocity – peak diastolic velocity) / Peak systolic velocity

Both PI and RI are measures of vascular resistance(Petersen et al. 1997). Using colour Doppler ultrasound the authors measured velocities of flap and recipient arteries and calculated resistance indices for 18 muscle flaps transferred to the lower limb at 2 weeks, 6 weeks, 3, 6 and 9 months post-operatively(Salmi et al. 1995b). They reported increased blood flow in the vascular pedicle of free muscle flaps for 6 months post-transfer caused by increased minimum velocity of the pedicle in diastole and decreased resistance index. These effects subsided after 6 months and were explained by the initial loss of vessel tone by denervation, though proposed mechanisms whereby the effects normalised were not proposed. Salmi et al. concluded that this initially high flow post-operatively could help explain the relatively high success of free flaps after micro-anastamosis.

Lorenzetti et al (2001) in a study of flow velocities of 25 TRAM flaps anastomosed to either the internal mammary or thoracodorsal vessels found that flow rates into flaps post-operatively were independent of the recipient artery pre-operative flow rates(Lorenzetti et al. 2001a). Using transit-time ultrasonic flowmeters, the pedicle flow rate post-transfer was measured and found to be the same as values measured for the TRAM flap pre-transfer and not similar to the higher values seen in the internal mammary pre-operatively or the lower values measured in the thoracodorsal vessels pre-operatively. This important piece of work indicates that the intake of blood into the flap is determined by the intra-flap tissue components, such as resistance in vessels, not the rate of flow of the vessel supplying it and as such is relatively independent of the flow rate of its recipient vessel.

Mahabir et al (2001) examined vascular resistance in an intra-operative group of muscle flaps, proposing that the different intra-muscular vascular architecture conveyed by the different anatomical pedicles they could be raised on may affect the resistances within the flap(Mahabir et al. 2001). They did not report a statistically significant difference in vascular resistance between flap types though found that vascular resistance was inversely proportional to muscle flap mass ($p<0.001$) and thus the larger flaps (rectus abdominis and lattisimus dorsi) tended toward lower resistances than the smaller gracilis flaps. The authors proposed that the inverse relationship between mass and vascular resistance might be attributed to choke vessels (describing choke vessels as small vessels with high resistance) but further explanation regarding mechanisms by which these were different in the larger and smaller flaps were not explored.

Ichinose et al (2003) measured vascular flow in arterial and venous pedicles pre and post-operatively in 8 free radial forearm flaps using colour Doppler ultrasound(Ichinose et al. 2003). Measurements were taken 1 hour and 1, 3 and 7 days post-operatively, with arterial inflow

(estimated by mean arterial pressure and heart rate) remaining relatively constant and measured. Using an hydraulaic analogy of Ohm's law where flow (Q) through a vessel can be defined as:

$$Q = P / R \qquad (Q = \text{flow}; P = \text{driving pressure}; R = \text{resistance})$$

and PI as a measure of vascular resistance the authors found that the initial decrease in flow seen in both arterial and venous pedicles after anastamosis increased steadily to 7 days post-operatively. At the same time, the PI steadily decreased. In the presence of a constant arterial pressure, this indicated that the vascular resistance was decreasing, improving the flow in the flaps during the time measured. Ichinose et al proposed that initial high resistance could be due to flap oedema, vasoconstriction and the removal of the vascular network surrounding the chosen pedicle. They suggest that as time progresses and some of these factors resolve, neovascularisation starting on day 4 post-operatively helps to speed the resolution, though the relatively steady increase in flow and resistance in PI would not fit with studies suggesting that swelling in flaps increases post-operatively and in muscle flaps peaks at 2 weeks(Salmi et al. 1995a, Salmi et al. 1996) and the explanation for the uniform recovery is likely to be more complex. Ichinose et al. concluded that the reduced flow immediately post-operatively explains why the early post-operative period is the most liable to thrombotic problems due to these factors and their timing.

Rubino et al. (2006) elegantly explain the differences in vascular anatomy and physiology in normal circulation, muscle flaps and perforator flaps, explaining that as the single perforator flap is only a segment of a normal vascular tree with all other ends sealed, it can be likened to a circuit with resistances in series (from a single supply), rather than in parallel(Rubino et al. 2006). The researchers also explain that the cross-sectional area of the vascular tree decreases in an isolated perforator flap, as opposed to increasing in a muscle flap or normal circulation with many perforators and their corresponding smaller branches. Using colour Doppler ultrasound in 10 patient perforator flaps Rubino et al. measured flow in flap pedicles and skin perforators both pre and post-operatively. They reported that as expected, pre-operatively blood velocity was higher in the pedicle and lower towards the periphery in the perforator. However, post-operatively the blood velocity was higher in the perforator than the pedicle ($p<0.01$). Though flow was still lower in the perforator than the pedicle post-operatively, it had increased to a much greater proportion of the pedicle flow ($p<0.01$). The researchers conclude that the blood velocity and flow reaching the skin is proportionally higher in a perforator flap than in normal circulation and term this as 'haemodynamic enhancement exploiting physiology of circulation'. This study suggests a significant difference in the vascular flow and physiology of perforator flaps compared to non-perforator flaps and normal circulation which could indicate higher flow rates in perforator flaps post-transfer. However, though good flow rates are undeniably a desired outcome in free flap transfer, whether this increase in perforator flap flow could have any significance clinically cannot be confirmed by these studies.

Patel and Keller (2008) proposed a mathematical model on the basis of the physics of flow through blood vessels(Patel and Keller. 2008). They remind the reader that via Poiseuille's law, resistance through any vessel is defined as:

$R = 8nl / (\pi)r^4$ (where R = resistance; n = a constant; l = length of a vessel; r = radius of a vessel)

Similar to Rubino et al. they proposed to look at flow through a perforator flap as a circuit with perforator vessels viewed as resistances. If only one perforator is used then this forms a single resistance (R_1) with the capillary bed perfusing the flap and providing a second resistance R_C, seen in Figure 2.8.

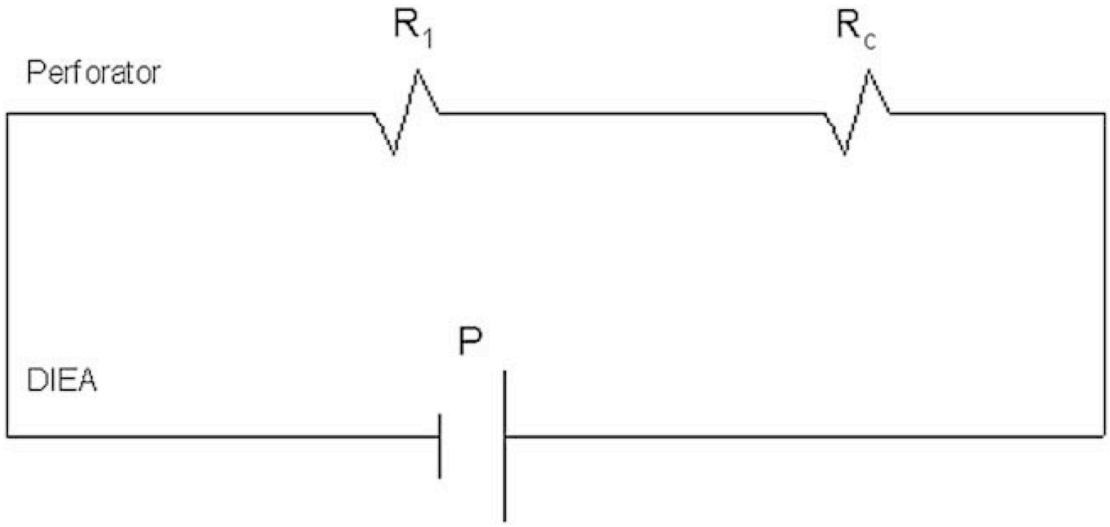


Figure 2.8: Circuit representing single perforator flap – R1 = resistance; Rc = capillary resistance; P = pressure. (Image permission granted by Churchill Livingstone. Patel and Keller. 2008)

In this model, a shorter vessel and larger vessel diameter would decrease the resistance and increase the flow. Patel and Keller then propose another model of two perforators to the flap, which would add resistance in parallel, not in series, shown in Figure 2.9.

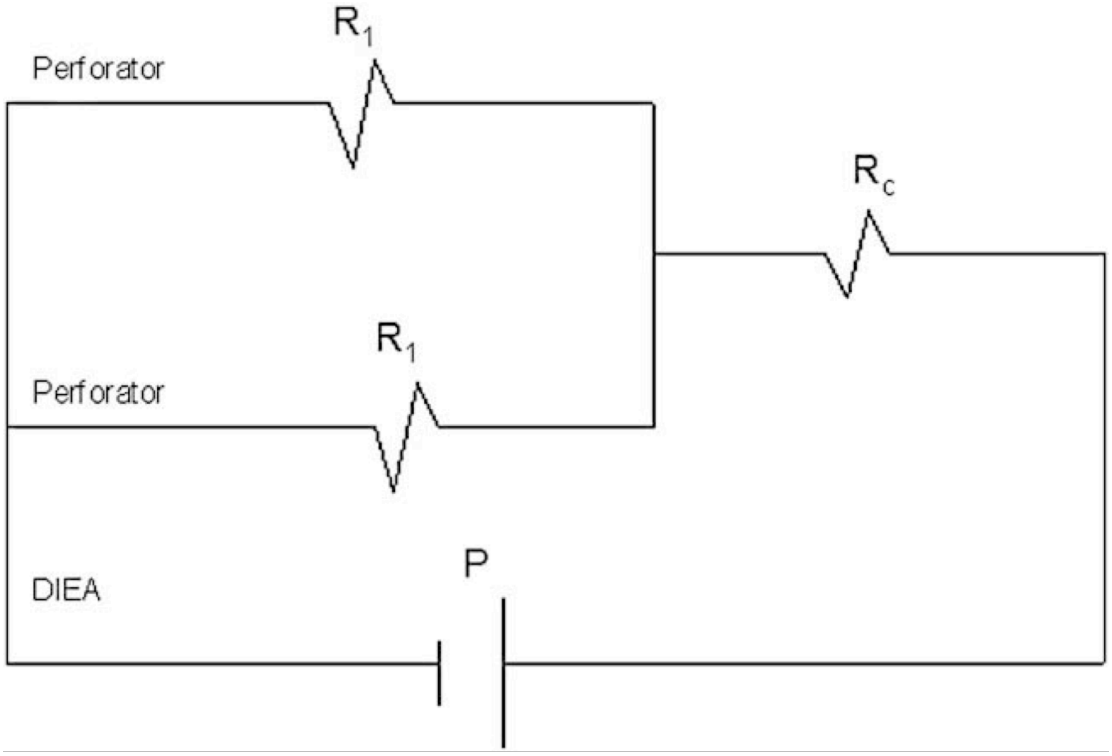


Figure 2.9: Circuit representing flap with two perforators of the same resistance as in Figure 2.9 – R1 = resistance; Rc = capillary resistance; P = pressure. (Image permission granted by Churchill Livingstone. Patel and Keller. 2008)

In this model, flap total resistance can be measured by: $1/R_{(total)} = 1/R_1 + 1/R_2 + \ldots 1/R_n$ (where $R_{(total)}$ = total flap resistance; R_1 = resistance through perforator 1; R_2 = resistance through perforator 2; and n = number of perforators in the flap).

Applying the above formulae to the proposed model, it is apparent that the effect of perforator radius on the equation will be much more than the linear relationship expressed by the length of the perforator vessel. The authors conclude that using their model the addition of another perforator would logically reduce resistance in the flap by 50% and thus double the flow rate, though they caution that this is highly dependant on the radius of the additional perforator vessel. In a series of elegant calculations, they show that unless the second perforator was of near-equal radius as the first perforator, the effect on resistance is minimal and the addition of multiple smaller perforators is likely to increase resistance by adding multiple smaller channels. In addition they caution that any increased benefit by adding another perforator of similar size would have to be balanced by the increased trauma to muscle, nerves and fascia by dissection. Patel and Keller also point out that though the venous flow through the system has been suggested to be of more importance, the same rules which govern the flow of arterial system will apply to the venous system; indeed the lower pressures in the venous system would make this even more applicable.

Figus et al (2008) examined the microcirculation in areas of 26 DIEP flaps, comparing blood velocity (measured using LDF as in their previous work) at the skin overlying perforators and skin furthest away from perforators immediately post-op and 3 months post-op and compared these values with those obtained preoperatively(Figus et al. 2008). They found that blood velocity

increased almost uniformly in flaps after transfer and this pattern was upheld at 3 months. In those flaps whose measurements did not show this pattern at the skin site furthest away from the perforator(i.e. those whose blood velocity stayed the same or decreased post-transfer) they clinically observed skin or fat necrosis. Though this was a very small sub-group, a fact the authors fully disclose and concede would need further investigation, Figus et al. wondered whether this may indicate that low blood velocity in DIEP microcirculation could indicate development of areas of skin/fat necrosis. This is very important, as it has linked the physiological measurements of LDF blood velocity and observed a pattern of lower measurements leading to clinical complications. What is not known is whether this is related to a reduction in arterial flow due to the lower velocity, the effect on venous return or a combination of the two.

Hanasono et al (2009) measured peak arterial and venous velocities using implantable Doppler probes in 32 free flaps(Hanasono et al. 2009). Blood velocity was measured prior to flap division, 20 minutes after flap anastamosis and then daily for 5 days. The authors reported increased peak arterial and venous flow velocities post-flap anastamosis, which steadily increased for the first 3 postoperative days and remained significantly higher than prior to flap division. These findings concur with the previous studies regarding post-anastamotic flow(Salmi et al. 1995b, Rubino et al. 2006) the clinically measured LDF skin flow velocities measured pre and post-flap inset by Figus et al in their clinical studies(Figus et al. 2006, Figus et al. 2008).

Rubino et al (2009) in a further study of perforator flap haemodynamics, prospectively evaluated blood flow in 25 perforator flaps(Rubino et al. 2009). By measuring the flap weight, the size of the perforating artery and vein diameters and the flow in the flaps studied, the researchers used linear regressions and Poiseuilles's law to produce a prediction model of the venous diameters required to drain a flap of any given weight. Referencing the work previously discussed by Lorenzetti et al(Lorenzetti et al. 2001a, Lorenzetti et al. 2001b) on the independence of free flap flow-rate to its recipient blood supply, Rubino et al. concluded that the bigger a flap is, the higher its flow rate will be and thus venous drainage has to be to support it. They provided an algorithm of flap weights, flow rates and the diameters of veins which would be required to support its venous drainage and provided examples with measurements of flap weights and vein diameters which may require a second venous (usually SIEV) anastamosis to support it. This is a very interesting regressional physiological study, and though limited in its clinical outcome application data, correlational findings and links can be made regarding the higher flow in larger flaps reported here and the lower resistance in large muscle flaps reported by Mahabir et al(Mahabir et al. 2001).

Kurita et al (2010) in a prospective study of 17 free latissimus dorsi muscle flaps anastomosed to the facial artery, using colour Doppler USS measured arterial pedicle flow, blood pressure and vascular resistance via PI(Kurita et al. 2010). Their findings concurred with those of the previously described studies of Ichinose et al.(Ichinose et al. 2003) and Hanasono et al.(Hanasono et al. 2009), that vascular resistance increases dramatically after flap anastamosis, and decreased gradually over 7 days and that after an initial drop in arterial flow volume

postoperatively, this steadily increased over 7 days. Kurita et al proposed that the slow but steady increase in arterial flap flow, despite the large decrease in vascular resistance and relatively stable blood pressure could indicate inherent flap autoregulation and feedback declination, keeping flap inflow relatively constant, though possible mechanisms for this are not explored.

2.6 Summary and null hypotheses

The evidence surrounding the choice of perforator row seems divided amongst researchers, and though it does appear that different territories exist for the two rows, the evaluation of zone IV and flaps in-vivo is limited. Additionally, within a row, there is a paucity of evidence regarding the effect of different numbers and locations of perforators chosen upon which to base the flap. Whilst clinically measurable outcomes of flap necrosis and fat necrosis are important, it was decided that a physiological study measuring flow and perfusion on different numbers and locations of perforators would be a good start in understanding the mechanisms by which flap tissue is perfused. The effect of venous drainage on flap survival has been discussed in this chapter and is of undeniable significance, though as previously discussed, the physics of flow within a vessel should govern both arterial and venous systems, arterial inflow should equate to venous outflow and the difficulty in measuring venous parameters due to artefact and problems with pressure effects of probes on this lower pressure system have been identified(Rubino et al. 2009, Kurita et al. 2010). As regards to perforator number and position, evidence exists regarding flow and resistance to indicate that altering the number, size and potentially the location of the perforators could affect the flow into and resistance within a flap and robust animal models of such physiological experiments have been used(Hallock and Rice. 2004, Patel and Keller. 2008). It is accepted that arterial flow into the flap needs to be sufficient to perfuse the flap with oxygenated blood and be of sufficient velocity to prevent thrombosis(Ichinose et al. 2003, Kurita et al. 2010). Clinically this can alter perfusion and drainage of flaps and thus flap survival and fat necrosis. The vascular territory supplied by a DIEP flap may be related to the filling pressure of the perforating vessel, opening linking vessels to adjacent perforasomes(Saint-Cyr et al. 2009). This filling pressure will be related to the arterial flow into the flap and could be affected by the variables of blood velocity, flow and vascular resistance, which have been previously discussed in this introduction.

This research will concentrate primarily on the flow and perfusion of DIEP flaps, to link with the previous work regarding blood supply to flap zones and perforator numbers and locations, with a view to examining the effect of venous drainage in further work.

Therefore the following null hypotheses are proposed:

1. There is no difference in the total pedicle flow of an epigastric flap rat model whether one or two perforators are used

2. There is no difference in the total pedicle flow of an epigastric flap rat model whether a superior or inferior perforator is used

3. There is no difference in the skin perfusion of an epigastric flap rat model whether a one or two perforators are used

4. There is no difference in the skin perfusion of an epigastric flap rat model whether a superior or inferior perforator is used

5. A single brief period of ischaemic preconditioning makes no difference to the total pedicle flow of an epigastric flap rat model

6. A single brief period of ischaemic preconditioning makes no difference to the skin perfusion of an epigastric flap rat model

7. There is no difference in the perfusion of zone IV fat of DIEP flaps whether a medial or lateral row perforator is used

8. There is no difference in the perfusion of zone IV skin of DIEP flaps whether a medial or lateral row perforator is used

9. There is no difference in the perfusion of zone IV fat of DIEP flaps whether one or two perforators from a single row is used

10. There is no difference in the perfusion of zone IV skin of DIEP flaps whether one or two perforators from a single row is used

11. There is no difference in the perfusion of zone IV fat of DIEP flaps whether a superior or inferior perforator from a single row is used

12. There is no difference in the perfusion of zone IV skin of DIEP flaps whether a superior or inferior perforator from a single row is used

Chapter 3: Outline of completed studies

This introduction has outlined the multitude of factors affecting microvascular free tissue transfer, in particular the DIEP flap and the selection of its perforating blood vessels. The different rows, numbers and locations of perforators from which to choose have been looked at previously, but the majority of the work has been completed in cadaveric studies and opinion is still divided. In addition, the perfusion of zone IV of the DIEP flap as an outcome of perforator selection and combination has not previously been reported.

Thus, the following studies have been outlined:-

3.1 The effect of perforator vessel choice in terms of number and position, ischaemic preconditioning and removal of zone IV on total pedicle flow and perfusion of an epigastric perforator flap in a rat model

The aim of this animal model is to attempt to characterise the differences in main vessel flow on the different numbers and locations of perforators, as well as assessing skin perfusion in all zones of the flap with LDF technology. Additionally, the effect of a short period of ischaemic preconditioning will be examined, on the variables of flow and perfusion. This will not only characterise any differences produced on main vessel flow on the different perforator combinations, but also assess whether a brief period of clamping intra-operatively may benefit flow and perfusion of the flap.

3.2 The effect of perforator number and location of the perfusion of fat and skin of zone IV of DIEP flaps

This study aims to establish the effect of different numbers and locations of perforating blood vessels on the perfusion of zone IV of a DIEP flap in-vivo, by examining the skin and fat perfusion using indocyanine green near infrared video angiography (ICG-NIR-VA) technology, using a Novodaq® system called a SPY scanner. To ensure accuracy of the skin perfusion data, a laser Doppler image (LDI) of zone IV of the flap will also be performed, as this technology is well validated in the assessment of blood perfusion in free flaps. SPY scans will be performed on the fat and skin of zone IV of DIEP flaps, isolated on different perforating vessels, and will be compared statistically to assess any differences. Any differences demonstrated may have implications on future flap planning, as if two perforators convey no extra benefit, then dissection time and muscle/nerve damage could be limited. Alternatively if one vessel is poorer than two, then surgeons requiring a larger flap may choose to take two perforators to include more of zone IV reliably.

Part II: Materials and Methods

The research projects discussed in this thesis are presented in separate chapters that provide details of the materials and methods used for each project. This section is provided to clarify some of the scientific details of the methods used in the following chapters, to allow brevity and clinical detail in their relevant sections.

Chapter 4: Laser Doppler Imaging (LDI)

4.1 Principles

LDI technology utilises the Doppler principle to measure blood flow in the skin; laser light directed towards a target area is scattered by the tissues, reflected and then detected by a receiver. Laser light reflected from moving objects, such as cells within blood, undergoes a frequency shift, unlike the light reflected by the static tissues surrounding it. This shift can be detected by the receiver, measured in relation to the static objects and translated into a waveform as a means of estimating blood flow in vessels and gives an indication of tissue perfusion in the skin (Stern. 1975). Stern (1975) was the first to demonstrate this principle to measure skin perfusion in humans, using a 633nm Helium-Neon laser(Stern. 1975). Laser Doppler systems frequently use visible red or near infrared light wavelengths, as these provide maximum light reflection from the blood cells, are poorly absorbed by melanin and penetrate deeply enough to identify blood flow in the dermal layers of the skin. The use of green light wavelengths has also been described, though are less commonly utilised in laser Doppler systems and there is evidence that the green light wavelengths do not identify venous flow elements as accurately as red wavelengths (Murray et al. 2004). This technology was extrapolated to design laser Doppler flowmetry (LDF), to assess blood flow and tissue perfusion over a single point and has been widely used in different experimental and clinical situations to measure changes in vascular circulation.

4.2 Laser Doppler Flowmetry (LDF)

The use of LDF most relevant to plastic surgery is the ability to assess tissue perfusion in situations where it is in question, such as the estimation of burn depth or the assessment of viability of tissues such as skin or composite flaps. The use of LDF in burn depth, to detect the presence or absence of blood flow in burned skin, has been well validated and shown to correlate accurately with histological analysis (Micheels et al. 1984b, Micheels et al. 1984a). LDF measurement of cutaneous blood flow, and its application in free flap monitoring and assessment is well recognised and utilised in clinical practice (Jones and Mayou. 1982, Smit et al. 2010, Yuen and Feng. 2000, Heller et al. 2001, Jones and Greenhalgh. 1983, Soderstrom et al. 1999). The flowmeter probe is held against the tissue to be assessed and a wavelength produced is converted into an audible sound wave, which can be compared for assessment of blood flow. Clinicians have highlighted the fact that this is a relatively difficult variable to interpret and

compare, and have devised computerised methods of converting the sound flow into a digital signal that can be quantified (Soderstrom et al. 1999).

4.3 Laser Doppler Imaging (LDI)

LDI was designed by Essex and Byrne in 1991, where laser light (visible red or infra-red) is scanned over an area of skin at a set distance from the target using a continuous scanning technique to provide multiple-point Doppler flow measurements over an area of tissue (Essex and Byrne. 1991). This technique provides a more reliable indication of vascular perfusion over a region of tissue, by taking many readings and thereby minimising the effect of erroneous or anomalous results generated by particularly high or low 'spot' readings within the area of tissue to be assessed (Choi and Bennett. 2003). In addition, the use of the remote scanning system positioned at a set distance from the tissue avoids the need for skin contact, reducing the chances of contamination of the area and compression of smaller skin capillaries, which can affect the readings obtained. This is particularly useful in the intra-operative situation, where sterility of the surgical field is paramount. Computer software available with the LDI systems provide perfusion measurements for the area of skin which is scanned, which are termed 'flux' measurements of perfusion and are given as values in arbitrary units. The LDI machine generates a visual image of the area scanned in colour, reflecting the perfusion or 'flux' of the area and can be analysed and compared using the available software. LDI scans have been used for various assessments of flap surgery, from detecting perforator vessels near the skin pre-operatively, perfusion of the flaps intra-operatively and monitoring of flaps post-operatively (Komuro et al. 2002, van den Heuvel et al. 2011, Ulusal et al. 2006, Hallock. 2001).

Criticisms of the LDI systems include the need for the area being scanned to be kept completely still during the measurements, as any movement can affect the ability of the scanner to detect the light reflected and therefore resulting perfusion measurements. Further disadvantages are introduced if the area scanned is not uniformly flat, as significant curvature in the scan area will affect the reflection of light to the receiver and thus the measurements obtained. Finally, a controlled environment in which to perform the scan is desirable, as the effect of any atmospheric ambient light could affect the values obtained, as the reflection of this light could be detected by the receiver also and falsely interpreted as higher flux measurements (Fullerton et al. 2002). The effect of temperature on any system measuring cutaneous blood flow is significant, and the maintenance of stable ambient temperatures between 20-25 degrees Celsius in a draft-free environment is recommended for measurement conditions(Bircher et al. 1994).

4.4 Project specifications

4.4.1 Machines and Software

Two different types of laser Doppler system were used in this research:

Human study

A Laser Doppler Imaging Scanner LDI2-IR (Moor Instruments, Axminster, Devon, UK) was used in this study to non-invasively measure blood flow to the skin of zone IV of the DIEP flap intra-operatively before division of the pedicle and tissue transfer. This system utilises a near infrared 733nm diode laser, produces a colour image of the scan area in terms of flux and takes a digital photograph image of the scan area at time of scanning to allow comparison of specific sites of the flap with the perfusion image obtained. A picture of the imager is shown in Figure 4.1.

Figure 4.1: Moor Laser Doppler Imaging scanner (Image permission granted by Moor Instruments Ltd. 2012)

This system was serviced and calibrated by Moor in August 2009, immediately prior to the commencement of this study. The scans were performed at a distance of 29cm from the patient on the large scan setting of 4 milliseconds/pixel. The software used to analyse the scans was Moor BDA software version 5.3 and could employ a 'region of interest' function, where specific areas of the tissue could be highlighted and analysed independently from other parts of tissue included in the scan, and this area copied between scans to allow for direct comparison of specific scan areas.

Animal study

A Moor VMS-LDF2 Laser Doppler Flowmetry system was used in this study to analyse skin perfusion in all zones of a cranially-based abdominal epigastric perforator flap created in a rat model as an analogue for a human DIEP flap. This system utilises a near infrared 785nm diode laser emitted from two optical probes, placed on the skin of the flap using a plastic flange with a disposable adhesive sticker to hold the probe in perpendicular contact with the skin. An image of the system is shown in Figure 4.2.

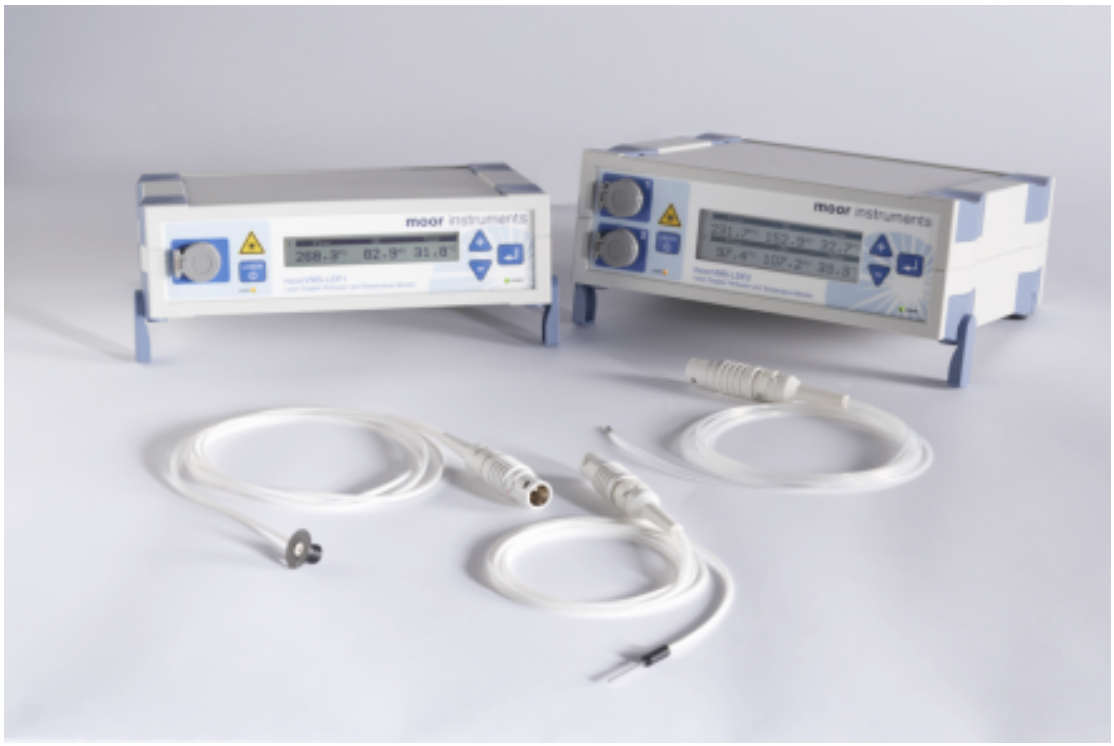


Figure 4.2: Moor Laser Doppler Flowmeter dual channel with temperature monitor and optical probes (Image permission granted by Moor Instruments Ltd. 2012)

This sytem was provided by Moor and calibrated prior to its use in this study in December 2010. The software accompanying this system was Moor-VMS-PC for Windows version 3 and recorded the flux values for each optical probe in flux perfusion units (PU) and the temperature of the skin at each probe position during recording. The system also has two analogue outputs to allow the data to be recorded simultaneously into different data acquisition systems, such as the Chart software for Macintosh described in Chapter 7. This allowed for duplication of data recording on two systems for accuracy and also simultaneous capture of flow, core temperature and perfusion

measurements. As recommended by manufacturer guidance in this experiment, the analogue outputs were set at 2.5V = 1000PU, therefore the minimum perfusion achievable was 0 PU and the maximum perfusion achievable was 1000 PU.

Chapter 5: Indocyanine green near infra-red video-angiography (ICG-NIR-VA)

5.1 Principles

Indocyanine green (ICG) angiography has been used in the field of ophthalmology for assessment of choroidal blood vessels since the early 1970's (Flower. 1973), though more recently it has been used for investigation of cardiac, neurological and reconstructive situations. The technology is based upon the principles of the fluorescein technique, which has been used in clinical practice since 1942 (Lange and Boyd. 1942) and uses detection of ICG fluorescent dye within blood vessels to assess blood flow. Raabe et al. first demonstrated the use of ICG-NIR-VA for the intra-operative assessment of vascular flow in their study of cerebral circulation in 2003 (Raabe et al. 2003). This technique has been compared with the gold-standard of x-ray angiography in an animal study by Matsui et al. and found 100% correlation between the imaging modalities, providing a measure of criterion validity for the ability of ICG-NIR-VA to detect perforating blood vessels (Matsui et al. 2009) supporting previous clinical studies in humans which found this to be the case (Azuma et al. 2008).

ICG is a fluorescent dye that is injected systemically and binds within a few seconds to intravascular plasma proteins (principally albumin) and has strong absorption spectra in the infrared wavelengths, with maximum absorption at 805nm. It remains in the intravascular space, with no transfer to interstitial fluid, allowing the vessels themselves to be assessed without any interference from surrounding signal. In these characteristics the advantages of ICG over fluorescein are several; the use of light from a longer wavelength (800nm vs. 350nm) allows deeper penetration of the tissue and imaging blood vessels in the deep dermis and the subcutaneous fat. Additionally the strong binding to intravascular proteins means that the dye is swiftly eliminated allowing for repeated measurements at short intervals, whereas fluorescein takes 12-18 hours to clear (Still et al. 1999).

To utilise ICG-NIR-VA technology and visualise ICG fluorescence in tissues an imaging system is required, which consists of an optical head on an articulating arm emitting laser light at a frequency of around 800nm. The light is absorbed by the ICG in the blood vessels of the tissues hit by the light and induces fluorescence, which is detected by a near infrared sensitive video camera inside the optical head, to give real-time video images of tissue perfusion.

Holm et al. (2002) in their prospective clinical study of 20 free flap procedures performed ICG-NIR-VA scans at the time of operation and flap inset and found a strong correlation between the findings of the scan and the clinical outcome (Holm et al. 2002a). Other studies have found it superior to clinical assessment in the intra-operative period for prediction of flap loss (Mothes et al. 2004). The technique has also been used to aid decision-making regarding skin viability in traumatic injuries such as de-gloving or crush injuries (Kamolz et al. 2006). More recently surgeons performing DIEP flap procedures have utilised the perfusion images obtained with ICG-

NIR-VA images intra-operatively to assess which blood vessels supply flaps more reliably and to aid decisions regarding which perforating vessel to choose (Francisco et al. 2010).

5.2 Project specifications

5.2.1 Machine and Software

The ICG-NIR-VA imaging unit used in this research was a SPY imaging system, loaned to our unit by Cardiologic, the UK face of Novodaq® (Novodaq Technologies Inc., Concord, Ontario, Canada) that developed the SPY machine. Also available was SPY-Q analysis software, which analyses and compares fluorescence in the image sequences captured. Using the software, the saved images can be analysed using perfusion algorithms to allow quantitative comparisons using levels and markers of perfusion in arbitrary units. A picture of the apparatus is shown in Figure 5.1.

Figure 5.1: SPY imaging system for indocyanine green near infrared video angiography (Image permission granted by Novadaq Corp. 2012)

This system was a year old, and had been calibrated by Cardiologic prior to its use in this study in August 2009.

5.2.2 Indocyanine green dye

ICG is a sterile nontoxic tricarbocyanine dye. Dosing regimens for ICG differ depending upon the tissue that is to be the focus of the vascular measurements. For flap perfusion measurements, a dose of 10mg per scan is recommended by Cardiologic, which is injected intra-vascularly via a central or peripheral line and is a dose used by other free flap studies using ICG-NIR-VA (Pestana et al. 2009), in line with the 0.1-0.2mg/kg dose suggested by earlier research (Cigna et al. 2010, Still et al. 1999). The UK Pharmaceutical Summary of Product Characteristics recommends that adult daily dosages should not exceed 5mg/kg. It is contraindicated in patients with hypersensitivity to iodine or sodium iodide and patients with increased thyroid activity such as hyperthyroidism or autonomic thyroid adenomas, though adverse reactions to ICG have been evaluated and reported as very low (Obana et al. 1994, Hope-Ross et al. 1994). It has a plasma half-life of 150-180 seconds, allowing it to be eliminated from the system and repeat measurements to be recorded within short periods of time.

The dye used in this study was ICG-Pulsion dye 5mg/ml in a powder form and reconstituted with water for injections immediately before the scan was performed.

5.2.3 Measurements

Depending on the central or peripheral route of dye injection, a period of time is allowed to elapse after which a measurement capture period is started which is 10 or 20 seconds respectively, as per manufacturer guidance. The camera captures fluorescent images from the tissue over a period of 38 seconds, after which the image is saved and can be reviewed at a later date. A time minimum of 10 minutes is allowed between injections and measurements to allow for dye elimination and the start of another image capture, similar to previous studies(Pestana et al. 2009).

Chapter 6: Measuring intra-vascular flow

6.1 Principles

Intravascular flow can be measured in different ways. The traditional use of clearance techniques has been well validated, by using markers that can be measured in blood and urine. However, these techniques are not appropriate to measure frequent changes in blood flow due to the time taken to process samples and clear markers. The use of electromagnetic flow meters (EFM) has been described and used in many experimental designs. This technology utilises Faraday's law of electromagnetic induction, where a magnetic charge is applied to an insulated non-magnetic steel flow tube, creating a potential difference proportional to the velocity of flow. However, reports of large error margins and fluctuating baseline flow measurements of up to 20mL/min have been reported(Nakayama. 1984), and in experiments involving low-flow models, this was not acceptable.

The most successful recent development in the measurement of intra-vascular flow is the ultrasonic transit-time flow meter, which measures the difference in transit time of ultrasonic pulses going in the same direction and the opposite direction of blood flow. The difference in these transit times creates an estimation of the volume of flow in the direction of the ultrasonic pulses. Each transducer emits an ultrasound beam in turn, which is reflected by the reflector to the other transducer, which receives the signal. This process is illustrated in Figure 6.1, showing a blood vessel within the body of a flow probe, as is performed during the measurements of flow.

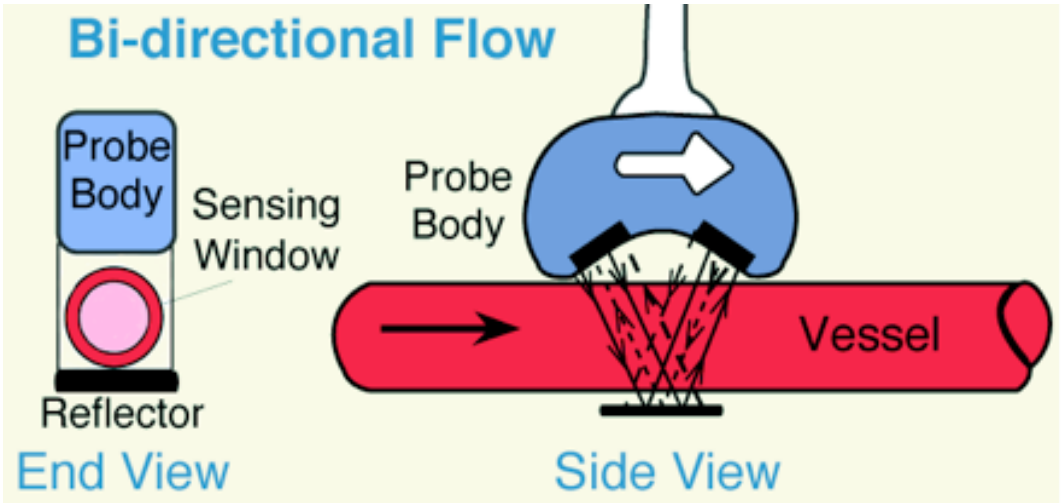


Figure 6.1: A blood vessel within a transit time flow probe (Image permission granted by Transonic Systems Inc. 2012)

The advantages of ultrasonic transit-time technology over the electromagnetic measurements are multiple; they have excellent zero stability which negates the need to clamp vessels to get a true zero reading, directly measures the volume rate of flow without the need to calculate across cross-sectional area or assume rotational symmetry of flow and the non-constrictive nature of the probe design minimises vessel spasm. In addition there is no direct electrical

stimulation and transit time flowmetry is not dependant on electrically charged molecules. The transit time technology has been well validated in the measurement of blood flow in rats, at different flow rates and haematocrit levels(Welch et al. 1995) and different anatomical sites(Shimura. 1986).

The flow probes are designed in different sizes for different calibre vessels, so as to contain the vessel diameter completely within the reflector, which constitutes the head of the probe. The size of the probe for the vessel is important, as the fixed distance of the reflective pathway is crucial to the accurate measurement of ultrasonic transit time and therefore flow.

6.2 Project specifications

6.2.1 Machine and Software

The flow probes used in this experiment were Transonic V-series microcirculation flow probes (Model 0.5V, Transonic Systems Inc., Ithaca, NY, USA. http://www.transonic.com), which are pre-calibrated flow sensors for use in small diameter vessels of 0.25-0.5mm with great precision. As the perforating vessels being used for measurements (rat epigastric vessels) are between 0.45-0.5mm(Zhang et al. 1993), this was appropriate. An acoustic couplant is required to transmit the sound, such as ultrasound gel, water or saline. To allow stable measurements to be taken a micromanipulator with a clamp is used to hold the probe in a stable position whilst recording flow, and the probe has a stainless steel handle to allow this. Scale is set at roughly 2.5mL/min for average flow volumes, but the probes can record between 0-50mL/minute of flow accurately. The probes have a zero offset of 0.2mL/min and a relative accuracy of +/-3%. These probes connect to the transit-time ultrasonic flowmeter (T204, Transonic Systems Inc.) that measures the signal produced from the probe and converts it into analogue data with a mean estimation of the flow in mL/min produced on a digital display at the front of the machine. The machine allows a low-flow setting to be selected, to correlate more accurately with the smaller probes and gives a volume flow measurement every 0.0025 seconds. The analogue data outputs on the flowmeter are connected to the PowerLab® 400 system (AD Instruments Pty Ltd), which converts the analogue signal to a digital signal. This PowerLab system is then connected via a USB lead to a Macintosh computer, where with the appropriate software, the data can be recorded. A photograph of the flowmeter, PowerLab® system and Moor LDF unit is shown in Figure 6.2.

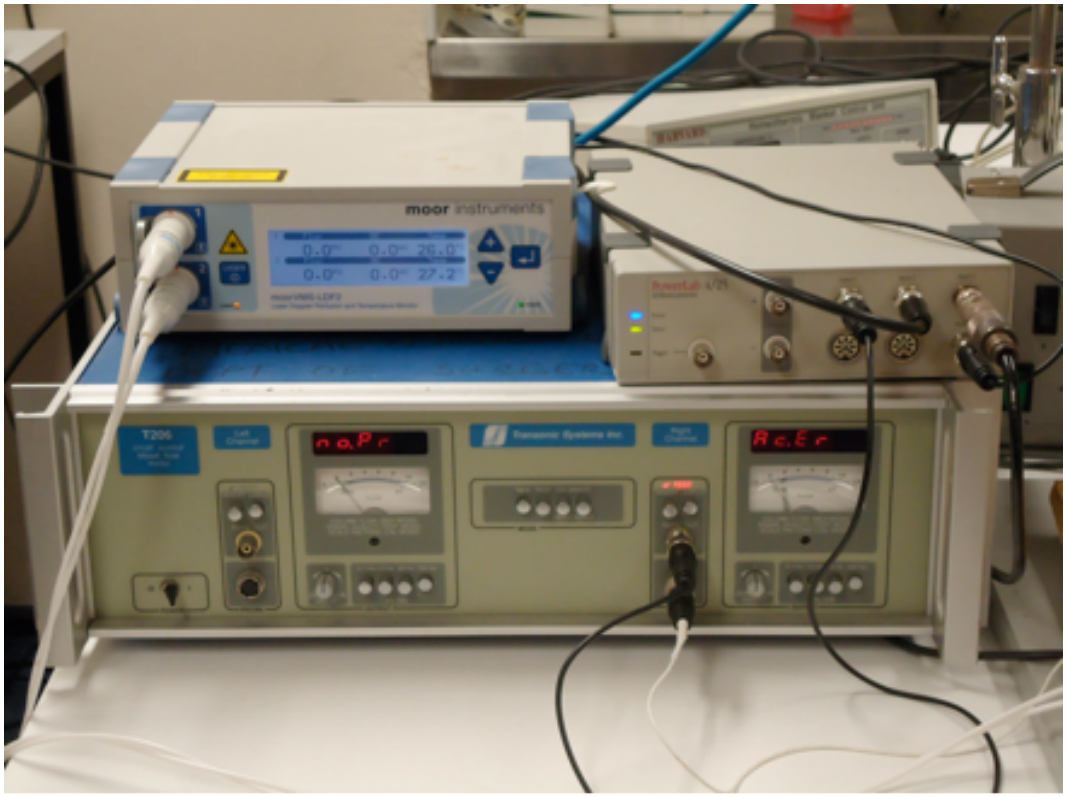


Figure 6.2: Operational set-up of machines for recording of flow and flux in the animal model

The same micro-vascular flowprobe was used throughout this study and was factory calibrated in 2008 at purchase. The flowprobe underwent bench calibration for received signal and zero offset using the flowmeter prior to the start of this experiment in December 2010. The flowmeter itself was calibrated before use in a previous experiment in 2003. Manufacturer guidelines recommend yearly service and calibration, but state no critical calibration period exists.

The software used in this study was Chart® version 5.5.6 for Macintosh (MLS013/M) via AD Instruments. This is an application program that allows the researcher to use PowerLab as a multi-channel recorder of digital data. The individual channel analogue data, processed through PowerLab, can be calibrated and a 'set-up' template of Chart created for the specific project being undertaken and recorded to masure flow and calculate the pulsatility index. As mentioned previously, the flexible functionality of PowerLab and Chart means that analogue data from the LDF machine could be integrated to the Chart window, to allow consistent and simultaneous analysis of the flow, perfusion and temperature data. In addition, the core temperature readings were recorded via Harvard apparatus machinery, which was calibrated to the PowerLab and Chart software prior to use, to allow concurrent measurements.

Part III: Results

Chapter 7: The effect of perforator number, location and ischaemic preconditioning on the total pedicle vascular flow and flap skin perfusion of epigastric abdominal perforator flaps in a rat model

7.1 Principles

The use of a rat perforator model as an analogue for human perforator flaps has been frequently utilised in research. Rat perforator flap models have been suggested for training purposes and indeed are used in live animal microsurgical courses(Kayano et al. 2010). However, some significant differences exist between the vascular dominance of the epigastric vessels in humans and rats. In humans, the deep inferior epigastric vessels arise from the external iliac vessels, just above the inguinal ligament. These vessels travel under the rectus abdominis muscle to anastomose with the smaller superior epigastric vessels, before continuing as the internal thoracic vessels under the ribcage. In the rat model, the epigastric artery follows the same anatomical pattern in terms of its origin and destination, however whereas in the human the inferior epigastric vessel is dominant (larger and more branches), in the rat model, the superior, or cranial, segment of the vessel is dominant. This can be seen by the rat injection study image in Figure 7.1 alongside the human analogue.

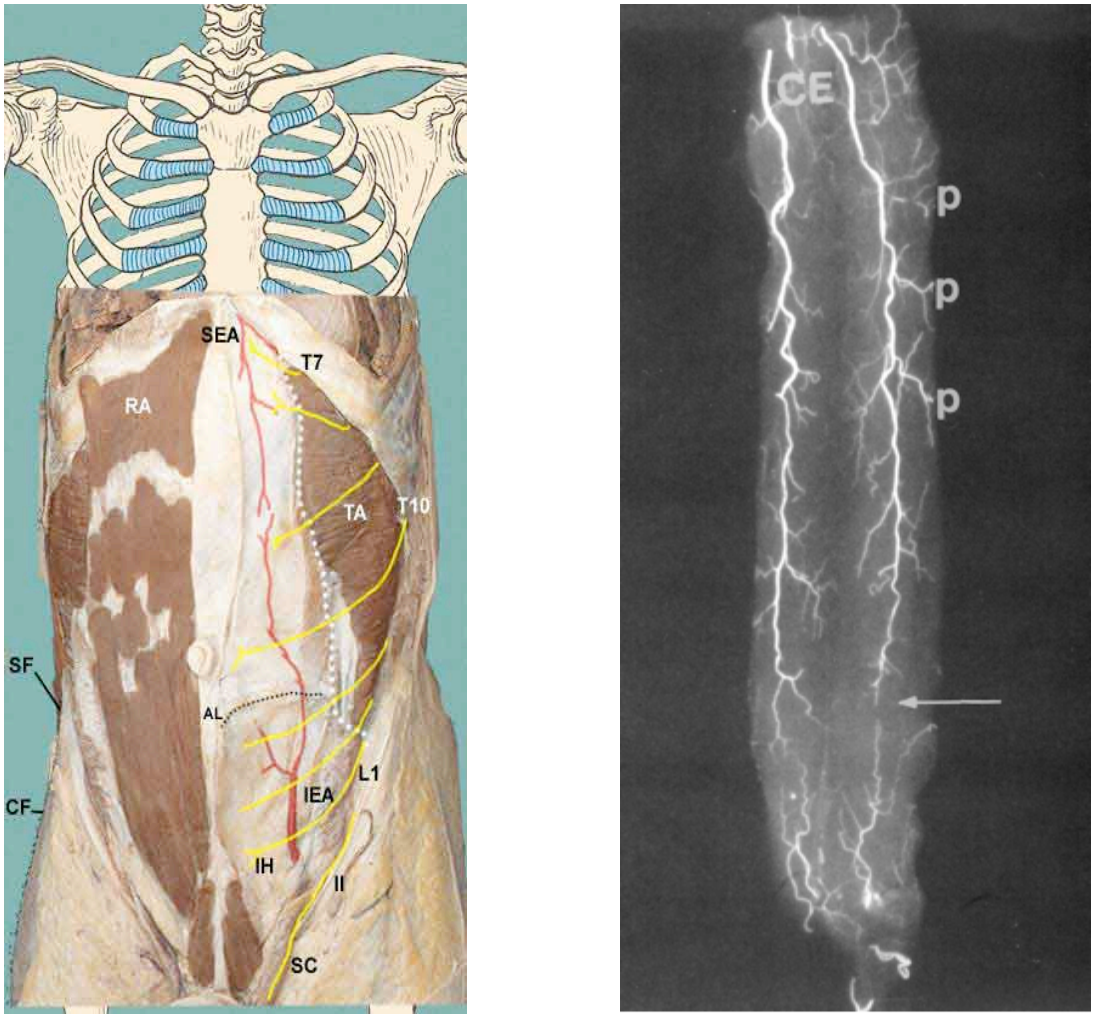


Figure 7.1: Epigastric vessel anatomy displayed in a human (left) and delineated in an injection study in a rat (right) displaying caudal and cranial dominance of the vessels respectively (Image permission granted by Wolters Kluwer Health. Hallock.1995)

This difference in the vascular dominance means that any flap models in rats made to represent human deep epigastric flaps need to take into account the cranial dominance of the rat epigastric vessel physiology and be based cranially accordingly(Hallock and Rice. 2003, Hallock and Rice. 1995). The model does have limitations, however earlier concern regarding the importance of the anatomical differences, particularly the fascial layers of panniculus carnosus in the skin of rats and panniculus adiposus in humans, has largely been allayed, due to findings that both may co-exist in species(Hallock. 1995). Differences in the perforator distribution also exist; there are no different rows of vessels in the rat flap model as the main vessel does not routinely divide into medial or lateral rows as in humans. In addition, in the human model, though the perforators can indeed be divided into inferior and superior, the distribution of dominant perforators tends to be clustered around the peri-umbilical region, whereas in the rat model a vertical equidistant perforator distribution is observed from xiphisternum to pubis(Hallock. 1995). However despite these differences, the use of a rat epigastric perforator model for DIEP flaps has been accepted as a relatively robust true perforator model to study for flap research(Oksar et al. 2001, Ozkan et al. 2006).

Clinical experiments of epigastric flaps in Wistar rats have shown that the traditional Scheflan zones apply, with the central two zones exhibiting significantly less necrosis than the lateral two

zones(Doncatto et al. 2007). In their study of TRAM and DIEP flaps in rats, Hallock et al. (2001) compared flap survival and LDF in zones I and II in DIEP flaps based on multiple perforators (numbers of which were not stated in the study) versus a single dominant cranial perforator. They found no difference in flap survival or LDF readings between the multiple perforator flaps compared with the single perforator flap(Hallock. 2001).

7.1.1 Ischaemic preconditioning

The phenomenon of ischaemic preconditioning (IP) was first defined by Reimer and Murry in 1986, and describes the situation where tissue exposed to a brief period(s) of ischaemia followed by an episode of reperfusion, thereafter provides the tissue with increased resistance to subsequent ischaemic injury(Murry et al. 1986, Reimer et al. 1986). This concept is of particular interest in the case of free flap surgery, where during transfer of tissues, there will be an inevitable period of ischaemia before the vascular flow is established by microsurgical anastomosis. Some studies suggest that ischaemia-reperfusion injury (IRI) is the main cause of partial flap loss(van den Heuvel et al. 2009). Providing the tissues with an inherent defence mechanism by conditioning them to ischaemia before flap transfer would be advantageous. Theories regarding how this protection is achieved revolve around the increased blood flow to the oxygen-starved tissues after the conditioning and molecular mechanisms initiated to prevent oxygen free radical injury post-reperfusion, which have been demonstrated experimentally(Adanali et al. 2002, Pasupathy and Homer-Vanniasinkam. 2005a). It has also been shown that the effects of ischaemic preconditioning are seen when a site distant to the operation site is used for the occlusion, such as a limb, creating systemic protection for flaps in animal models(Pasupathy and Homer-Vanniasinkam. 2005a, Kuntscher et al. 2002). However, the transition of these findings to the human operating theatre has been limited, as most tissues are able to withstand short periods of ischaemia easily (1-2 hours for skeletal muscle), therefore surgeons primarily concentrate on reducing ischaemia time during operations to avoid the possibility of ischaemic injury(Pasupathy and Homer-Vanniasinkam. 2005b). With that in mind, it must be remembered that in the specific case of flap surgery, if an anastomosis is difficult or requires repeating, the ischaemia time can be unwittingly prolonged. In addition, whilst the transferred tissue will have re-establishment of vascular flow performed with microsurgical anastomosis, the vascular supply has inherently changed and is now reliant on one or two perforating blood vessels from a single pedicle, as opposed to the rich blood supply it had previously received. It is possible, therefore that in addition to the ischaemia time the flap will spend with no vascular supply, the actual process of free flap surgery will provide some level of ischaemic insult to parts of the flap tissue in terms of the modulation of blood flow.

Different tissues can withstand different periods of ischaemia, after which irreversible damage is apparent. These times for muscle, fat and skin have been quoted as 4, 13 and 24 hours respectively(Blaisdell. 2002). Therefore it would seem that as DIEP flaps do not take muscle, the ischaemia time would be less significant. However, a small number of studies have shown that flaps of different composition have different reactions at a metabolic level to ischaemic injury

and reperfusion(Röjdmark. 2002) and some studies have suggested that fat is more sensitive than previously thought to ischaemic injury than other tissues(Paschen et al. 1986, Nishikawa et al. 1992). If true, this could perhaps explain the observed differences in flap fat necrosis when skin integrity is maintained.

Ischaemic preconditioning has been shown to increase blood flow and flap survival in animal models of skin and muscle flaps(Zahir et al. 1998a, Kinnunen et al. 2002, Coskunfirat et al. 2006, Shah et al. 2009). Patterns of ischaemic preconditioning can include one longer period of ischaemia, or several short periods of ischaemia, followed by reperfusion(Wang et al. 2008, Kinnunen et al. 2002, Coskunfirat et al. 2006). The time periods of ischaemic preconditioning to which tissues are subjected in the literature varies in the literature from 5 minutes to 60 minutes(Wang et al. 2008). Research suggests that cycles of ischaemia are more effective the longer they are and the more cycles performed(Zahir et al. 1998b). However, it must be remembered that in a theatre setting time is limited, and the operation cannot be prolonged more than can be justified.

To explore the differences in flap perfusion when varying the number and location of the perforating blood vessels supplying the flap, a rat epigastric perforator model was proposed to observe the effect of different numbers and locations of perforating vessels on the main pedicle flow and the perfusion of the skin in the different zones of the flap. The additional factor of subjecting the flap to a short period of ischaemic pre-conditioning was also proposed to gain as much information from the flap model as possible. As research equipoise seems to exist in the literature regarding the most effective ischemia time to apply in either single or repeated periods of ischaemic preconditioning, a single period of 15 minutes was selected, followed by a 15-minute rest period to allow effects of any ischaemic preconditioning to occur. This time selection, in the absence of any strong evidence to suggest other action, was chosen as it is a time period that could coincide with a natural comfort break in theatre without significantly extending the operation time.

Aside from ischaemic preconditioning, when modulating blood flow in a flap, such as sequential clamping of vessels, a period of vascular stabilisation needs to occur, where the clamped vessel no longer admits blood to the region it supplies, leaving the flap based on any unclamped vessel. Additionally, when a vessel is un-clamped, a period of time needs to be observed to allow that vessel to overcome any spasm and to re-perfuse its region of tissues reliably again before any assessment of that vascular territory can commence. This auto-regulation of blood flow after any occlusion is termed ‘reactive hyperaemia’, an inherent mechanism to control local blood flow and increased the perfusion of tissues many times above normal following any reperfusion(Guyton. 1996) to repay any oxygen deficit accumulated in the tissues(Ganong. 1997). A recent study by Tollan et al (2012) investigated the time required for this period of stabilisation and reperfusion to occur using DIEP flaps raised in different perforating blood vessels, clamped for varying periods of time and assessed using LDI scanning(Tollan et al. 2012).

The authors found that a period of 5 minutes was sufficient to overcome the period of hyperaemia and allow reliable reperfusion to occur to perform any measurements.

7.2 Materials and Methods

7.2.1 Study plan

In the approach to this project and before planning the statistical design, a study plan was formulated summarising the elements to be investigated in the animal model. An unknown number of animals were required, in all of which total pedicle flow and perfusion of the flap was to be measured on different perforator vessel combinations. Although this was the primary objective, to gain the maximum information from these subjects, the effect of ischaemic preconditioning on total pedicle flow and flap skin perfusion was also examined. Therefore the following flowchart was designed upon which to style the experiment.

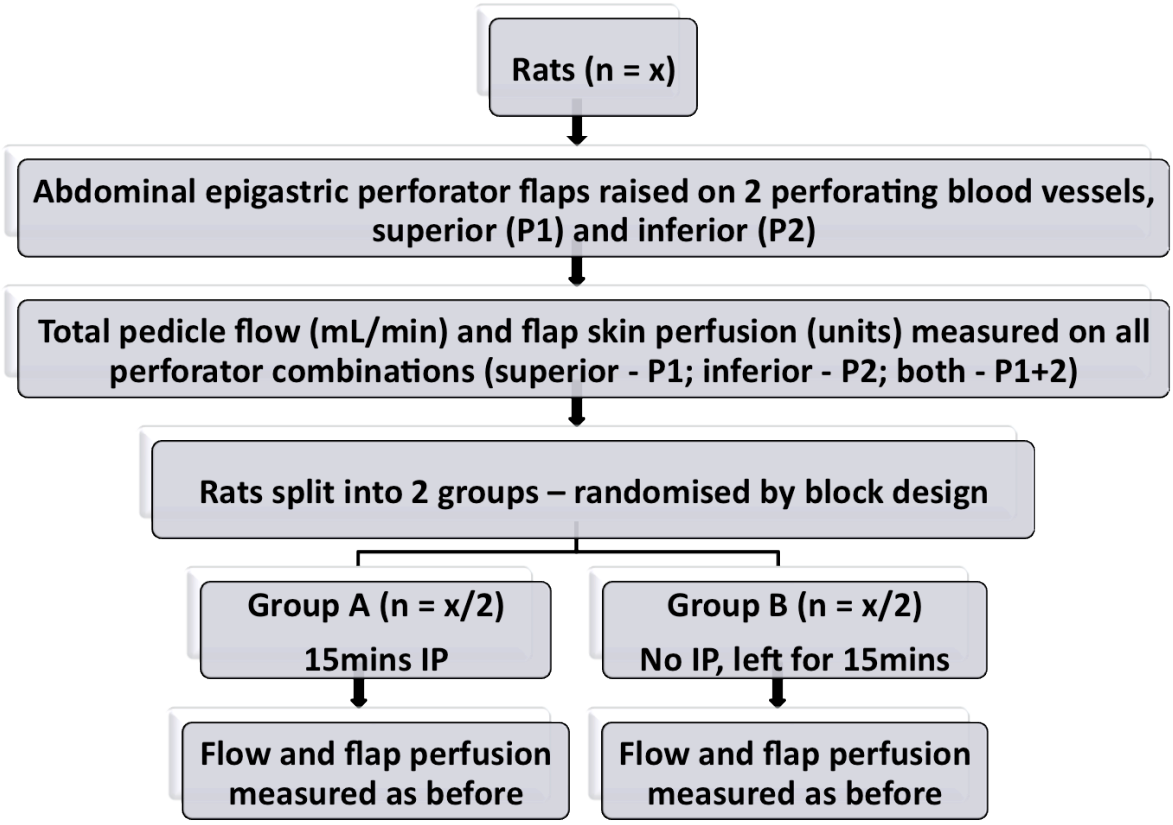


Figure 7.2: Flowchart of the study design of the rat epigastric flap model study

7.2.2 Statistical design

Sample size

To estimate the sample size required for this study, a statistician allied to animal research and the Home Office was consulted. A formal power calculation was not feasible due to inadequate knowledge of perforator vessel blood flow or reported rat flap LDF tissue perfusion in the literature, making standard deviations difficult to calculate. In most pilot studies to determine

these standard deviation estimates, a minimum of ten animals is required. A resource equation was therefore calculated using the following equation, in line with Home Office guidance:

E = N (number of animals per treatment group x number of treatment groups) – T (number of treatment groups)

Using this equation, E should be between 10-20, as stated in the NC3R's guidance (www.nc3rs.org.uk/category.asp?catID=7). This equation is used for smaller, more complex experiments where analysis of variance is likely to be used to analyse the data. A value of E<10 is likely to have inadequate numbers of animals, whereas a value of E>10 may be too large and waste animal numbers, introducing ethical issues to the project. Applying this to the data from the flowchart, where there are only two treatment groups (ischaemic preconditioning (group A) versus no ischaemic preconditioning (group B)) a treatment group size of ten animals was proposed and entered into the equation as follows:

E = (10 x 2) – (2) = 18.

Where N = total number of subjects (e.g. individual animals or groups/cages of animals) and T = the number of treatment combinations, E (the sample size) should be approximately between 10 and 20. Therefore the number of 20 rats in total (10 in each group A and B) was decided upon.

Randomisation

The next part of the design to be addressed was the randomisation of the animals, which was performed as restricted block randomisation with time as the blocking factor. The potential animals were numbered 1-20 and divided into blocks of four. Remembering that group A represents the ischaemic preconditioning group, and group B the non-ischaemic preconditioned group, any single block of 4 animals would have either the pattern of A B B A, or B A A B. The block pattern for each of the 5 blocks was decided using a random number generator, resulting in the following pattern of a 2x2 Latin square replicated 5 times:

B A A B; A B B A; A B B A; A B B A; A B B A

This ensured an equal number of A's before a B and equal numbers of B's before an A. This block design of randomisation was performed so as to keep the animals of a similar age and size. They could not all be ordered at once from the facility, as the operation and measurements would take the majority of a day and access to the facility meant a maximum of 2-3 animals could be operated on per week. Thus if the entire project took ten weeks, there would be animals at the end of the experiment whom were significantly older and heavier than those at the start. In addition, housing social animals such as rats on their own is ethically questionable due to the increased stress a rat faces in isolation. This meant ordering individual rats was not an option, and therefore each block of 4 rats could be kept in a similar environment for a similar time

before the experiments. Having a similar number of group A and group B at the start and end of the study means that uncontrollable factors which may bias results such as increased skill levels of the operator and reduced time for the operation would be spread evenly amongst both groups.

Allocation concealment

Concealed allocation of the experiment was performed by matching the animal number to the block sequence pattern of A's and B's shown above, with each being contained in a data file, to be opened only when the flap had been dissected and the event of ischaemic preconditioning, or no ischaemic preconditioning, was about to commence. This was performed to avoid any unwitting bias and differences in flap raising. In concordance with the advice from the statistician, this was felt to be the most realistic method to attempt to remove bias from the study design.

The anatomical side of the flap to be used, the order in which the perforating blood vessels would be assessed using clamps for the other vessels, and the order in which the perfusion of the zones would be measured with each optical probe were all randomised using a random number generator and concealed until the start of the operation in a data file as outlined above.

7.2.3 Animals

The animals used in this study were male out-bred Wistar strain rats, obtained from Harlan UK Ltd., Bicester, Oxfordshire, UK. All animals weighed between 295-350g. An out-bred strain of rat was used as these animals are larger with bigger blood vessels, making dissection easier and error less likely. The genetic variability of this out-bred strain was accepted, as performing microsurgery on smaller in-bred animals would be technically more difficult. This particular strain of animal was chosen under advice from the Named Veterinary Surgeon for Glasgow University animal research facilities, as Wistar rats are known to be easier to intubate than Sprague Dawley rats, and the use of intubational anaesthesia in the study was being considered. In addition, previous studies involving flow measurements had used Wistar rats successfully(Rickard et al. 2009b, Rickard et al. 2009a).

Ethical review of this project was conducted by the University of Glasgow Ethical Review Panel. The study was licensed by the UK Government Home Office under the terms and conditions of the Animals (Scientific Procedures) Act 1986 (Project License 60/4214).

7.2.4 Anaesthesia

All animals were weighed and underwent gaseous induction with isofluorane in a containment box. The first 4 animals underwent oro-endotracheal intubation with a modified 16G/45mm venous cannula using a trans-tracheal illumination technique. The following 16 animals received

a thiobutylbarbitole (Inactin) intra-peritoneal injection. This initial choice of anaesthesia and subsequent change was performed under guidance from the named Veterinarian in liaison with the project and reasons for this will be detailed in the results section.

All anaesthesia was maintained by an N_2O / isoflurane / O_2 gaseous mixture at 0.4-1%. In the case of the 4 intubated animals a rodent ventilator (Model No.7025, Ugo Basile, Comero, Varese, Italy. http://www.ugobasile.com) was used at 60 breaths per minute and a ventilator stroke volume of 4 – 5mL. In the non-ventilated animals, a nose cone was used, to which the animal was secured using tape to allow continuous anaesthetic maintenance through voluntary respiration. All animals received an intra-peritoneal injection of atropine at the start of the anaesthesia, to minimise endotracheal secretions.

The operating environment temperature was maintained at 21-24.5°C at all times. Heart rate and arterial oxygen saturations were measured throughout the experiment using a Nonin Model No 8500AV pulse oximeter attached to a hind limb (Nonin Medical, Inc., Plymouth, MN, USA. http://www.nonin.com) and were recorded at the start of the procedure and before the data collection interval.

Animal core body temperature was continuously monitored by a rectal probe, and maintained between 37.0 and 38.0 degrees centigrade by the use of a homoeothermic warming blanket (Harvard model 50-7061, Harvard Apparatus Ltd., Edenbridge, Kent, UK. http://www.harvardapparatus.co.uk) in the case of the first 4 animals, and by a radiant heat source for the last 16 animals. Temperature was recorded by analogue voltage output through an analogue-to-digital interface (PowerLab® 400, AD Instruments Pty Ltd., Castle Hill, NSW, Australia. http://www.adinstruments.com) linked to an Apple® MacBook computer (Apple Computer Inc., USA. http://www.apple.com) running Chart® v5.5.6 software (AD Instruments Pty Ltd.).

7.2.5 Experimental procedure

Animals were positioned supine below the operating microscope, and the abdominal skin shaved using fine electric hair clippers. The abdominal flap was drawn onto the abdominal skin using indelible pen, and the zones marked using the original Scheflan zone classification. Figure 7.3 shows an animal randomised to have a left-sided flap and the zones marked accordingly.

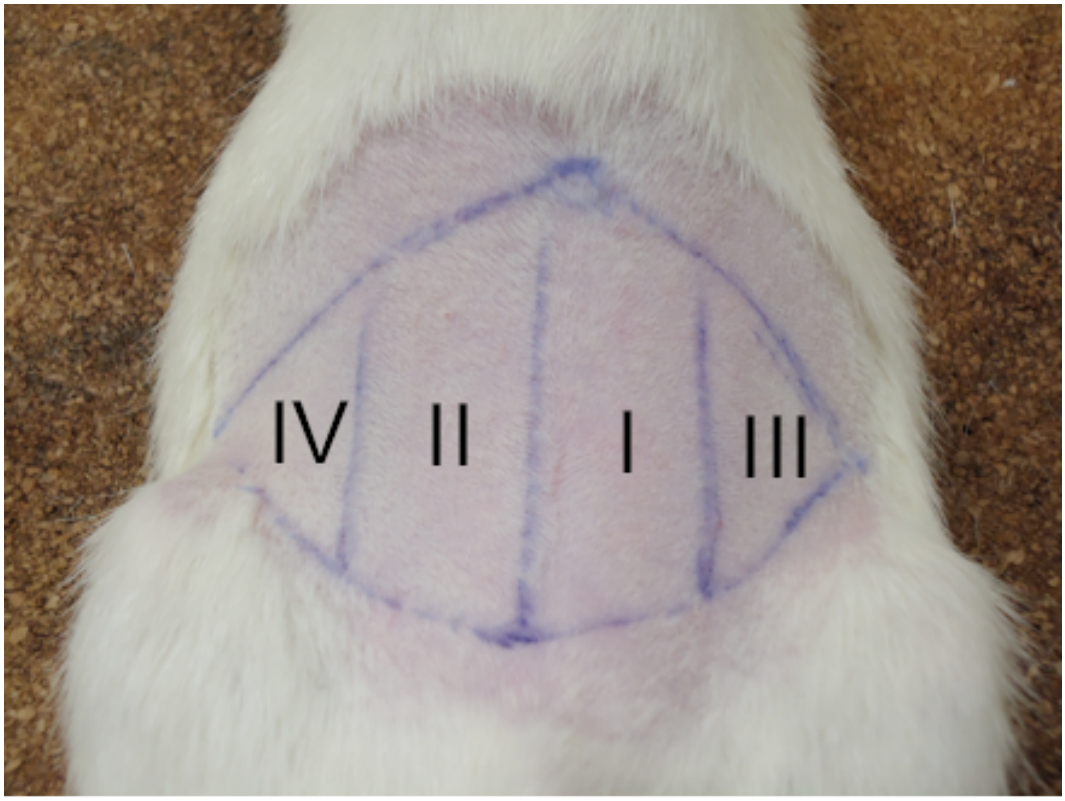


Figure 7.3: Scheflan flap zones marked on a left-sided abdominal epigastric flap

The flap was raised from lateral to medial, taking the subcutaneous tissue under the flap also. Dissection was performed using microsurgical scissors and any vessels encountered at the periphery of the flap before reaching the perforators cauterised using diathermy. The perforator row was identified, as shown in Figure 7.4, and the second and third cranial perforators (the most central to the flap) were chosen upon which to base the flap.

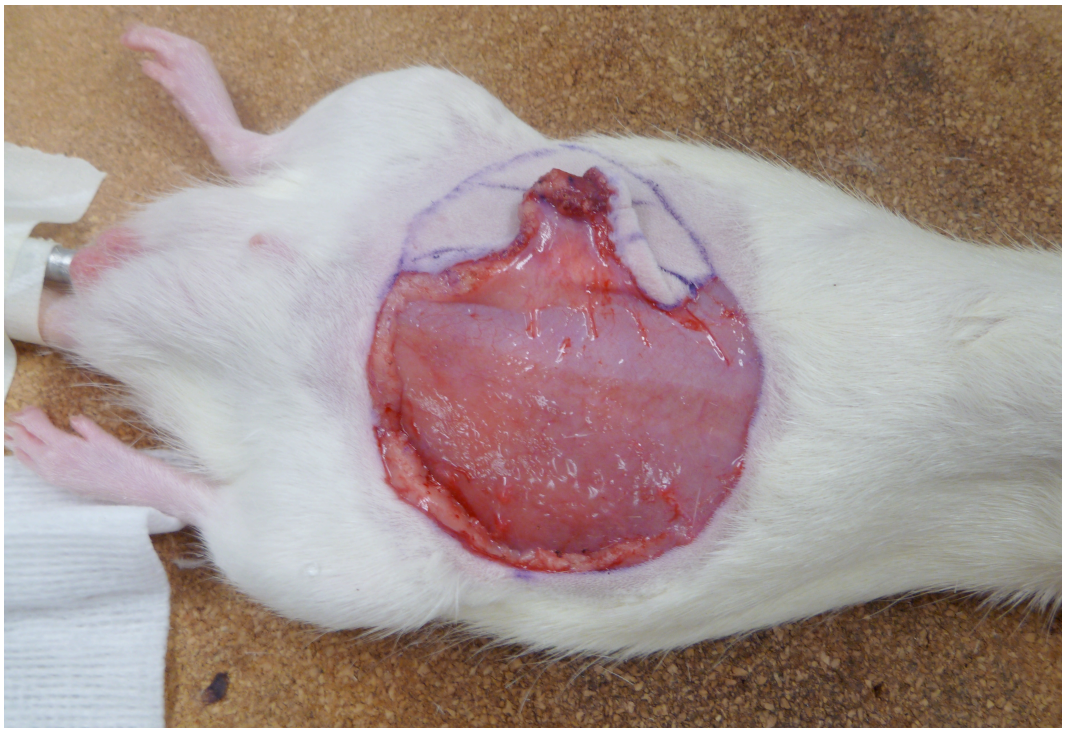


Figure 7.4: Right-sided abdominal epigastric flap with row of 4 perforating blood vessels displayed

These perforators were dissected in an atraumatic fashion using microsurgical instruments, making a linear cut in the rectus sheath to allow the main pedicle between the perforators to be dissected, and the distal end of the epigastric vessels ligated using a 9/0 nylon suture. A small amount of sheath and muscle was taken with the intramuscular perforator, to avoid trauma and skeletonisation of such a small vessel, however this tissue was minimised and any vascular connections within this tissue were cauterised using diathermy on a microsurgical setting. The flap was then dissected cranially up to the level of the xiphisternum, where the main vessels disappear under the ribcage, seen in Figure 7.5. The flap vessels were then carefully separated from each other and from the muscle, using sharp dissection and the rest of the flap raised, cauterising the contralateral perforators to ensure the flap was raised entirely on the pedicle.

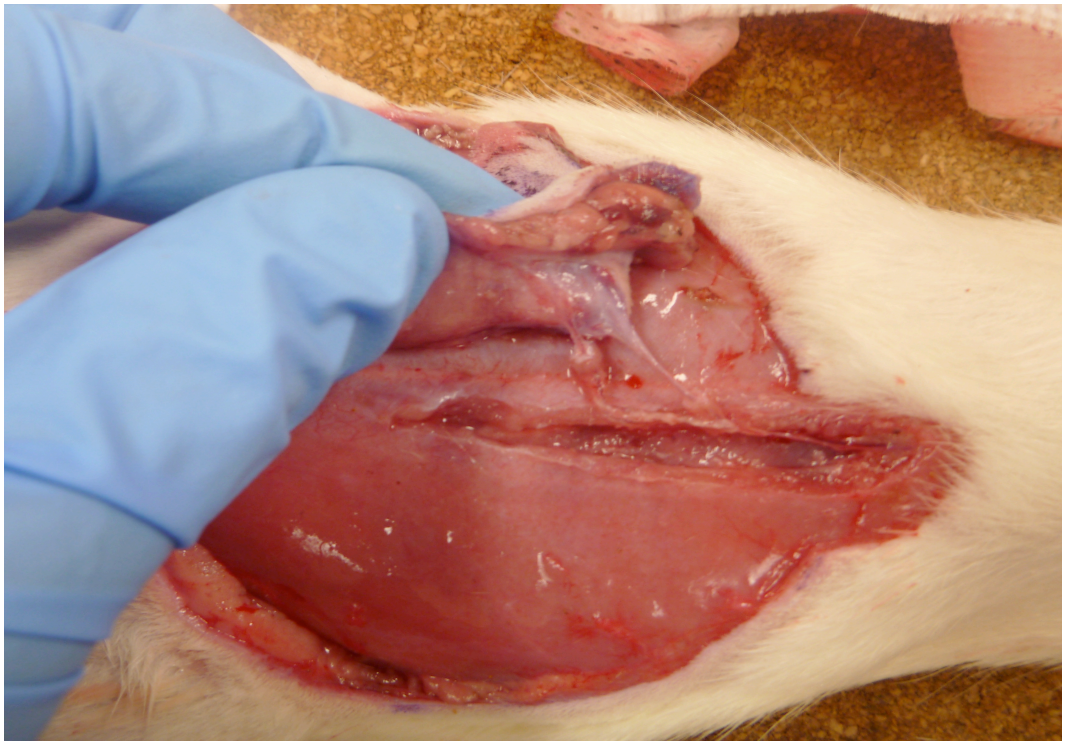


Figure 7.5: Right-sided abdominal epigastric flap completely raised on 2 perforators, dissected through the rectus sheath and muscle to the main pedicle (shown)

The flap was then sutured back into its anatomical position using a non-absorbable suture fixed at eight points and measurements of flow and perfusion taken as described below on the different combination of perforating vessels. After these primary measurements, depending on the group randomisation, the main pedicle was either clamped using a single Acland B1-V vessel clamp for 15 minutes, or left unclamped for the same period of time. After 15 minutes any clamp applied was removed and both groups were left for a further 15 minutes. The previous sets of measurements were repeated and recorded exactly as before.

7.2.6 Measurements

For measurements individual animal data files containing the randomised treatment group and perforator order were opened. If, for example, the randomised perforator order was superior perforator (P1), both perforators (P1+2) and inferior perforator (P2), then the following

sequence events are described. The inferior perforator was clamped using a single Acland B1-V vessel clamp and 5 minutes was then observed to allow the effects of reactive hyperaemia to stabilise and the flap to be based on the superior perforator (P1). Flow and perfusion measurements were taken and recorded and the clamp released, to allow the flap to be based on both perforators (P1+P2). After a further 5 minutes, the next set of flow and perfusion measurements were recorded and the superior perforator (P1) clamped, a further 5 minutes allowed to elapse, and final flow and perfusion measurements recorded. A photograph displaying the beginning of this protocol is shown in Figure 7.6.

Figure 7.6: Superior perforator clamped, with flap based on the inferior perforator

Flow measurements

Flow measurements were performed using the techniques previously described in Chapter 7. A transit-time ultrasound flowprobe (Model 0.5V, Transonic Systems Inc.) was placed around the main arterial pedicle of the cranially dissected epigastric vessels as proximally as possible. Normal saline, maintained at 37^0C in a waterbath, was used as the acoustic couplant. The flowprobe was held in place using a microclamp secured in a stand, to avoid artefact from movement during measurements. Flow rate was measured by the transit-time ultrasound flowmeter (T204, Transonic Systems Inc.) and converted to a digital signal by the PowerLab 400 (AD Instruments Pty Ltd.). Flow was recorded for a minimum of one minute. Time-averaged pulsatility index (PI) was calculated using Chart® version 5.5.6 for Macintosh.

Perfusion measurements

Perfusion was measured using the techniques previously described in Chapter 6.2.2 using a Moor VMS-LDF2 Laser Doppler Flowmetry system with two optical probes attached to the different

zones of the flap using adhesive circles of tape (provided by Moor Systems) on the underside of the plastic flanges holding the probes in position, shown below in Figure 7.7. All perfusion measurements (termed ‘flux’ measurements) were taken at the same time as flow measurements to ensure concurrent measurement accuracy. As the individual flap zones were so small, only one LDF reading could be taken from a flap zone at a time, therefore two readings were taken consecutively from each zone to try to increase the level of accuracy of the LDF measurements. As the skin of the flap was occasionally wet from the fluid couplant of the flow measurements, it was observed that if the flanges did not adhere tightly to the skin, ambient light could enter and affect the readings taken. To avoid this, if a secure seal was unable to be made with the small adhesive rings, a length of tape was placed over the probes to allow them to rest on the skin, taking care to ensure no pressure was exerted upon them, as this could affect readings. Perfusion readings were also taken over a time period of at least a minute, recorded directly onto a laptop via Moor-VMS-PC software for Windows version 3 and also analogue outputs (2.5V = 1000 PU) digitised by PowerLab 400 to allow import into Chart software as well.

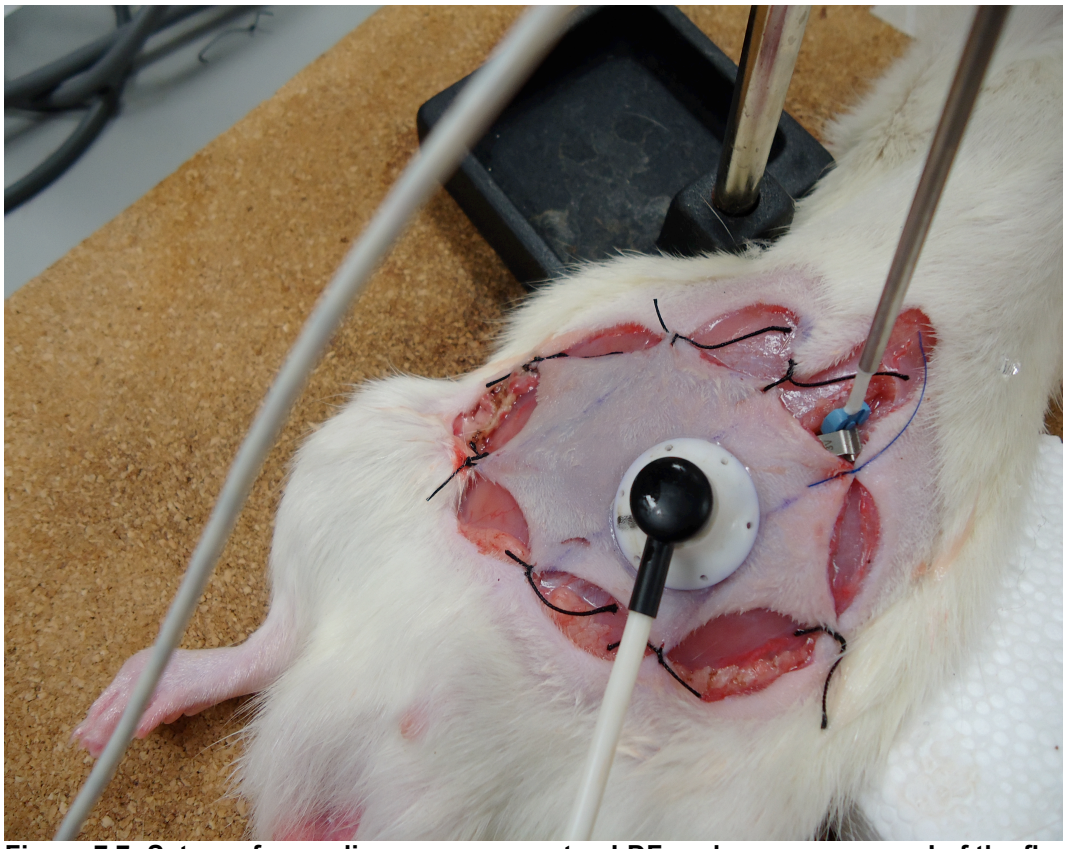


Figure 7.7: Set up of recording measurements - LDF probe seen on zone I of the flap and flowprobe in-situ measuring main pedicle flow, supported by the clamp seen at the top of the picture

7.3 Results

A table of animal characteristics, pre and intra-operative measurements is shown in Table 7.1.

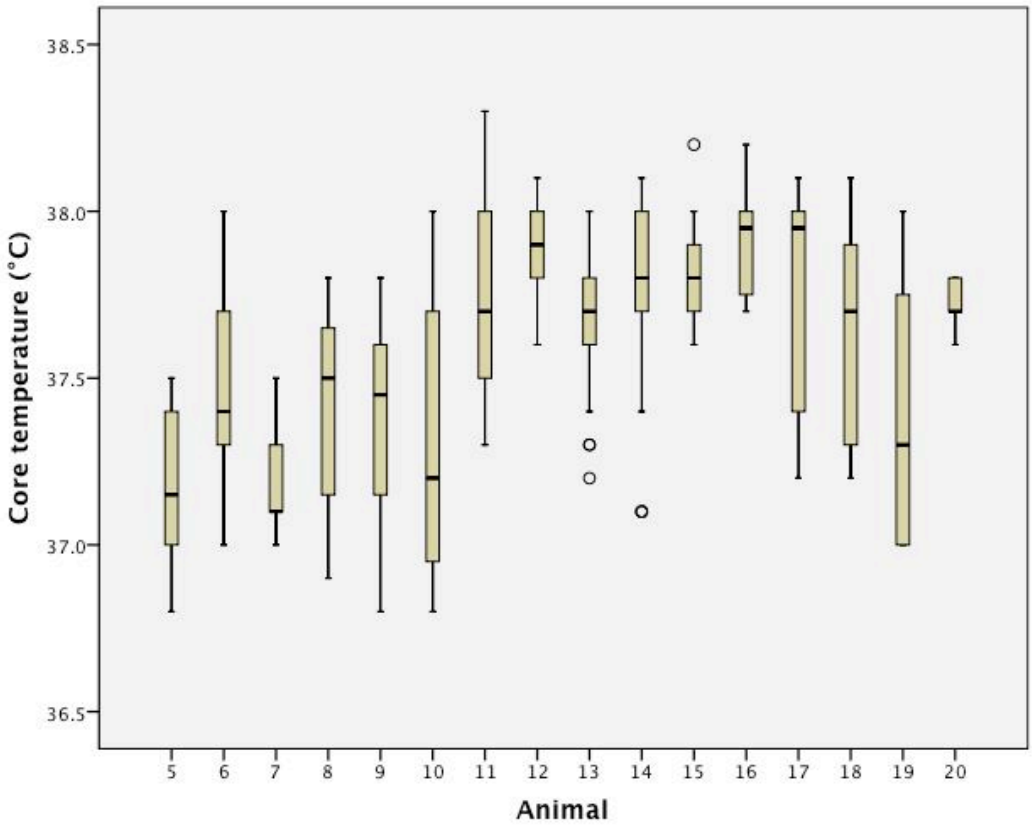
Animal	Weight (g)	Flap side (L/R)	IP / No IP	Mean heart rate (bpm)	Mean oxygen saturations (%)	Mean core temperature (°C)
1	315	Left	No IP	411	92	37.0
2	335	Right	IP	423	96	37.2
3	337	Left	IP	407	95	37.1
4	320	Right	No IP	398	94	37.2
5	336	Right	IP	374	94	37.2
6	334	Right	No IP	401	97	37.5
7	308	Left	No IP	398	96	37.2
8	294	Right	IP	393	97	37.4
9	309	Right	IP	383	96	37.4
10	314	Left	No IP	422	98	37.3
11	324	Left	No IP	397	95	37.7
12	320	Left	IP	406	94	37.9
13	333	Left	IP	422	98	37.7
14	326	Right	No IP	395	95	37.8
15	340	Right	No IP	418	97	37.8
16	304	Left	IP	403	94	37.9
17	320	Left	IP	375	96	37.8
18	319	Left	No IP	410	98	37.6
19	324	Left	No IP	402	96	37.4
20	330	Left	IP	409	97	37.7
Mean Values	322			402	96	37.5

Table 7.1: Table of animal characteristics and pre- and intra-operative measurements

Animals 1-4 were excluded from the data interpretation, as they were treated slightly differently. As mentioned in the Anaesthesia section of the methods, the first 4 animals had anaesthesia maintained via intubation and inhalational anaesthetic and temperature maintained using a heat blanket. Unfortunately, these animals were more difficult to keep stable under anaesthetic and indeed Animals 1-3 died shortly after the period of ischaemic preconditioning was performed. Post-mortem examination revealed that they had likely overheated and also that their lungs were congested. Animal 4 survived the experiment, but was still relatively difficult to maintain temperature and stability. Therefore, although their basic measurements and characteristics have been included in the table above for completeness, the limited data from these animals has been removed from the results analysis. After our Named Veterinary Surgeon took the decision to change the anaesthetic maintenance to the Inactin thiobutylbarbitole injection, and the heat source to a radiant lamp, we had no further problems with temperature maintenance or instability under anaesthetic.

This exclusion of data obviously affects the number of animals in each treatment group for the IP/NoIP data, however, as our resource equation was followed (See 7.2.2), the number of animals in each treatment group (now minimum 7) should still be enough to power our experiment, as E = N (7x2=14) – T(2) = 12, which is within the required range of 10-20.

As temperature is known to influence vascular flow and particularly cutaneous flow, animal core temperature was monitored continuously throughout the experiment, recorded throughout the data collection interval and as mentioned, maintained between 37 and 38°C. The mean temperatures of the animals are displayed in Table 7.1 and graphically in box-plot Graph 7.1 depicting the medians and ranges for each animal.



Graph 7.1: Box-plot of median core animal temperatures with 95% confidence intervals

7.3.1 Flow

Mean flow values on both and each of the perforators individually is shown in Table 7.2.

Perforator combination	Mean flow (mL/min)
Superior	0.21
Inferior	0.25
Both	0.13

Table 7.2: Table of mean total pedicle flow and pulsatility index results for different perforator combinations (superior; inferior; both)

It can be seen from the data table that total pedicle flow was highest on the inferior perforator, followed by the superior perforator and lowest on both perforators.

Effect of IP on Flow - IP group

Table 7.3 displays the average flow values recorded before and after the period of IP on each of the perforator combinations.

Perforator combination	Mean flow pre-IP (mL/min)	Mean flow post-IP (mL/min)
Superior	0.20	0.26
Inferior	0.22	0.27
Both	0.12	0.17

Table 7.3: Table of mean total pedicle flow results pre-IP and post-IP on different perforator combinations (superior; inferior; both)

As with the previous results, total pedicle flow was highest on the inferior perforator, followed by the superior perforator and lowest on both perforators. When examining the effect of IP, it can be seen that all mean flow values (regardless of perforator combination) increased after the period of IP.

Effect of IP on Flow – No IP group

Table 7.4 displays the average flow values recorded before and after the period of No IP on each of the perforator combinations.

Perforator Combination	Mean flow pre-No IP (mL/min)	Mean flow post-No IP (mL/min)
Superior	0.22	0.28
Inferior	0.28	0.23
Both	0.14	0.15

Table 7.4: Table of mean total pedicle flow results pre-No IP and post-No IP on different perforator combinations (superior; inferior; both)

Again, a similar pattern is observed in the results, with total pedicle flow highest on the single perforators and lowest on both perforators for pre- and post-No IP. There does not however appear to be a clear pattern between the flow values pre and post-No IP.

7.3.2 Perfusion

A summary table displaying the mean LDF perfusion values for each flap zone, on each of the different perforator combinations is shown in Table 7.5.

Perforator combination	Mean perfusion (units)			
	Zone I	Zone II	Zone III	Zone IV
Superior	34.55	34.63	19.06	15.85
Inferior	39.71	32.50	18.20	15.27
Both	38.51	29.80	19.89	15.52

Table 7.5: Table of mean perfusion results in each flap zone (I-IV) on different perforator combinations (superior; inferior; both)

It is clear that highest and higher perfusion values are observed in zone I and II of the flap respectively, with lower and lowest values recorded for zone III and zone IV respectively, fitting with the original zoning allocation of Scheflan. There does not appear to be a clear pattern as regards to the perforator combination and perfusion.

Effect of IP on perfusion – IP group

Table 7.6 displays the mean LDF perfusion values for each flap zone pre and post-IP, on each of the perforator combinations.

Perforator combination	Mean perfusion (units)							
	Zone I Pre-IP	Zone I Post-IP	Zone II Pre-IP	Zone II Post-IP	Zone III Pre-IP	Zone III Post-IP	Zone IV Pre-IP	Zone IV Post-IP
Superior	38.20	33.86	32.80	34.99	16.86	17.70	15.85	13.10
Inferior	45.19	38.54	34.30	33.97	17.16	18.50	15.27	16.20
Both	40.84	42.38	30.00	32.64	18.20	25.30	15.52	17.60

Table 7.6: Table of mean perfusion for Pre and Post-IP in each flap zone (I-IV) on different perforator combinations (superior; inferior; both)

Effect of IP on perfusion – No-IP group

Table 7.7 displays the mean LDF perfusion values for each flap zone pre and post-No IP, on each of the perforator combinations.

Perforator Combination	Mean Perfusion (units)							
	Zone I Pre-No IP	Zone I Post-No IP	Zone II Pre-No IP	Zone II Post-No IP	Zone III Pre-No IP	Zone III Post-No IP	Zone IV Pre-No IP	Zone IV Post-No IP
Superior	32.80	33.30	32.60	30.20	19.60	19.70	15.70	15.60
Inferior	37.40	28.80	34.40	28.90	22.00	16.70	15.30	13.12
Both	37.90	34.10	29.70	31.60	23.50	17.40	15.60	14.30

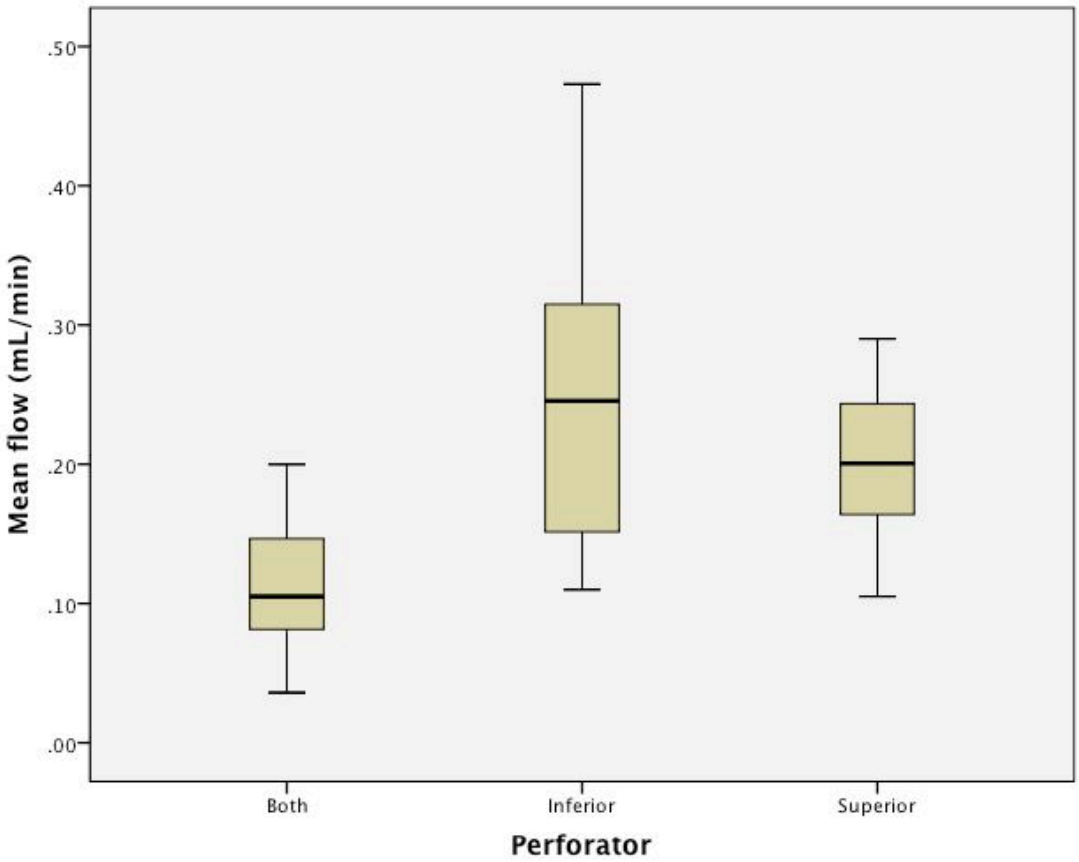
Table 7.7: Table of mean perfusion results for Pre and Post-No IP in each flap zone (I-IV) on different perforator combinations (superior; inferior; both)

7.4 Statistical analysis

The data collected in this study was not normally distributed (confirmed by the plotting of a scattergraph) and therefore non-parametric tests were performed with SPSS® 21 for Mac® (SPSS IBM, NYC, USA) using the Kruksal-Wallis test of multiple unrelated variables and the Mann Whitney test of 2 unrelated variables. Box-plots and line graphs were created in SPSS to illustrate the statistical relationships and interactions between the variables and the values obtained.

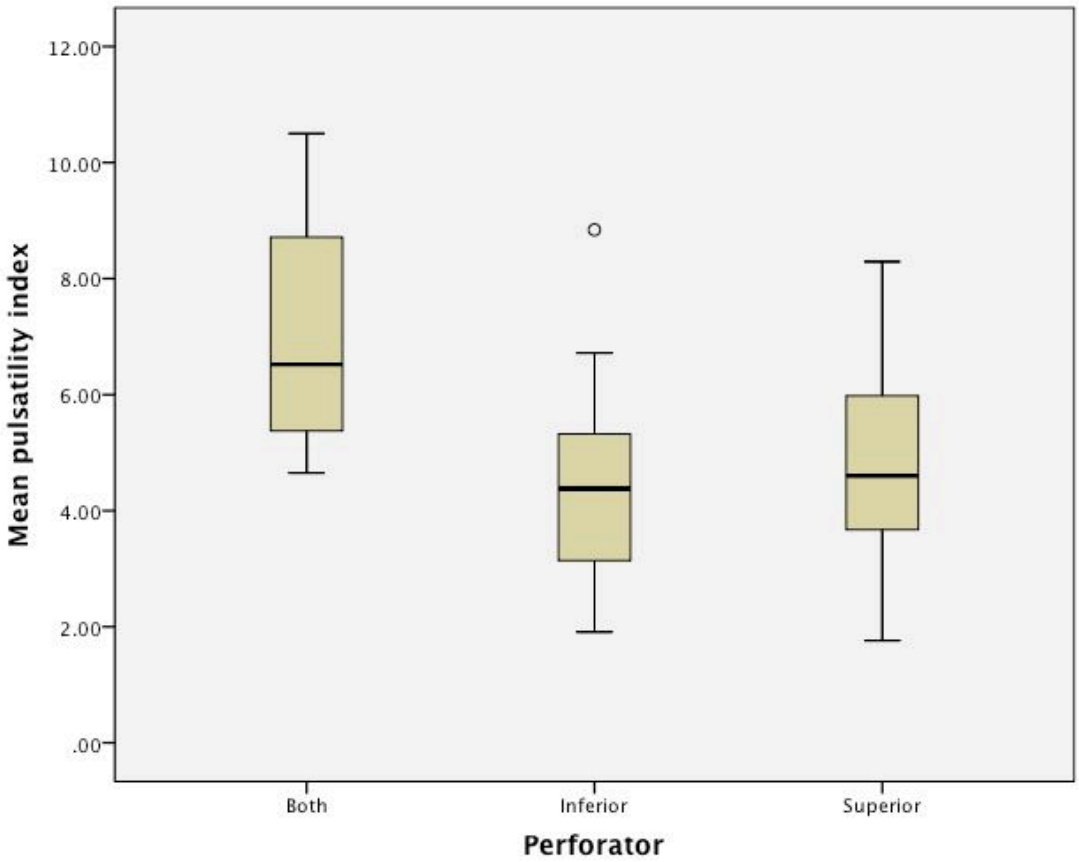
7.4.1 Flow

This analysis confirmed the observed differences seen in the tabulated data in the results section, that total pedicle flow was significantly ($p<0.001$) higher on a single perforator, whether inferior or superior, compared to both perforators. The higher flow observed on the inferior perforator was not statistically different to that observed on the superior perforator ($p=0.259$). These results are displayed in the box-plot Graph 7.2, where the median flow on each perforator combination is plotted along with 95% confidence intervals. The higher values on the single perforators are clearly displayed.



Graph 7.2: Box-plot of median total pedicle flow rates with 95% confidence intervals for all perforator combinations (superior; inferior; both)

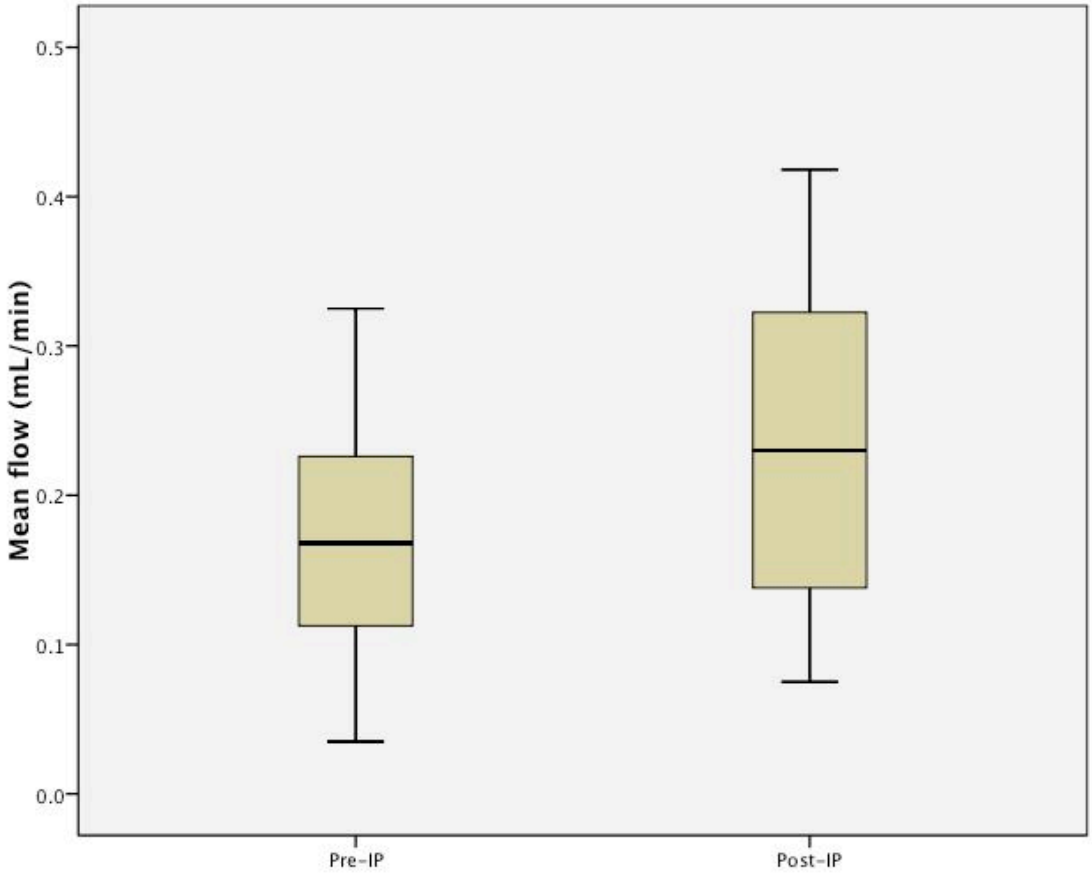
The pulsatility index of the single perforators, whether superior or inferior was significantly less (p=0.01) than that of both perforators. These results are displayed in Graph 7.3, where the median PI of each each perforator combination is plotted along with 95% confidence intervals. The smaller values on the single perforators are clear to see.



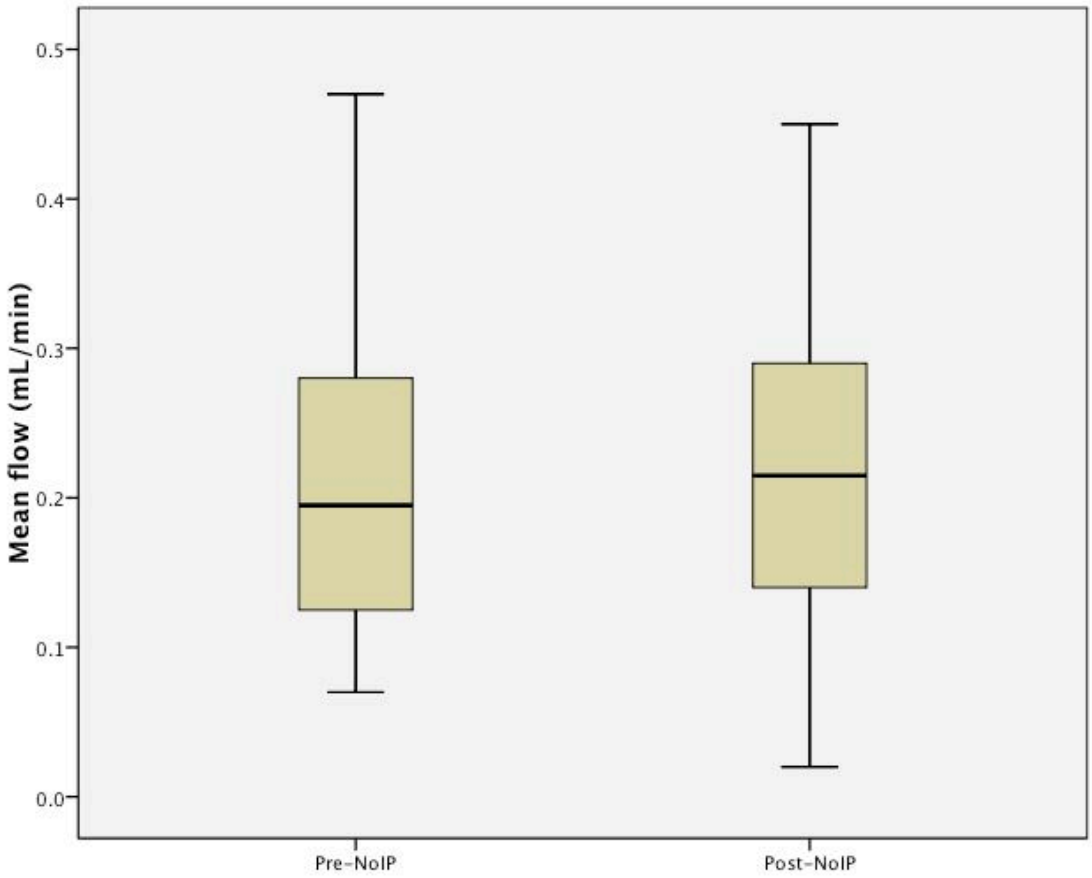
Graph 7.3: Box-plot of mean pulsatility indices with 95% confidence intervals for all perforator combinations (superior; inferior; both)

Ischaemic Preconditioning

The rats exposed to the brief period of ischaemic preconditioning (IP) had a significant increase in total pedicle flow (p=0.05) observed across all perforator combinations. The rats not exposed to the brief period of ischaemic preconditioning (No IP) had no significant change in flow (p=0.820). These analyses are displayed for the effect of IP and No IP on flow in box-plot Graphs 7.4 and 7.5 respectively.



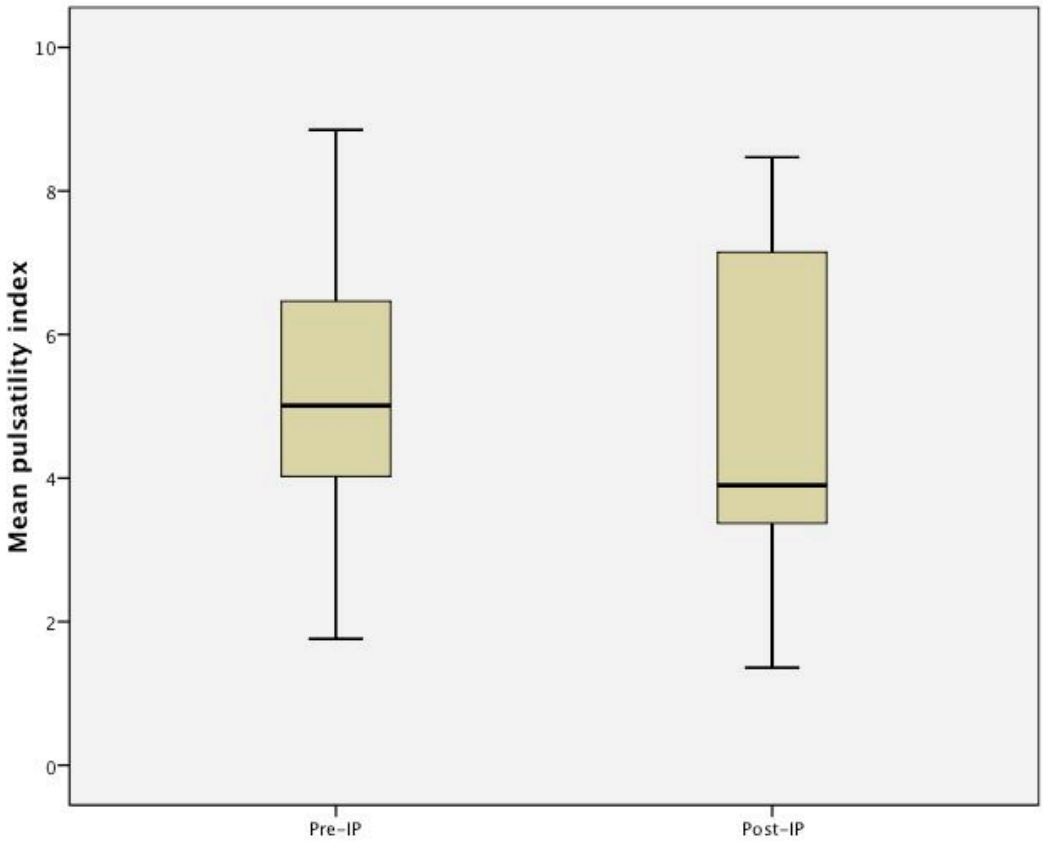
Graph 7.4: Box-plot of median total pedicle flow rates with 95% confidence intervals Pre and Post-IP



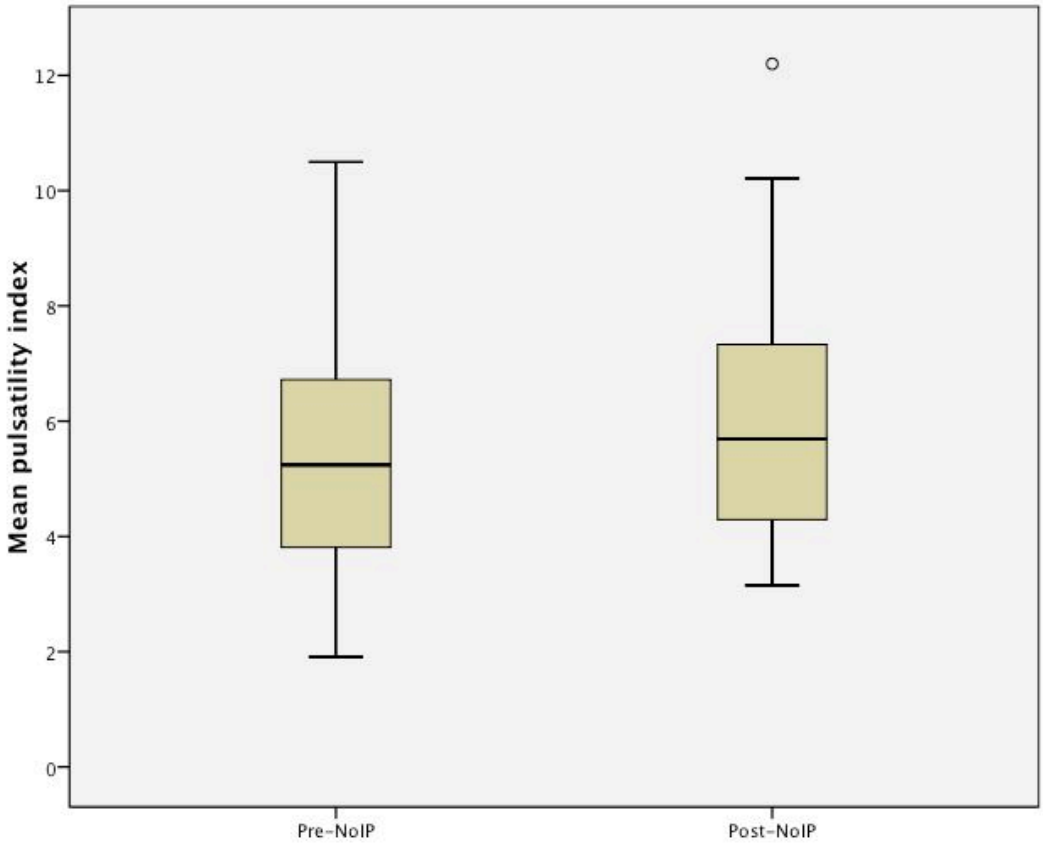
Graph 7.5: Box-plot of median total pedicle flow rates with 95% confidence intervals Pre and Post-NoIP

The higher flow value on the Post-IP plot in Graph 7.4 can be observed, though the substantial overlap of the confidence intervals of the two plots is reflected in the fact that this difference only just reached statistical significance. In the No-IP flow data, in Graph 7.5 the very wide overlapping confidence intervals are clear to see.

Pulsatility indices for the IP and NoIP overall flow were also calculated and though a trend existed for a decrease in pulsatility index after the period of IP, this did not reach statistical significance (p-0.068). The No IP group had no significant difference (p=0.296) in PI before and after the period of NoIP. These relationships are shown in box-plot Graphs 7.6 and 7.7.



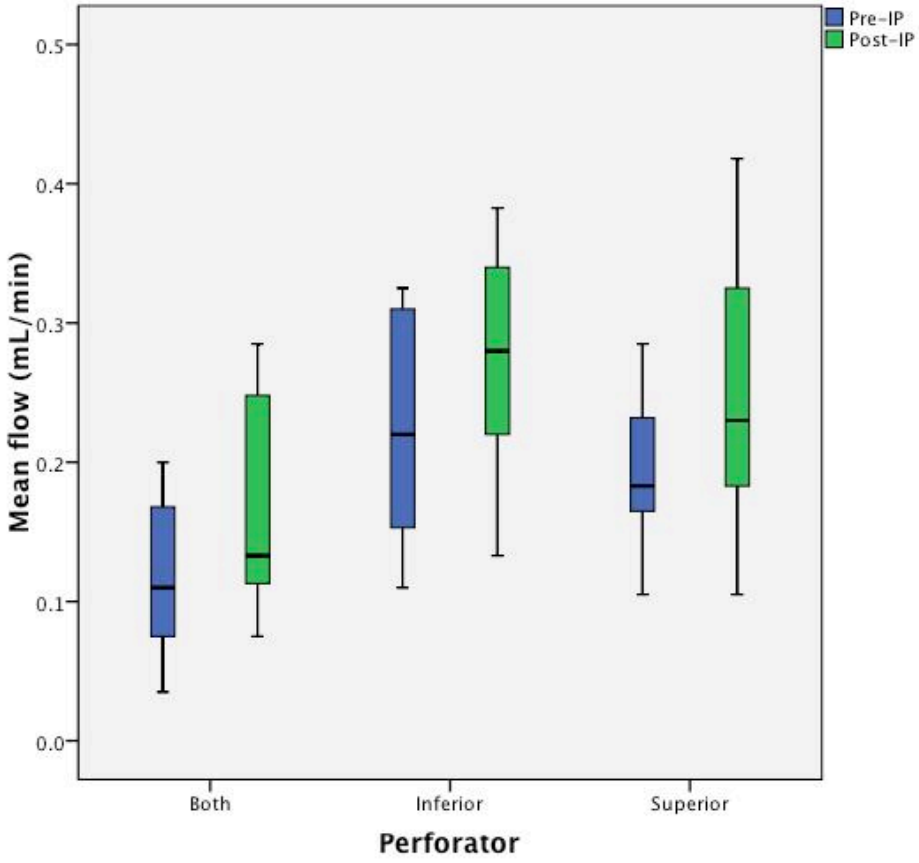
Graph 7.6: Box-plot of mean pulsatility indices with 95% confidence intervals Pre and Post-IP



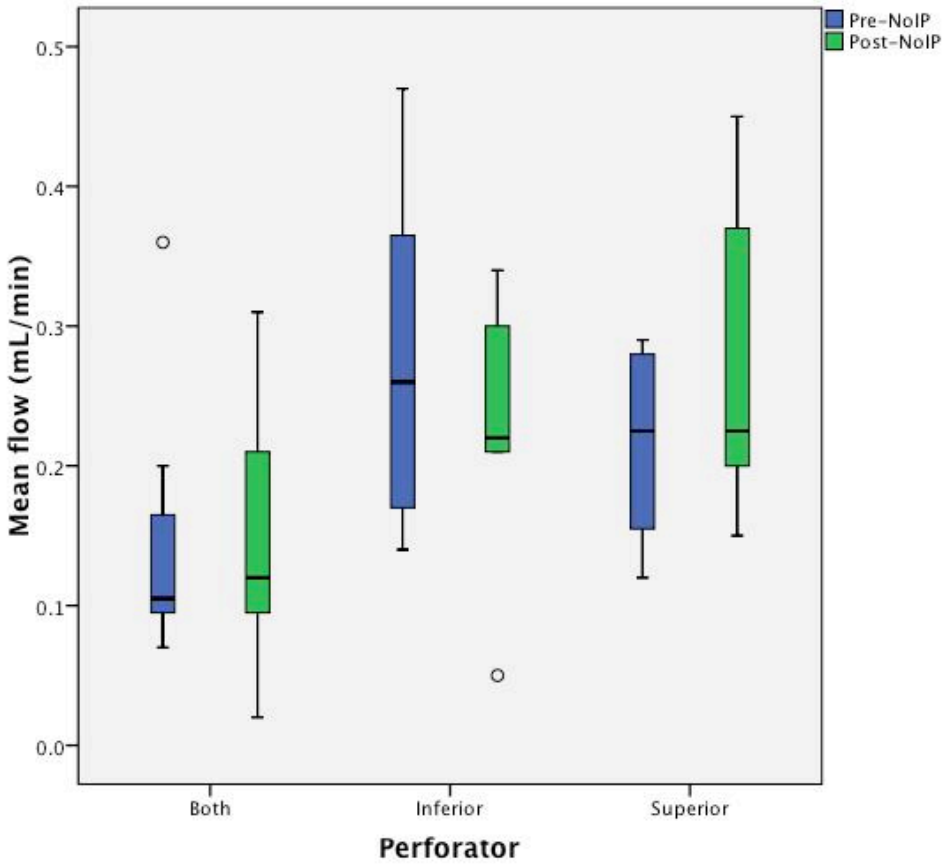
Graph 7.7: Box-plot of mean pulsatility indices with 95% confidence intervals Pre and Post-NoIP

The similar values, wide overlapping confidence intervals and ranges seen indicate the lack of significant difference.

The relationships between the IP and No IP data in relation to perforator combination are shown in Graphs 7.8-7.9 using box-plots of median values and 95% confidence intervals. It can be observed that the rats subjected to the period of IP had an increase in mean total pedicle flow on all perforator combinations, whilst the rats exposed to No IP displayed no such pattern, with the flow slightly increasing, decreasing or remaining the same for both, inferior and superior perforator combinations respectively.



Graph 7.8: Box-plot of median total pedicle flow rates with 95% confidence intervals pre and post-IP for all perforator combinations (superior; inferior; both)



Graph 7.9: Box-plot of median total pedicle flow rates with 95% confidence intervals pre and post-No IP for all perforator combinations (superior; inferior; both)

As the IP and No IP groups were subjected to a full second set of unaltered flow measurements before and after a time period of 30 minutes (15mins of IP or No IP and 15mins to stabilise), the effect of perforator number and location on flow were checked again, to gain increased evidence for the observations previously recorded. The results obtained for this second set of measurements at a different time period showed the exact same pattern was the same as the previous flow measurements recorded for both the IP and No IP groups.

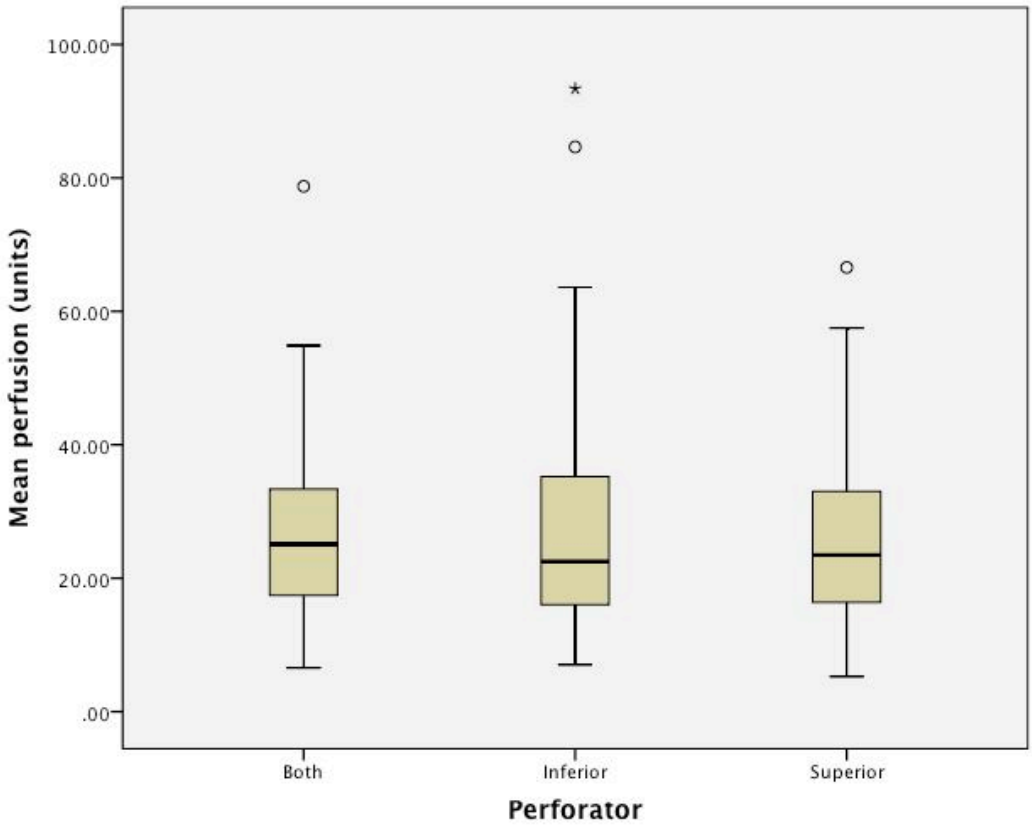
In the IP group there was significantly higher flow on a single inferior perforator ($p=0.014$) when compared to both perforators, and significantly higher on a single superior perforator ($p=0.024$) when compared with both perforators. The difference between inferior and superior perforators was not significant ($p=0.796$).

In the No IP group there was significantly higher flow on a single inferior perforator ($p=0.014$) when compared to both perforators, and significantly higher on a single superior perforator ($p=0.038$) when compared with both perforators. As with the IP group, the difference between inferior and superior perforators was not significant ($p=0.328$).

Therefore it has now been demonstrated at different times with three different sets of measurements that a single perforator provided significantly higher flow than both perforators together.

7.4.2 Perfusion

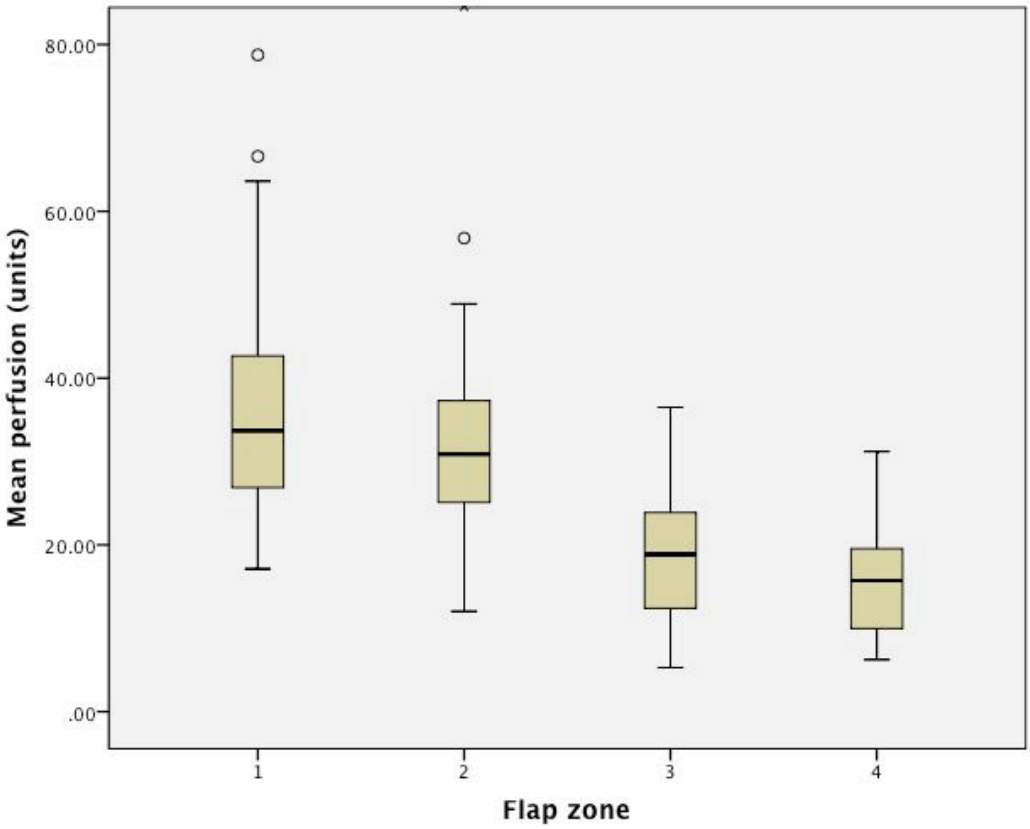
This analysis revealed no significant difference in the mean values obtained for overall perfusion on any particular perforator combination ($p=0.991$), which can be seen more clearly in box-plot Graph 7.10.



Graph 7.10: Box-plot of median perfusion with 95% confidence intervals measured on all perforator combinations (superior; inferior; both)

The similar median values, overlapping confidence intervals and ranges highlight the lack of significance of any small differences.

Differences observed between the perfusion values of the flap zones were confirmed as statistically significant; with zone I significantly higher than zone II ($p<0.05$); zone II significantly higher than zone III ($p<0.001$); which was again significantly higher than zone IV ($p<0.024$). Perfusion differences between all zones were significant, however the difference between zone II and zone III was the largest in size and significance, giving weight to the original Scheflan zoning of this model, supporting the findings of Doncatto et al(Doncatto et al. 2007). These relationships between the data can be seen in the box-plot Graph 7.11.

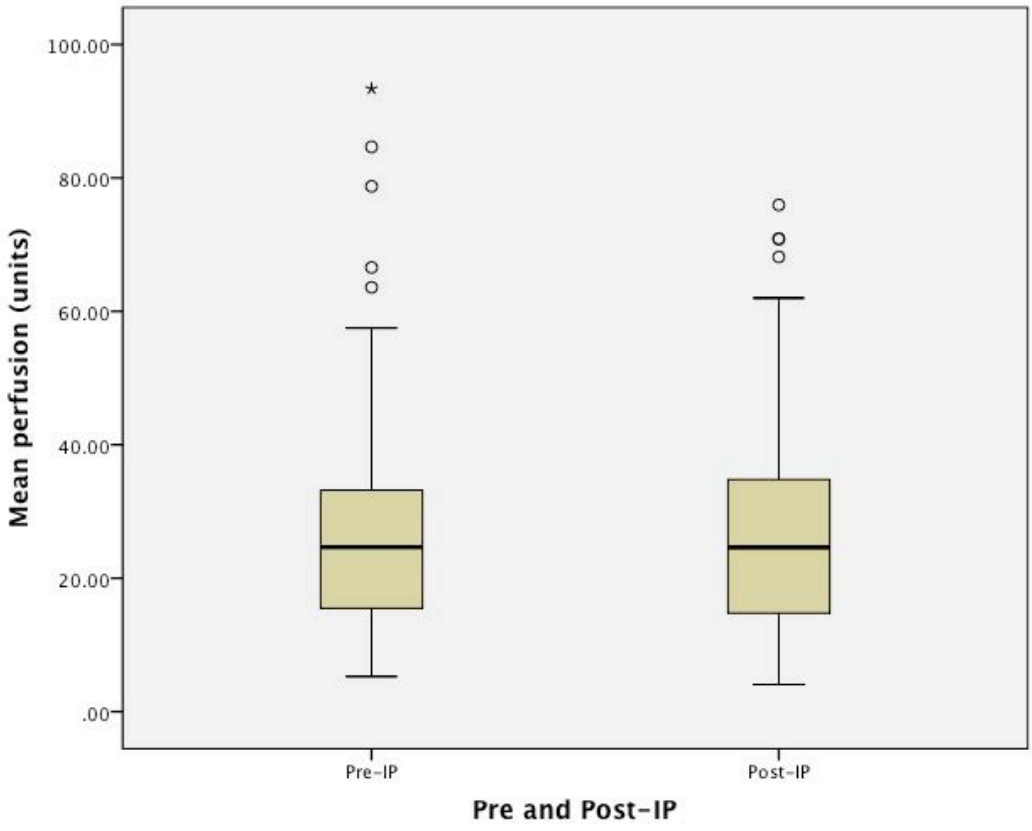


Graph 7.11: Box-plot of median total perfusion with 95% confidence intervals for all flap zones (regardless of perforator combination)

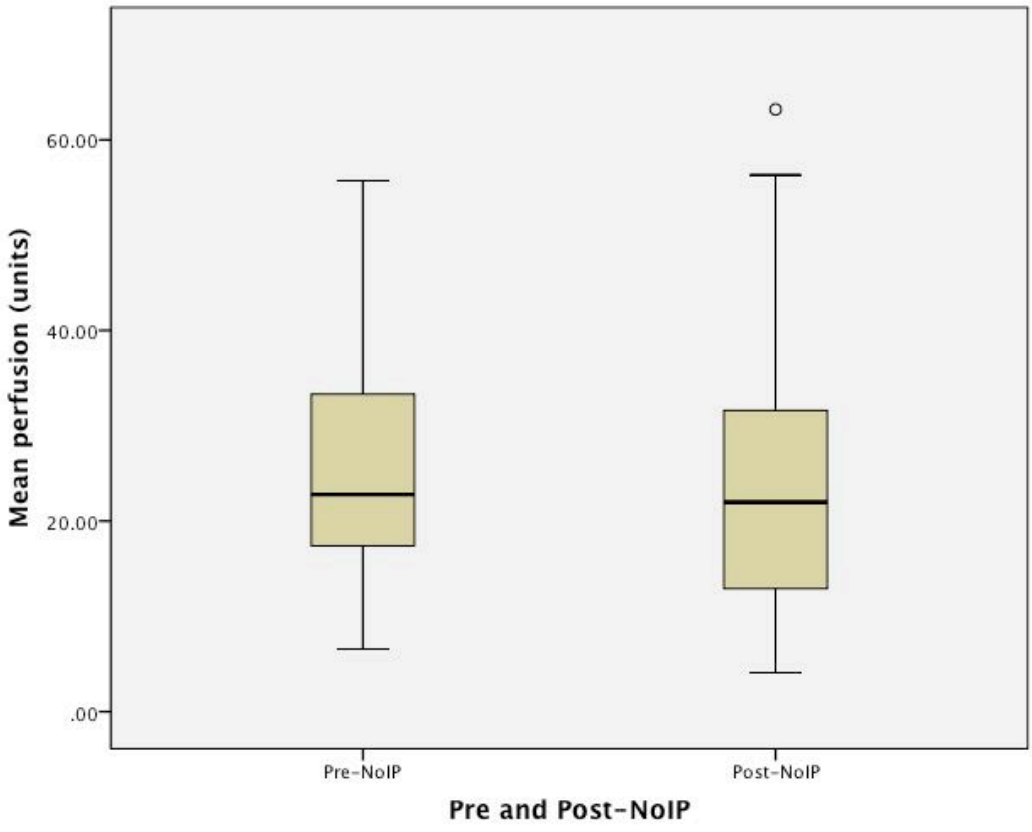
These statistically significant differences in zone perfusion highlight the flap skin perfusion distribution in this cranial epigastric perforator flap model as being concordant with the original Scheflan flap zoning and add to the construct validity of this as a flap model for DIEP flap perfusion, as found in other studies of flap LDF perfusion(Hallock. 2001).

Ischaemic Preconditioning

The rats exposed to the brief period of ischaemic preconditioning had no significant difference in overall mean perfusion values obtained pre and post-IP (p=0.919). The rats not exposed to the brief period of ischaemic preconditioning also had no significant difference in overall mean perfusion (p=0.26). These relationships can be seen more clearly in the box-plots below. Graph 7.12 shows the effects of IP on the mean total perfusion, regardless of perforator combination, whilst Graph 7.13 shows the same effects but for the rats that were not exposed to IP (No IP).

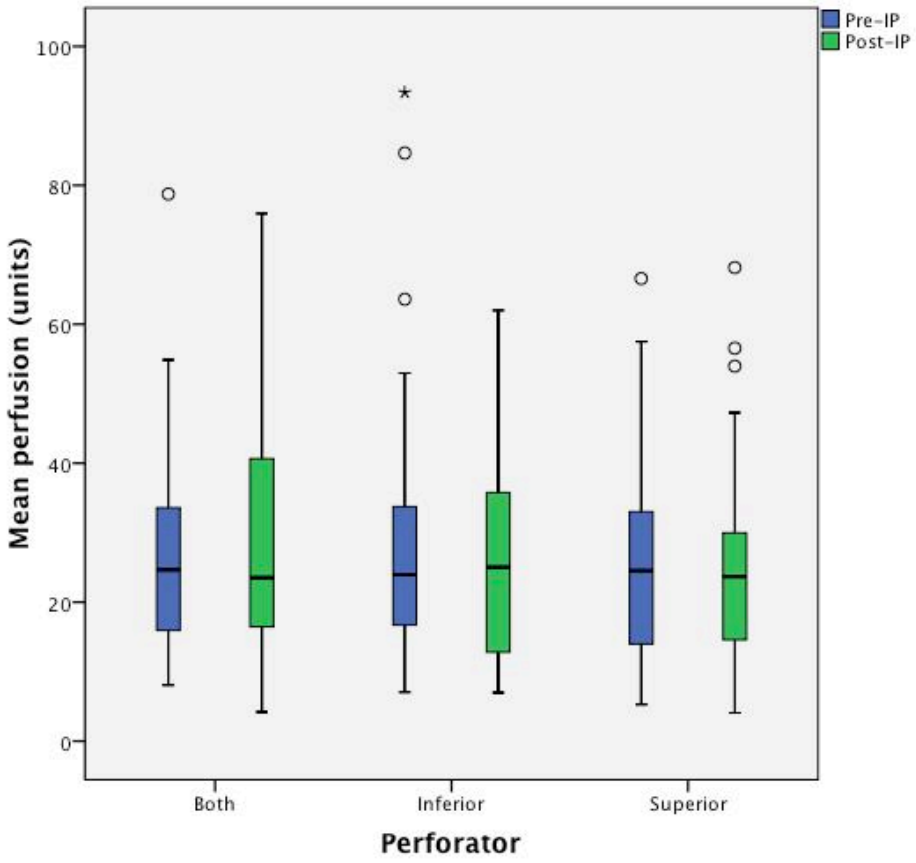


Graph 7.12: Box-plot of median overall perfusion with 95% confidence intervals pre and post-IP

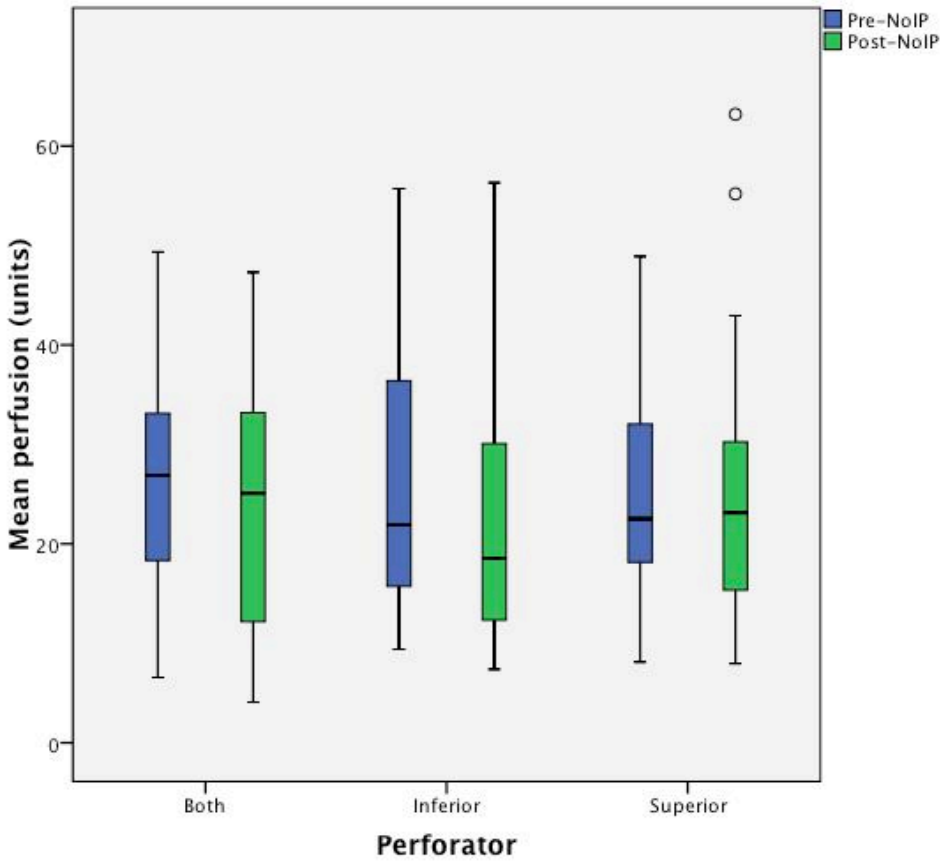


Graph 7.13: Box-plot of median overall perfusion with 95% confidence intervals pre and post- NoIP

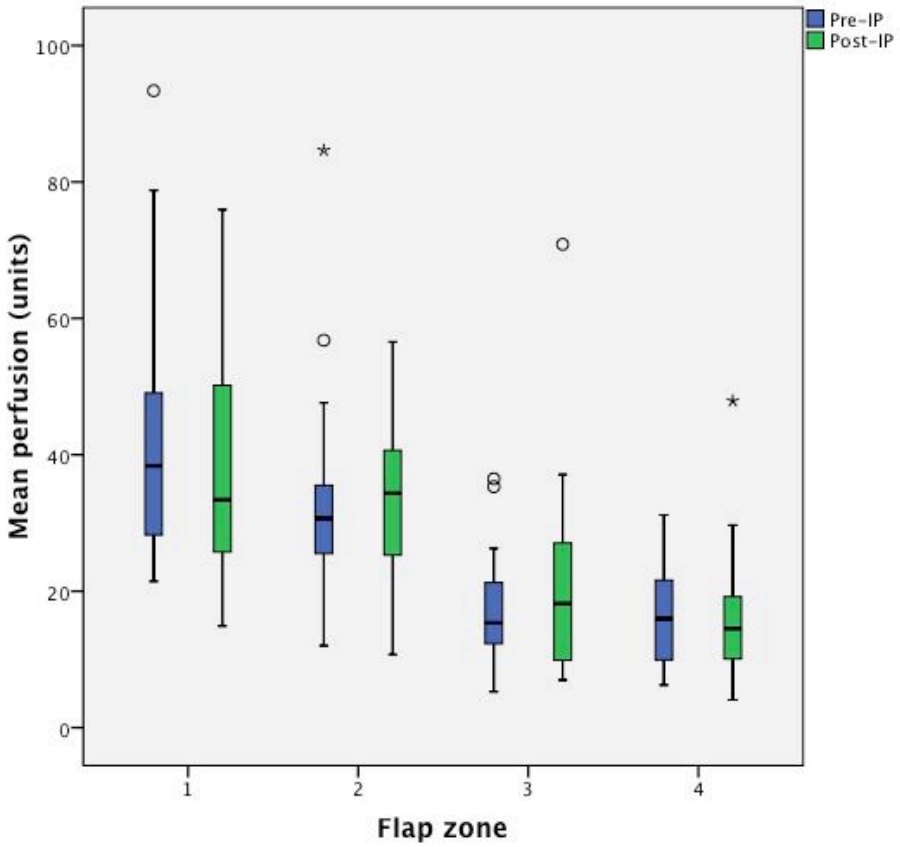
The changes in perfusion pre and post IP or No IP are shown on the different perforator combinations and flap zones in the box-plots below (Graphs 7.14-7.17). On all perforator combinations, the change between pre and post IP was not significant (p=0.602) nor the change between pre and post-No IP (p=0.582). The significant differences in perfusion between flap zones were as previous.



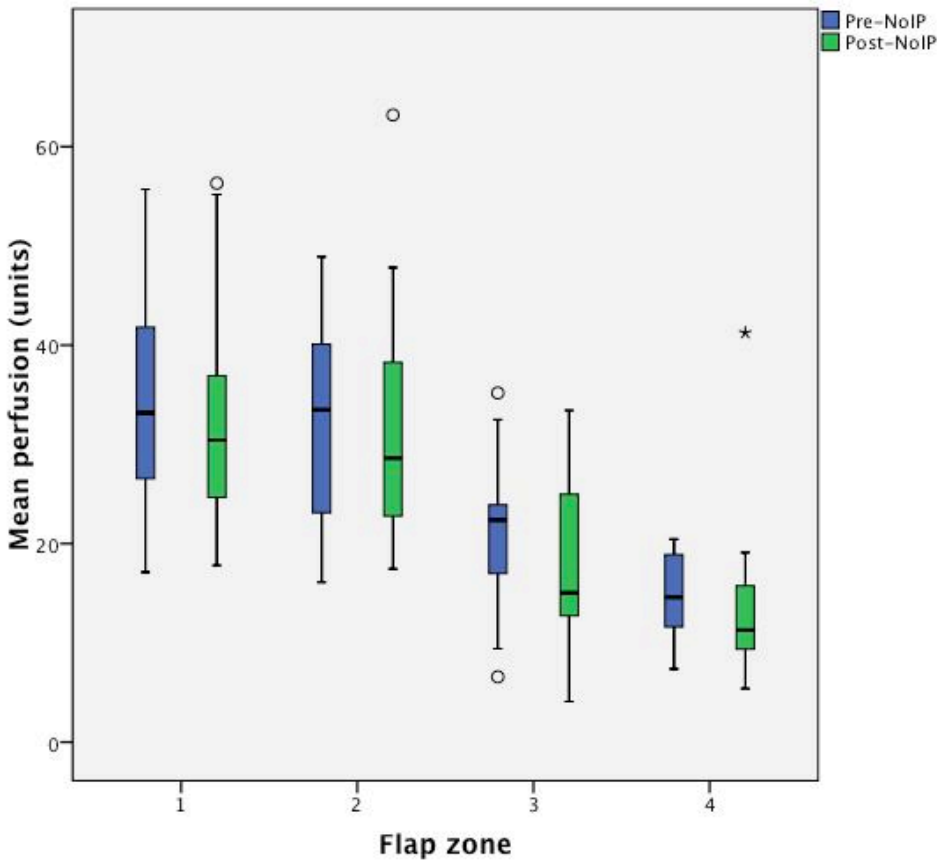
Graph 7.14: Box-plot of median perfusion Pre and Post-IP with 95% confidence intervals for all perforator combinations (superior; inferior; both)



Graph 7.15: Box-plot of median perfusion Pre and Post-NoIP with 95% confidence intervals for all perforator combinations (superior; inferior; both)



Graph 7.16: Box-plot of median perfusion Pre and Post-IP with 95% confidence intervals for all flap zones



Graph 7.17: Box-plot of mean perfusion Pre and Post-No IP with 95% confidence intervals for all flap zones

Again, as the IP and No IP groups were subjected to a full second set of flow measurements before and after a time period of 30 minutes (15mins of IP or No IP and 15mins to stabilise), the effect of perforator number and location on perfusion were checked again, to gain increased evidence for the observations previously recorded. The results obtained for this second set of measurements at a different time period showed the exact same pattern was the same as the previous perfusion measurements recorded for both the IP and No IP groups.

In the IP group there was no significant difference in flap skin perfusion between any perforator combination ($p=0.602$) and in the No IP group there was also no significant difference in flap skin perfusion on any perforator combination ($p=0.582$). Therefore it has now been demonstrated at different times with two different sets of measurements that there appears to be no relationship between perforator and skin perfusion in this model.

7.5 Discussion and Conclusions

7.5.1 Model validation and potential confounders

Number reduction

The exclusion of the data from animals 1-4 was performed for the reasons discussed in the Results section. As discussed, the resource equation supports the use of 16 animals for the model as being adequate to detect differences, however this must be frankly acknowledged as a potential confounder and potential source of selection bias.

Flow data

As discussed in the Introduction section of this chapter, the rat epigastric perforator flap model is well validated in the literature with previous researchers using the model to study flap viability, flap zones and flap flow measurements. The results obtained for the flow measurements on the epigastric vessels in our animals were compared with those reported for a previous study by Rickard (2010) which examined flow rates on the femoral vessels of rats of the same species. These flow values were reported as between 1-4mL/min. The same study also measured the diameters of the femoral (mean 0.95mm) and caudal epigastric (mean 0.54mm) arteries in these species reporting a ratio of 1:1.7(Rickard. 2010). Applying the Poiseuille's law formula ratios outlined in Chapter 2 as a crude method of comparing the data, means that the reduction in the radius in the smaller vessel used in our study would decrease the flow rate, which fits with the flow data measurements gathered in this experiment (0.09-0.4mL/min) and supports the data findings. As stated above, the vessel diameters of femoral vessels in Wistar rats are approximately twice that of the branch they give off, the caudal epigastric artery, this reduction in flow observed in our study is expected and this supports the values found in this experiment.

Perfusion data

The validation of the model perfusion data is more difficult, as output is measured in arbitrary perfusion units, which differ between machine manufacturers and can be dependant upon software analogue outputs of voltage to perfusion unit. Studies identified of murine models measuring skin perfusion using LDF were either using different machines, software and measurement systems(Patel et al. 1999, Sonmez et al. 2013, Marks. 1985). Therefore it is not possible to correlate perfusion data obtained in our animal model to provide validity for the model. We do however have model data in our study to suggest that the perfusion measurements exhibited construct validity (i.e. measures what it is supposed to measure) as the zone differences in perfusion followed that of Hallock's model in terms of the higher perfusion values on zones I and II and lower on zones III and IV, further discussed in section 7.5.3.

Temperature/Arterial inflow

Core animal temperature was kept constant between 37 and 38°C, as seen in Table 7.1 and Graph 7.1. and though variation existed between animals, this range was maintained throughout. There was a tendency towards slightly higher animal temperatures towards the end of the experiment (animals 10-20) and this possibly reflects slightly increased user confidence with the radiant heat source, and must be acknowledged as a potential confounder, however the maintenance of the temperature within one degree and within the recommendations for monitoring of cutaneous blood flow was observed(Bircher et al. 1994). Though animal heart rate, temperature and oxygen saturations were measured throughout the experiment, measurement of animal blood pressures was not performed and it is accepted that invasive arterial monitoring of the animals or waveform analysis would have provided better information regarding the constant arterial input into the flap. This is therefore accepted as a potential confounder.

Venous drainage/Contralateral anatomy

The effect of venous drainage of the flap on the measurement of flow into the flap is not known and thus this is accepted as a potential confounder as the time taken to raise the flap was likely to have decreased across the study period due to increased confidence with the technique. This could have meant that the earlier flaps were subject to a longer period of potential venous congestion than later flaps. However, as all animals had the flap isolated on the pedicle prior to the start of any data collection and the same time interval was left between measurements and interventions, this was kept as consistent as possible between the animals. The effect of any differences in the sealed contra-lateral anatomy of the other side of the flap is unknown and accepted as a potential confounder. The effect of any difference in preload is also acknowledged as having an effect on flow and could be a potential confounder, however all animals were housed in similar conditions and fed/watered similarly and of the same weight and thus this was felt to have been kept to a minimum, accepting that central venous access (not performed) would have given more information regarding this.

Observer error

A single surgeon performed all operations and collected all data to reduce inter-observer error, though as with most experiments the possibility of intra-observer error is also accepted as a potential confounder.

7.5.2 Summary of results and Discussion

The results of our study showed that total pedicle flow was significantly higher and pulsatility index significantly lower, on a single perforator, whether superior or inferior, compared to both perforators. No significant difference could be demonstrated in perfusion of the flap skin on any perforator combination, though perfusion in the flap zones were significantly different, giving

weight to the accuracy of the model. A single 15-minute period of ischaemic preconditioning significantly increased total pedicle flow across all perforator combinations, an effect which was not oberved in the control 'No IP' group, though it is accepted that this increase in flow only just reached statistical significance. No significant difference could be identified in the pulsatility indices between the periods of IP or No IP. The period of ischaemic preconditioning had no effect on perfusion of the flap skin.

Recalling Ohm's law, which states that flow = driving pressure / resistance, then if a steady driving, or arterial, pressure is assumed, then any change in flow is indicative of an effect on resistance. The increased flow values and decreased pulsatility indices obtained on the single perforator would appear logical, because as discussed pulsatility index is a measure of vascular resistance.

However, the findings of increased flow and decreased pulsatility index on a single perforator compared to both are counter-intuitive, because as discussed in the Introduction it would be expected to find decreased resistance (and therefore increased flow) with circuits in parallel (2 perforators) than in series (1 perforator). The significantly higher pulsatility index obtained on both perforators compared to the lower values on single perforators would indicate that indeed vascular resistance is higher on both perforators compared to one.

Additionally, the higher flow on a single perforator did not translate to increased perfusion of the flap skin. As the higher perfusion values in zone I and II and the lower perfusion values in zones III and IV were accurately detected in the study, the model appears valid, thus it is likely that no change in skin perfusion occurred, despite the increase in flow. This could be because the increase in flow was not enough to create a measureable clinical change in perfusion of the flap skin. It could also be a more complex reason, due to multifaceted intra-flap anatomical and physiological mechanisms, as increased flow may not automatically translate to increased skin perfusion and as previously discussed, few papers have linked higher flow rates in flaps to any clinical benefit or outcome measure(Figus et al. 2008). One possible explanation is that with a single perforator there is a physiological decrease in resistance due to shunting. In short, it is possible that when a single perforator is used, arterio-venous communications open at the level of the subdermal plexus, meaning that the skin is bypassed as the blood circulates the subcutis. This would explain the increased total pedicle flow observed on a single perforator but no effect seen on the perfusion of the flap skin.

The increased flow observed with the single period of IP only just reached statistical significance and there was no significant difference in pulsatilitity index after the period of IP. The lack of any effect on flap skin perfusion could be due to the small increase in flow not creating any clinically measureable effect on skin perfusion. It is possible that the period of IP caused subdermal vascular channels to open within the flap subcutis, decreasing resistance and increasing flow, however this was not supported by any significant change in pulsatility index and thus indicated no significant change in resistance.

Chapter 8: The effect of perforator vessel choice in terms of number and position, on perfusion of zone IV of a human DIEP flap.

8.1 Introduction

Breast reconstruction post-mastectomy has been shown to confer significant psychosocial benefit to patients, improving quality of life and self-esteem(Atisha et al. 2008, Reavey and McCarthy. 2008). Breast reconstructions can be performed as immediate or delayed procedures and for active malignant disease or as a prophylactic measure for high-risk patients. The use of a patient's own tissue (autologous) for breast reconstruction post-mastectomy produces a natural feel to the reconstruction, the appearance of which improves over time and unlike implant reconstructions will not require replacement at some stage(Visser et al. 2010). Studies have shown that particularly in unilateral breast reconstructions, patients rate the aesthetic outcome of autologous reconstructions significantly higher than implant-based reconstructions(Shaikh-Naidu et al. 2004, Kroll and Baldwin. 1992). A DIEP flap is a popular choice of autologous breast reconstruction, as there is usually adequate donor tissue to recreate the breast mound and the donor site scar is favourable. However, as previously discussed in Chapter 3, the concern regarding fat necrosis means that some surgeons routinely discard zone IV of the flap, meaning that a size-match for a large breasted patient may be more difficult to achieve.

As previously discussed in Chapter 5, the use of laser Doppler technology for the monitoring of cutaneous blood flow(Choi and Bennett. 2003), and specifically free flaps is well established(Smit et al. 2010, Hirigoyen et al. 1995). The use of LDF to assess flaps intra-operatively is less routine, though has been used experimentally(Ulusal et al. 2006, Hallock. 2001, Yoshino et al. 1996). LDI technology has been used successfully to identify perforating blood vessels pre-operatively(Komuro et al. 2002) and to assess flap perfusion intra-operatively and after flap transfer to assess perfusion on perforating vessels(van den Heuvel et al. 2011).

Indocyanine green near infra-red video-angiography (ICG-NIR-VA) technology is a more recent trend in flap assessment and monitoring and has been used experimentally and clinically in free flaps(Still et al. 1999). It has been shown to be successful in identifying perforator vessels preoperatively(Azuma et al. 2008) and intra-operatively, both assessing flap territories of perfusion and viability(Holm et al. 2006, Pestana et al. 2009) with a strong correlation reported between scan findings and clinical outcome(Holm et al. 2002b, Mothes et al. 2004).

When assessing flap tissues intra-operatively, the need for timely approach to any scan assessment of the flap is paramount, so as to avoid lengthening the procedure and anaesthetic, which can increase complications(McKenzie et al. 1985, Pedersen et al. 1992). Thus the time left to elapse between any intervention (such as vessel clamping) should be as short as possible whilst long enough to eliminate the effects of the previous intervention. As mentioned in the introduction of Chapter 7, a recent study by Tollan et al (2012) investigated the time required

for resolution of reactive hyperaemia and found that a period of 5 minutes was sufficient(Tollan et al. 2012).

In summary, decision-making regarding perforator selection when raising a free flap has previously been discussed in Chapters 1 and 3. Whilst some researchers believe that a medial row perforator will supply a larger portion of the flap more reliably, others have found lateral perforators to be larger and to have less tortuous intra-muscular routes than medial. However, the majority of this research has been in cadaveric experiments, and zone IV of flaps not assessed in detail, if at all. LDI and ICG-NIR-VA technology has been used successfully to assess and monitor free flaps and the time between such scans has been shown to be reduced to an acceptable level to permit intra-operative research scanning within an acceptable time to permit ethical research.

8.2 Materials and Methods

8.2.1 Study plan

In the approach to this study a design plan was formulated to outline the elements of the research to be investigated. An unknown number of patients were required, to study the perfusion of the skin and fat of zone IV of a DIEP flap when altering the following variables:

- medial versus lateral row perforators
- one versus two perforators
- superior versus inferior perforators

A flowchart of the initial study plan is shown in Figure 8.1.

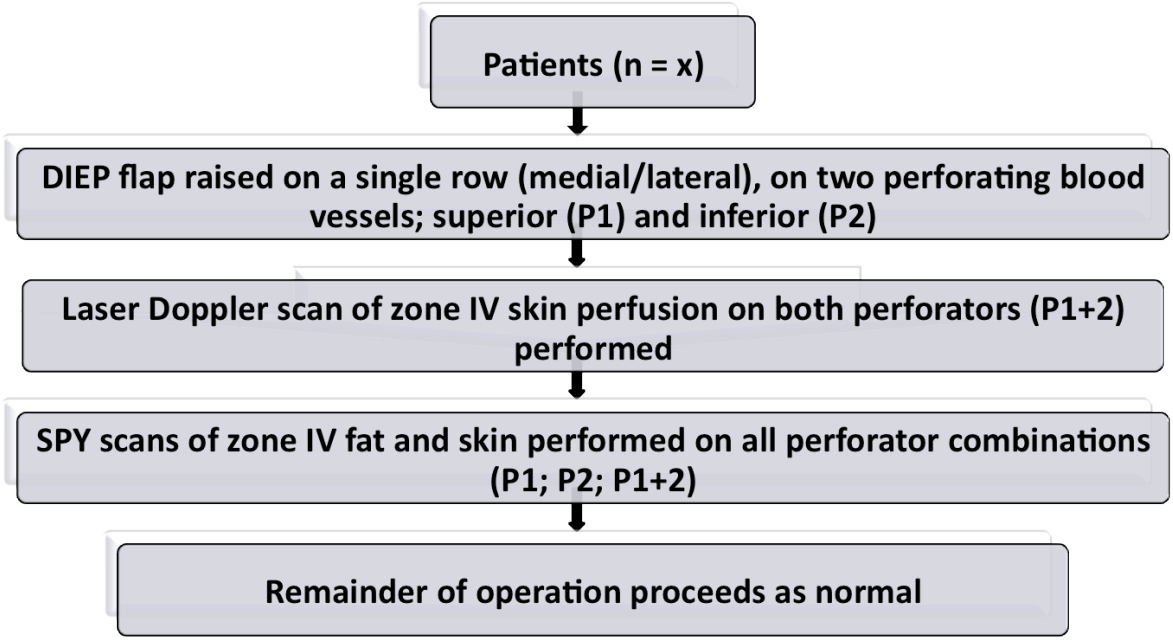


Figure 8.1: Flowchart of the study design of the human DIEP flap study

8.2.2 Statistical design

Sample Size

A professor of statistics was consulted to discuss the number of patients required for the study. It was decided that a formal power calculation was unfeasible, due to limited knowledge of standard deviations in perfusion measurements using the ICG-NIR-VA equipment. It was decided therefore that a sample of 10-15 patients would be recruited initially, with statistical analysis performed after 6-8 patients to assess the inter-patient variability. Whilst the medial vs. lateral row element of the study would be dependant upon this unknown variability, the assessment of one versus two perforators and superior versus inferior perforators would be an intra-patient study, using each patient as their own control and therefore producing valuable data regardless.

Selection of medial vs. lateral row in each patient

It was decided that randomising each patient to receive either medial or lateral row perforators was not ethically justifiable, as although research equipoise exists regarding the selection, the surgeon's judgement regarding the ease of dissection and the calibre of the vessels in the row remains the best decision for the patient undergoing the flap procedure for their flap. Therefore it was decided that the patient would have the perforator row decided by the surgeon at the time of operation based on clinical grounds, as with any other DIEP flap operation. It was discussed that this may result in a particular 'row group' having more patients than the other, but it was agreed that this should be reviewed after the first 5 patients to check approximately equal numbers were being gathered.

Selection of one vs. two perforators and superior vs. inferior perforator

As the primary operating surgeon in this study usually bases DIEP flaps on two perforators, it was not necessary to design any statistical variation into these factors. Two perforating vessels would be raised on a particular row for each flap and one vessel would be clamped using a microvascular clamp whilst assessing perfusion on the other perforator, allowing assessment of the variables of one vs. two and superior vs. inferior.

8.2.3 Patients

Patient inclusion criteria were as follows: female patients between the ages of 18-70 requesting a breast reconstruction post-mastectomy for cancer or high-risk prophylaxis.

Patient exclusion criteria were as follows: Abdominal scarring, ASA score of 3+, iodide allergy/autonomic thyroid adenomas/hyperthyroidism (in accordance with the contraindications stated in the Summary of Product Characteristics of ICG dye – 09/2004)

Therefore the patients recruited for this study were female patients undergoing a DIEP flap for breast reconstruction post-mastectomy. Both immediate and delayed DIEP patients were included in the study, as were cancer patients and high-risk prophylactic mastectomy patients.

Thirteen patients were recruited for this study, which was performed from 09/2009 to 06/2011.

- age ranges from 41-57 (median of 51)
- 9 immediate DIEPS; 4 delayed DIEPS
- 2 smokers; 11 non-smokers
- 10 patients underwent pre-operative chemotherapy, of which 4 also received pre-operative radiotherapy

Ethical review was granted by the West of Scotland Research Ethics Service, Western Infirmary, Glasgow (REC reference number: 09/S0709/64).

8.2.4 Anaesthesia and environment

Anaesthesia details

In line with guidance regarding measurement of cutaneous blood flow by laser Doppler(Fullerton et al. 2002, Bircher et al. 1994), room temperature in theatre was maintained between 25 and 27°C in theatre at all times, recorded during scans again specifically to check at a constant level

whilst measurements were being recorded. This relatively small temperature range is very much in line with the recommendations by Bircher et al (1994), who showed that capillary blood flow in the skin of extremities is very stable between room temperatures of 17-28°C. Patient core body temperature was recorded throughout the operation and maintained with the use of Bair-hugger air-warming blanket between 36 and 37°C. Blood pressure and heart rate were monitored at 5-minute intervals and core temperature recorded at 15 minute intervals.

8.2.5 Experimental procedure

All operations were performed by the same surgeon (IRM). A DIEP flap was drawn over the abdomen of each patient and divided into zones. As zone IV of the flap was the only zone being scanned, the decision of Scheflan vs. Dinner zone classification was not relevant.

The flap was raised from lateral to medial and the perforators on the appropriate side identified. The surgeon then decided which row of vessels to base the flap on clinical grounds and dissected two perforating vessels on the appropriate row and isolated the flap upon these vessels. At this point the measurements were taken (with any perforator vessel not being assessed at that time clamped with a single Acland B2-V clamp) and afterwards the flap operation continued with anastomoses made to the internal mammary vessels under the 4^{th} rib, the flap inset and the abdomen closed.

8.2.6 Measurements

In line with guidance regarding measurement of cutaneous blood flow(Fullerton et al. 2002), all measurements were performed after checking the temperature of the theatre was constant and between 25-27°C and with the overhead lights switched off to avoid any interference from ambient light. The distance of the scanner from the patient was standardised for each system used (29 cm for LDI and 40cm for SPY) as was the image size and measurement speed.

The first six patients underwent scans in the following order:

- LDI scan of zone IV skin on both perforators (P1+P2)

- After a single intra-vascular injection of ICG (10mg) a SPY scan on zone IV fat on both perforators, followed by a SPY scan of zone IV skin on both perforators.

- After a single intra-vascular injection of ICG (10mg) a SPY scan on zone IV fat on P1 or P2 (randomised order using a random number generator), followed by a SPY scan of zone IV skin on the same perforator.

- After a single intra-vascular injection of ICG (10mg) a SPY scan on zone IV fat on the remaining perforator, P1 or P2 (randomised order using a random number generator), followed by a SPY scan of zone IV skin on the same perforator.

After review of the results with a statistician (detailed in the Statistical Analyses section below), the remaining six patients underwent scans in the following order:

- LDI scan of Zone IV skin on both perforators (P1+P2)

- After a single intra-vascular injection of ICG (10mg) a SPY scan on zone IV skin on P1, P2 or P1+P2 (randomised order using a random number generator), followed by a SPY scan of zone IV fat on the same perforator.

- After a single intra-vascular injection of ICG (10mg) a SPY scan on zone IV skin on P1, P2 or P1+P2 (randomised order using a random number generator), followed by a SPY scan of zone IV fat on the same perforator.

- After a single intra-vascular injection of ICG (10mg) a SPY scan on zone IV skin on P1, P2 or P1+P2 (randomised order using a random number generator), followed by a SPY scan of zone IV fat on the same perforator.

A minimum of 5 minutes was left between clamping or releasing a perforator before any measurements were made, to allow for the effects of hyperaemia to settle, as mentioned earlier in this chapter(Tollan et al. 2012). A minimum of 10 minutes was left between ICG injections to allow for elimination of dye from previous scans from the blood vessels in the flap. A further patient, separate to the 12 patients detailed and termed 'control' patient, underwent 3 scans in an identical manner to those detailed above, but with no vessel clamping performed. This patient underwent 3 repeated measurements of the fat and skin respectively of zone IV, to assess reliability of the scan measurements and to ensure that venous congestion and thus retention of dye was not a cause of increased fluorescence seen in the flap tissue, which could emulate increased perfusion.

LDI scan

A single scan of the skin of zone IV was performed using the Moor Laser Doppler Imaging Scanner LDI2-IR on the large scan setting.

SPY scans

The Novodaq ICG-NIR-VA unit SPY scanner was used to scan the fat and skin of zone IV. Thee injections of dye were given for the three different perforator combinations and for each, a scan of the fat and skin of zone IV were taken consecutively (each scan is 38 seconds in duration).

8.3 Results

Moor BDA Software V5.3 was used to analyse data from LDI scans of zone IV skin perfusion on both perforators, producing a static image of the area scanned and a colour visual image of the same region showing areas of low perfusion (dark blue) to areas of high perfusion (red), with green and yellow colours corresponding to areas of moderately low and moderately high perfusion respectively. An example of an LDI scan image of a flap is shown in Figure 8.2.

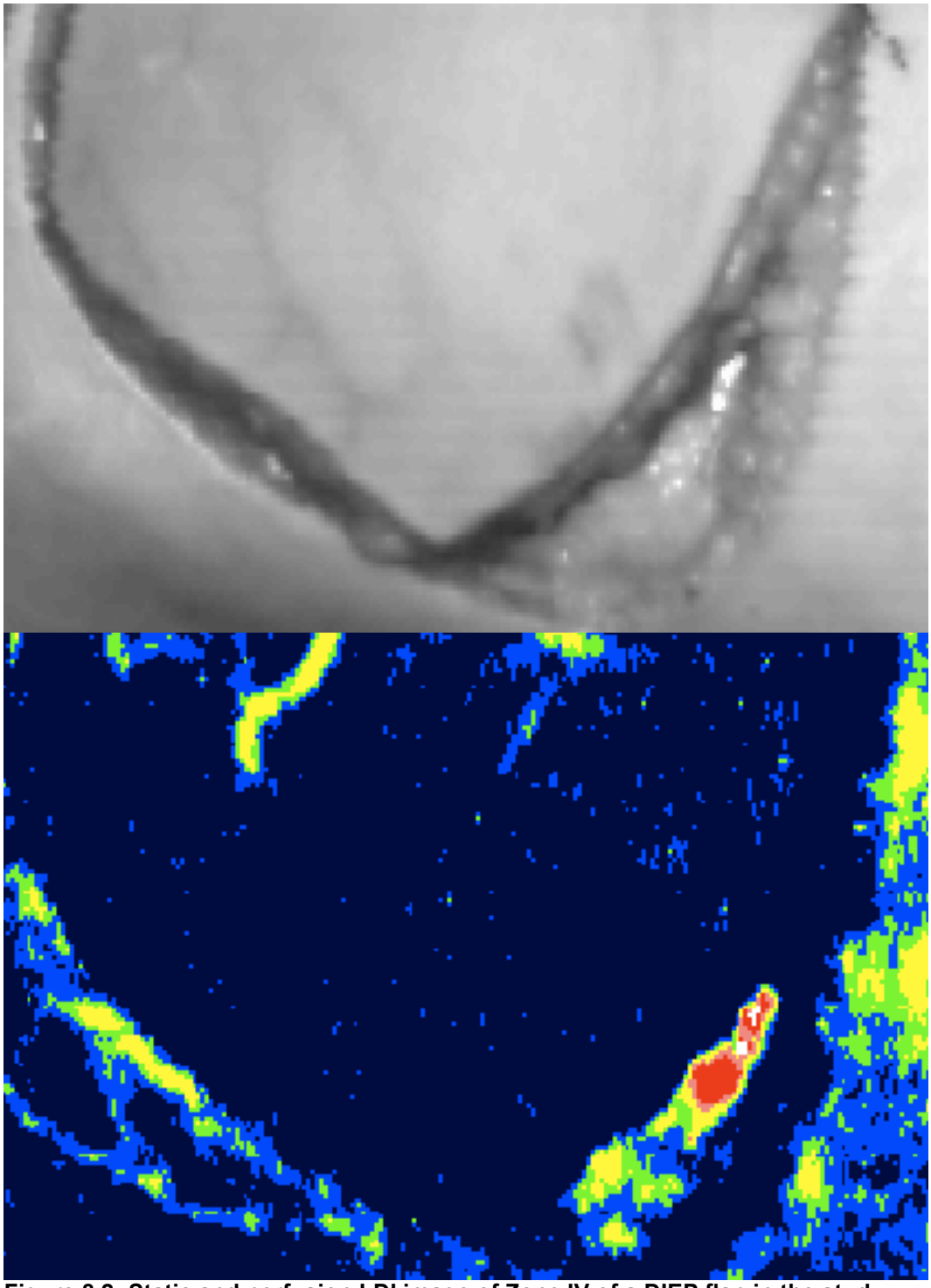


Figure 8.2: Static and perfusion LDI image of Zone IV of a DIEP flap in the study

LDI data perfusion units on a scale from 0-800 can obtained by putting the mouse over any one area of the perfusion image of the scan. SPY-Q analysis software was used to analyse data from the SPY scans, comparing fluorescence between the image sequences recorded using perfusion algorithms to quantitatively compare perfusion values in arbitrary units. As with the LDI software, the SPY software allows a photographic image of the area scanned to be viewed alongside an identical image of the area displayed in coloured perfusion units; again ranging from low perfusion (dark blue) to high perfusion (red). An example of a skin flap image is shown in Figure 8.3.

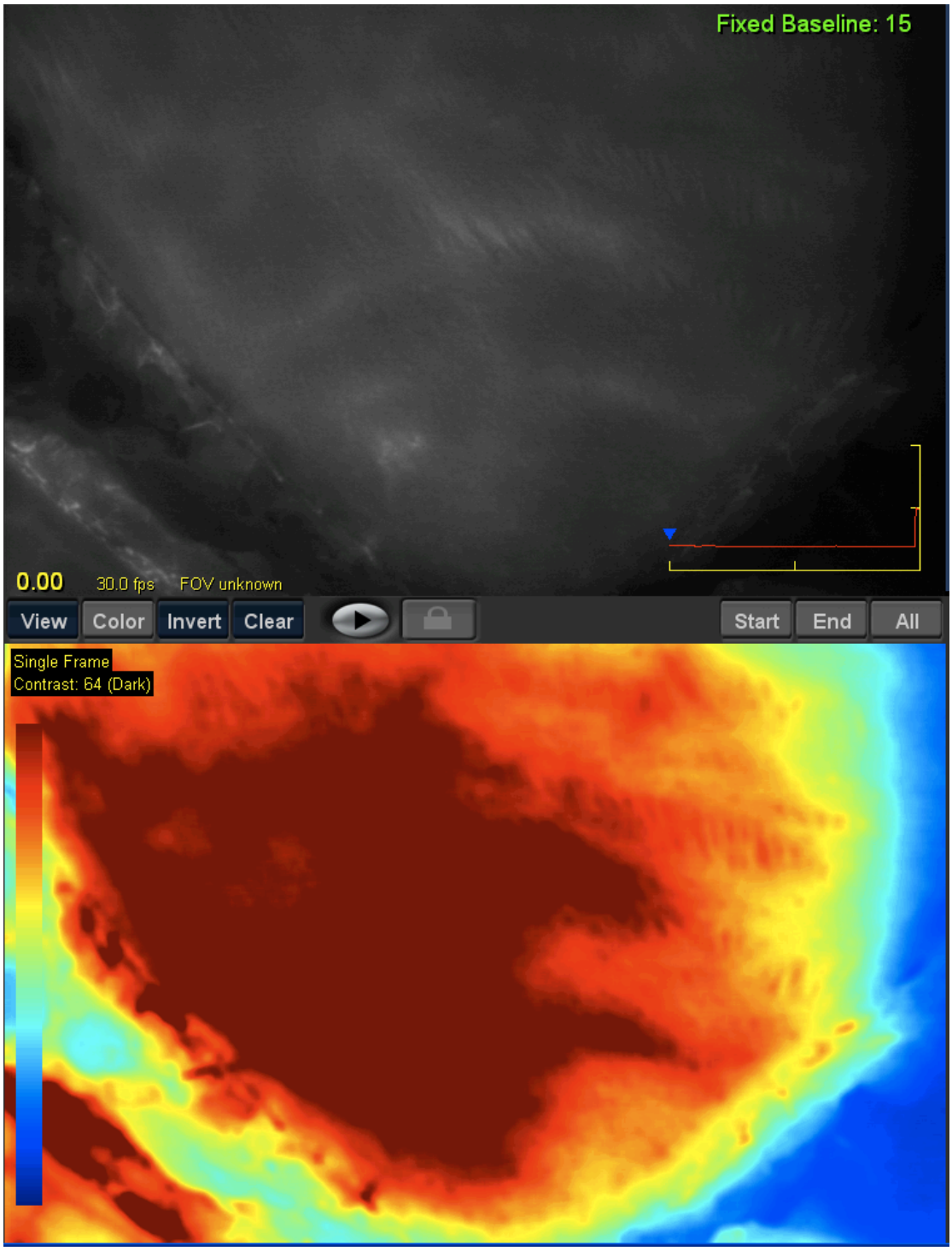


Figure 8.3: Static and perfusion SPY image of zone IV of a DIEP flap in the study

Again, individual perfusion units of any area of the scan can be measured by holding the mouse of the computer unit over the individual area, giving an arbitrary value for perfusion in that specific area, range 0-1000. As previously mentioned, each scan included zone IV of the DIEP flap and an area of 'normally' perfused tissue i.e. an un-dissected area below the flap. The software allows a marker value to be placed on the un-dissected tissue, which is given the value of 100 PU, from which all other values can be measured against relative to this value. An example of this performed in a scan of flap fat can be seen in Figure 8.4, with the '100' marker in the top left hand corner in un-dissected tissue and corresponding data values on the flap area measured.

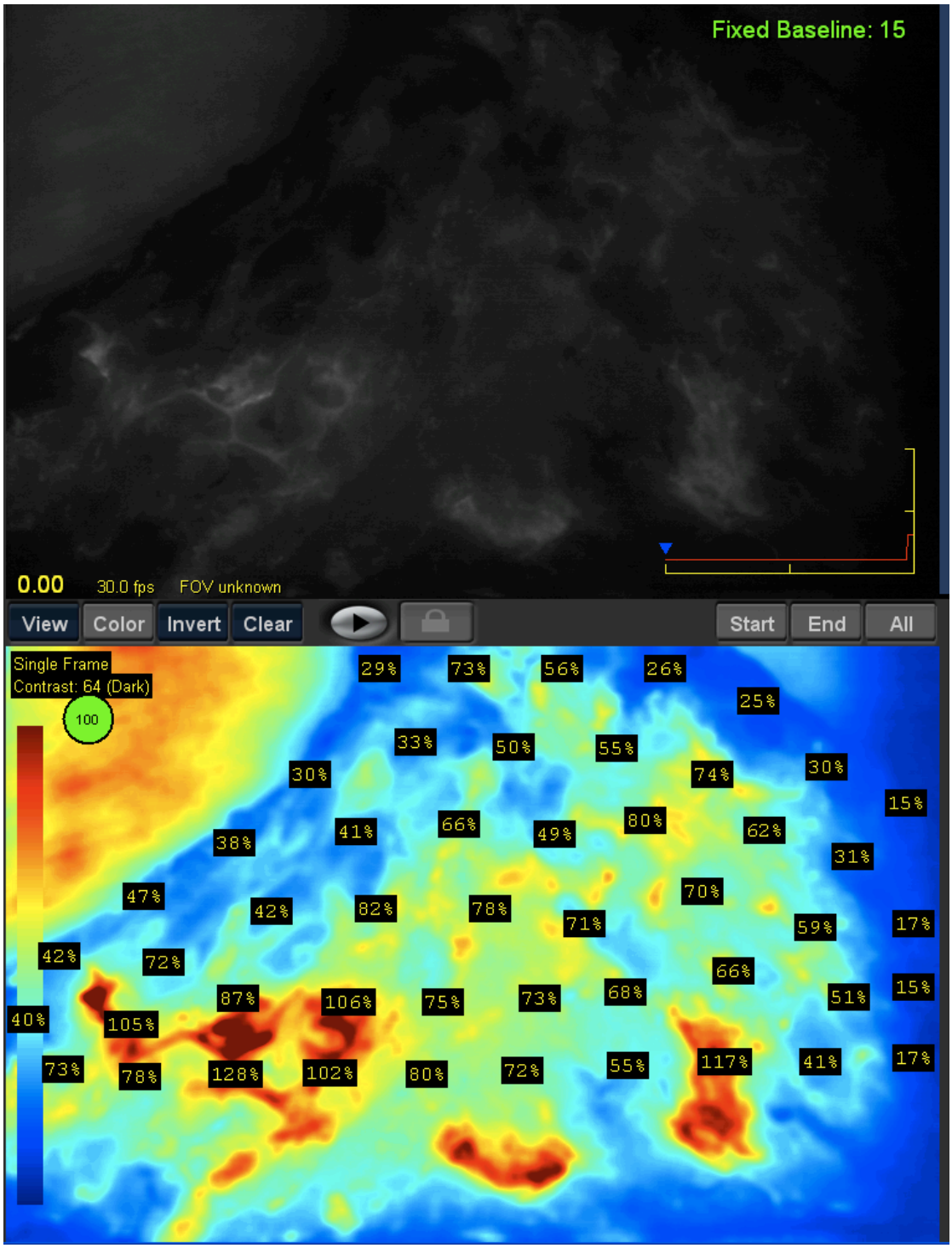


Figure 8.4: Static and perfusion SPY quantative analysis image of zone IV of a DIEP flap in the study

This allows assessment of individual differences in perfusion values at specific points on the flap between the scans obtained, ensuring that the same area of tissue and 'normal' tissue is scanned each time. As the SPY-Q software does not have an average area function to allow summation of perfusion in a drawn area (as is available with the Moor laser Doppler) each scanned area of zone IV had 50 equally spaced reference points chosen and drawn onto a sheet of tracing paper, to attempt to analyse the same points in each flap with each scan, along with the '100' reference marker to ensure this was placed at the same point each time. To create consistency, the same technique of selecting 50 data points on the area scanned was employed for the LDI scans obtained for zone IV skin perfusion on both perforators.

8.3.1 Patients

Table 8.1 summarises patient and flap pre- and intra-operative characteristics.

Patient	Age	Smoker	Previous Chemo	Previous Radio	Immediate / Delayed DIEP	DIEP flap side (L/R)
1	45	Yes	Yes	No	Immediate	Left
2	45	No	Yes	No	Immediate	Right
3	54	No	Yes	No	Delayed	Left
4	43	No	Yes	No	Immediate	Right
5	50	No	Yes	No	Delayed	Left
6	57	No	No	No	Immediate	Right
7	41	No	Yes	Yes	Delayed	Right
8	57	No	Yes	Yes	Immediate	Left
9	52	No	No	No	Immediate	Right
10	56	Yes	Yes	No	Immediate	Right
11	50	No	No	No	Immediate	Left
12	51	No	Yes	Yes	Immediate	Left
Control	54	No	Yes	Yes	Delayed	Left

Table 8.1: Table of patient and flap characteristics

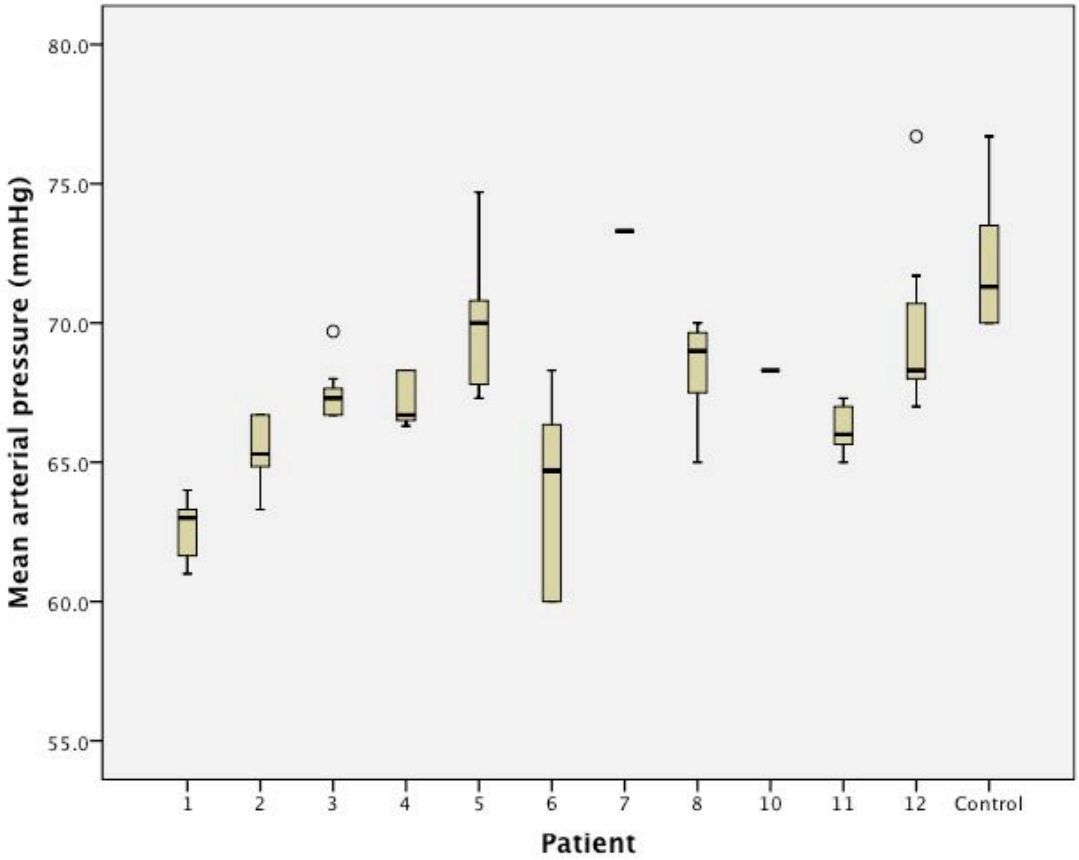
As temperature, blood pressure and heart rate affect cutaneous blood flow and driving pressure (thus blood flow) respectively these data were recorded and assessed to ensure that no significant fluctuations occurred during the data collection period. Mean blood pressures, mean arterial pressures, heart rate and core temperatures during the 30 minute data collection period

are displayed in Table 8.2 and graphically in box-plots in Graph 8.1 and 8.2 for all patients except Patient 9, whose data could unfortunately not be retrieved due to missing notes.

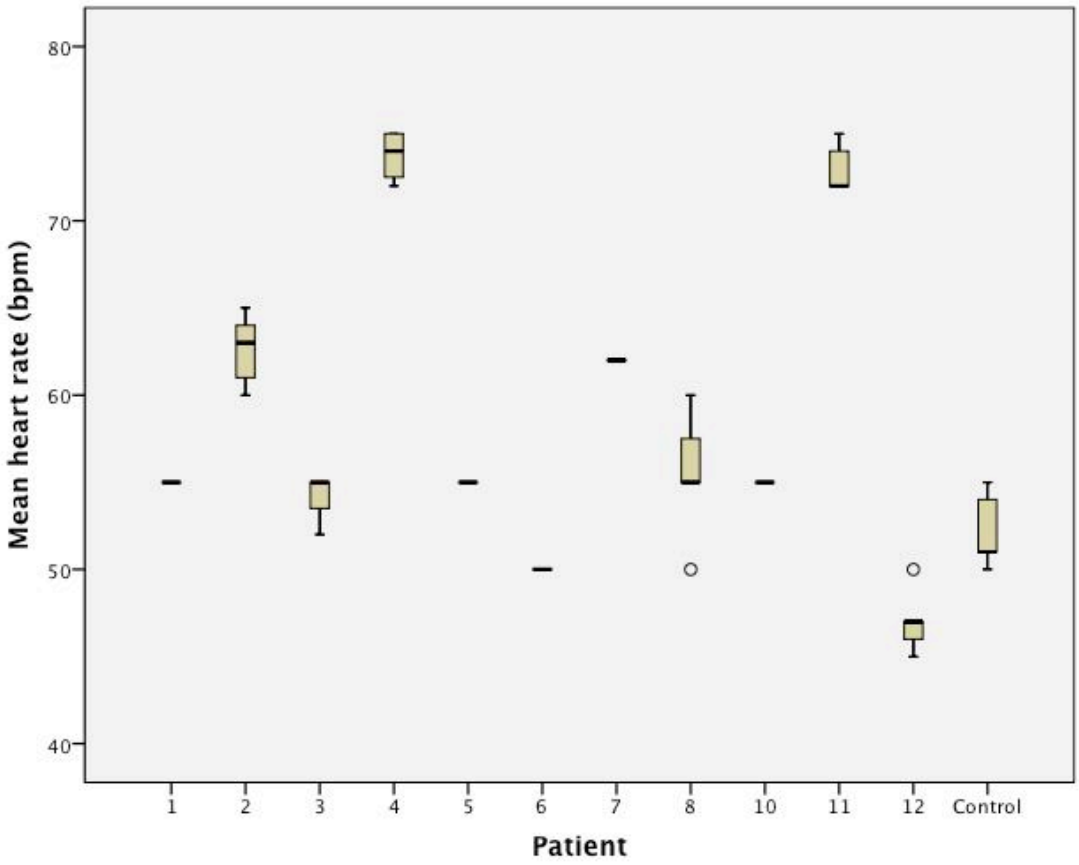
Patient	Mean systolic blood pressure (mmHg)	Mean diastolic blood pressure (mmHg)	Mean Arterial Pressure (mmHg) and range	Mean heart rate (bpm) and range	Mean Core temperature (°C)
1	91	48	62.6 (61.0-64.0)	63(60-65)	36.7
2	99	49	65.5 (63.3-66.7)	63 (60-65)	36.8
3	101	51	67.6 (66.7-69.7)	54 (52-55)	37.4
4	96	53	67.3 (66.3-68.3)	74 (72-75)	36.7
5	96	57	69.9 (67.4-74.7)	55 (55-55)	36.5
6	96	47	63.7 (60.0-68.3)	50 (50-50)	36.2
7	100	60	73.3 (73.3-73.3)	62 (62-62)	36.8
8	115	45	68.3 (65.0-70.0)	55 (50-60)	36.8
10	95	55	68.3 (68.3-68.3)	55 (55-55)	36.3
11	97	51	66.2 (66.0-67.3)	73 (72-75)	36.4
12	106	52	68.9 (67.0-71.7)	47 (45-50)	36.3
Control	112	52	72.1 (70.0-76.7)	52 (50-55)	36.0

Table 8.2: Table of patient and flap characteristics

These data for MAP and heart rate (recorded every five mintes throughout the data collection period) have been plotted into the following boxplot Graphs 8.1-8.2. The narrow ranges for the vast majority of patients can be seen, indicating little variation throughout the study period.



Graph 8.1: Mean arterial pressures with 95% confidence intervals and ranges of patients 1-8 and 10-13 during 30-minute period of data collection



Graph 8.2: Heart rates with 95% confidence intervals and ranges of patients 1-8 and 10-13 during 30-minute period of data collection

8.3.2 Patients 1-6

After the first 6 patients, the perfusion values obtained were analysed to discover if any patterns emerged relative to each of the variables described above (medial vs. lateral row; one vs. two perforators; superior vs. inferior perforators).

Medial vs. Lateral row

This variable was the only one that assessed data between patients, rather than within patients, as each patient could only have one row selected and to compare rows meant therefore comparing the values obtained between different patients. Table 10.2 summarises the mean perfusion values obtained when comparing all perfusion data obtained from all 18 scans for the first 6 patients (6 patients x 6 scans (3 fat + 3 skin)).

Patient	Row	Mean zone IV SPY fat perfusion (units)	Mean zone IV SPY skin perfusion (units)
1	Lateral	18.5	44.0
2	Medial	72.3	133.1
3	Medial	12.3	65.4
4	Lateral	39.3	105.3
5	Medial	13.4	100.2
6	Lateral	65.1	85.2

Table 8.3: Table of mean zone IV SPY fat and skin perfusion on different perforator rows (lateral; medial) for patients 1-6 plus control

Immediately obvious from the average perfusion results above, is the equal numbers of Medial and Lateral rows which had been selected upon clinical ground when looking at both rows of vessels, indicating insignificant clinical variation in the size and calibre of the vessels seen at surgery. The large variation between patients is also apparent, as is the trend for skin perfusion to be higher than fat perfusion in the majority of cases.

In order to reduce time spent scanning during the operation, and to comply with the ethical constraints of this study, the first scans of fat and skin had been performed on both perforators (superior and inferior) in the first instance, with the selection of the next perforator being randomised to either superior or inferior. After discussion with the statistician allied to this project, to rule out any order effect, it was decided that for our next 6 patients a 3-way randomisation block should be performed, as shown in Table 8.4.

Patient	1st SPY Scan perforator	2nd SPY Scan perforator	3rd SPY Scan perforator
7	Superior	Inferior	Both
8	Inferior	Superior	Both
9	Both	Superior	Inferior
10	Superior	Both	Inferior
11	Inferior	Both	Superior
12	Both	Inferior	Superior

Table 8.4: Table of statistical study design for randomisation of patients 7-12 to order of perforator combination measurements with SPY scanner

In addition, prior to this review of results we had always scanned the fat of zone IV of the flap first, followed immediately by the skin. Although only around 40-50 seconds existed between these scans (the length of time taken to perform a scan of the fat and then reposition the flap to scan the skin) our statistical advisor suggested that since there appeared to be a difference between the perfusion values of the skin and fat, we should reverse this order for the next 6 patients, scanning skin perfusion first and fat second. It was decided that these two changes to our design based upon the observed results so far, would allow us to assess whether the effects observed in the first set of patients was due to an order effect or was in fact true. The control patient, as previously mentioned, was included to ensure any increased perfusion measurements were not due to venous congestion and dye retention and to add a measure of reliability to the data. This patient data was not included in any calculations regarding row or perforator number data, and was used purely to assess reliability and repeated accuracy of measurement and to ensure repeated measurements were not accumulating dye in tissues due to venous congestion. Thus, these changes to the design were made and the next 6 patients and the control patient were scanned as advised. These results are summarised in Table 8.5.

Patient	Row	Mean zone IV SPY fat perfusion (units)	Mean zone IV SPY skin perfusion (units)
Control	Medial	45.8	62.0
7	Medial	13.4	32.9
8	Medial	55.5	64.4
9	Medial	37.5	84.1
10	Lateral	14.0	30.6
11	Lateral	35.2	50.6
12	Lateral	49.7	77.1

Table 8.5: Table of mean zone IV SPY fat and skin perfusion with 95% confidence intervals on different perforator rows (lateral; medial) for patients 7-12

Again, an equal number of medial and lateral rows were selected at the time of surgery, indicating no clinical difference in rows at time of theatre and giving us a total of 6 patients in each row study group. Again, skin perfusion appears to be greater than fat perfusion, with large variability between patients.

One vs. Two perforators and Superior vs. Inferior perforators

All patients were also examined irrespective of row, for the differences in perfusion of the fat and skin of zone IV when a single perforator was used compared to both perforators, and in addition, if the position of a single perforator, superior vs. inferior, exerted any effect upon perfusion. The mean perfusion values relating to these variables are shown in Table 8.5 and 8.6 for each patient and also overall.

Patient	Perforator combination	Mean zone IV SPY fat perfusion (units)	Mean zone IV SPY skin perfusion (units)
1	Both	16.8	52.6
	Superior	15.6	49.9
	Inferior	23.0	29.3
2	Both	17.8	130.2
	Superior	106.0	147.1
	Inferior	93.0	122.0
3	Both	8.9	45.2
	Superior	20.4	82.8
	Inferior	7.7	68.1
4	Both	12.4	108.8
	Superior	31.0	112.9
	Inferior	74.4	94.3
5	Both	15.9	57.3
	Superior	17.0	141.2
	Inferior	7.3	102.1
6	Both	20.5	70.1
	Superior	110.2	94.9
	Inferior	64.6	90.6
7	Both	22.1	48.0
	Superior	11.2	41.7
	Inferior	6.8	9.1
8	Both	52.0	54.0
	Superior	46.1	21.4
	Inferior	68.5	117.9
9	Both	29.0	67.0
	Superior	36.5	112.1
	Inferior	46.9	73.3
10	Both	11.2	23.9
	Superior	22.5	63.9
	Inferior	8.3	4.1
11	Both	5.5	17.3
	Superior	43.8	74.1
	Inferior	56.3	60.4
12	Both	35.2	49.0
	Superior	67.1	110.3
	Inferior	46.8	71.9

Table 8.6: Table of mean zone IV SPY fat and skin perfusion on different perforator combinations (both; superior; inferior) for patients 1-12

Perforator combination	Mean zone IV fat perfusion (units)	Mean zone IV skin perfusion (units)
Both	20.61	60.28
Inferior	41.97	70.26
Superior	43.95	87.69

Table 8.7: Table of overall mean zone IV SPY fat and skin perfusion on different perforator combinations (both; superior; inferior)

The higher overall mean values on the superior perforator and the inferior perforator compared to both perforators are clearly seen in Table 8.7.

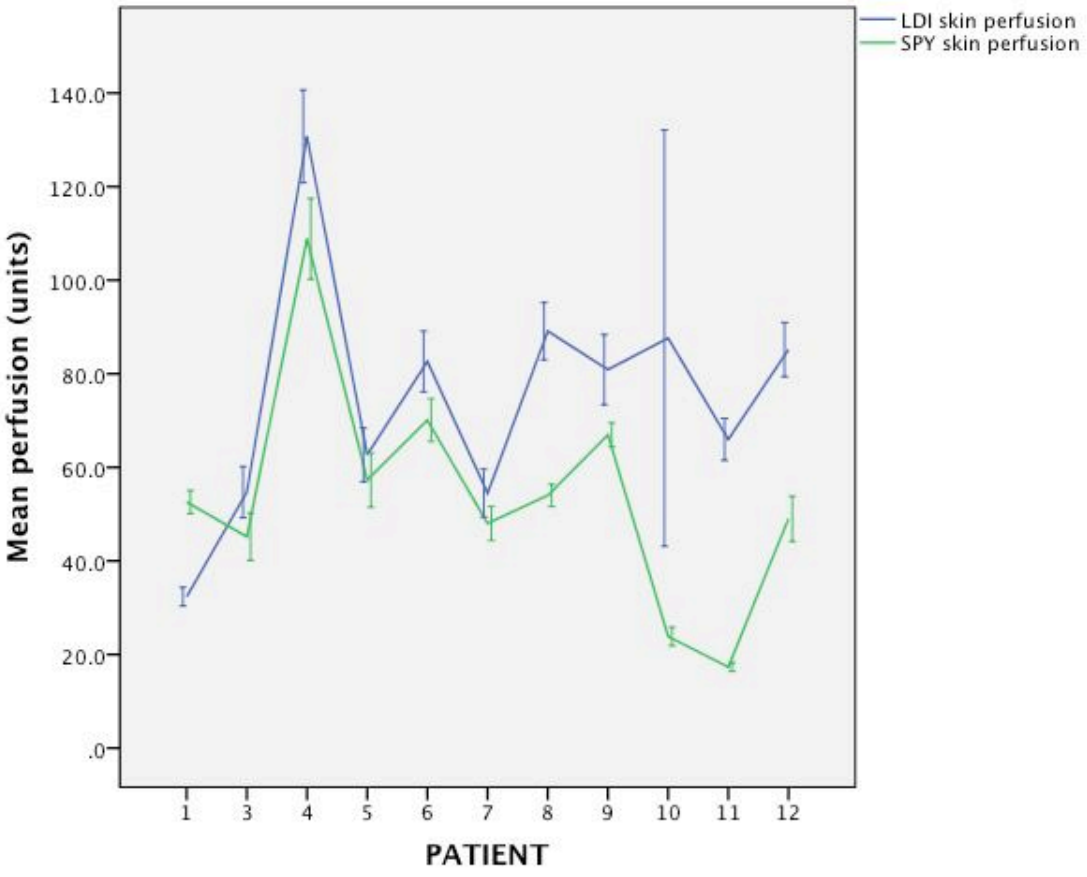
The results from the LDI scan of the skin of zone IV of the flap on both perforators are shown below. This was taken as a measure of skin perfusion that could be compared to the skin perfusion of the SPY scanner to roughly assess how similar the results obtained were. As there was a technical problem with the machine during the second patient's operation, regrettably no LDI scan could be performed for that patient. Therefore scan data for 11 patients is shown in Table 8.8.

Patient	Mean LDI perfusion of zone IV skin (units)
1	32.4
3	54.7
4	130.8
5	62.7
6	82.7
7	54.5
8	89.1
9	80.9
10	87.6
11	66.0
12	85.2

Table 8.8: Table of mean zone IV LDI skin perfusion on both perforators with 95% confidence intervals for patients 1-12

The comparison of the skin perfusion values obtained from the SPY scans of skin on both perforators and the LDI scans (which were performed on both perforators) is difficult to compare directly or apply statistical analysis to, as both machines employ arbitrary units to convert images into quantitative values on different scales which do not correspond. For this reason it

was decided to make a graphical representation of the two machine's measurements to observe if the gross graphical trend was similar in the two methods, lending a degree of comparison. A graph of the relationship between the SPY and LDI values is displayed in Graph 8.3 along with 95% confidence intervals displayed in error lines above and below the mean reference point.



Graph 8.3: Graph of mean zone IV LDI and SPY skin perfusion with 95% confidence intervals on both perforators for patients 1,3-12

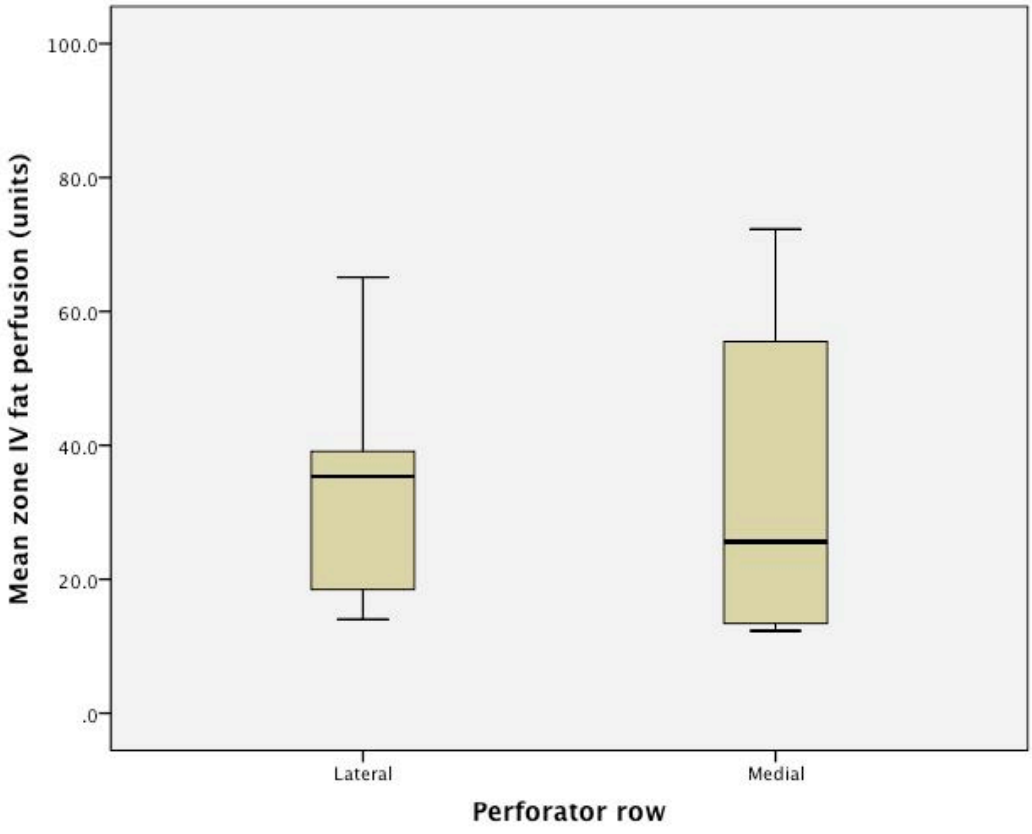
As mentioned, no formal statistical analysis has been performed to compare the SPY and LDI values, as they are both arbitrary in nature and not on the same scale. However, Graph 8.1 shows both systems have small 95% confidence intervals for results making the values obtained more reliable. The only exception was Patient 10's LDI results, which were more varied and probably represent erroneous LDI measurement. The correlation between the two scanning systems is high, particularly when the scans are performed immediately after each other in the first 6 patients. Though the LDI scan was always performed first, the sequence of all SPY scans was randomised for the last 6 patients, therefore a time period of up to 25 minutes was possible between the two scan methods for these patients, reflecting the slightly increased variation seen in this group. It is concluded from these graphical analyses that the SPY and LDI scans of zone IV skin on both perforators had a good concordance, particularly when performed at a similar time.

8.4 Statistical analysis

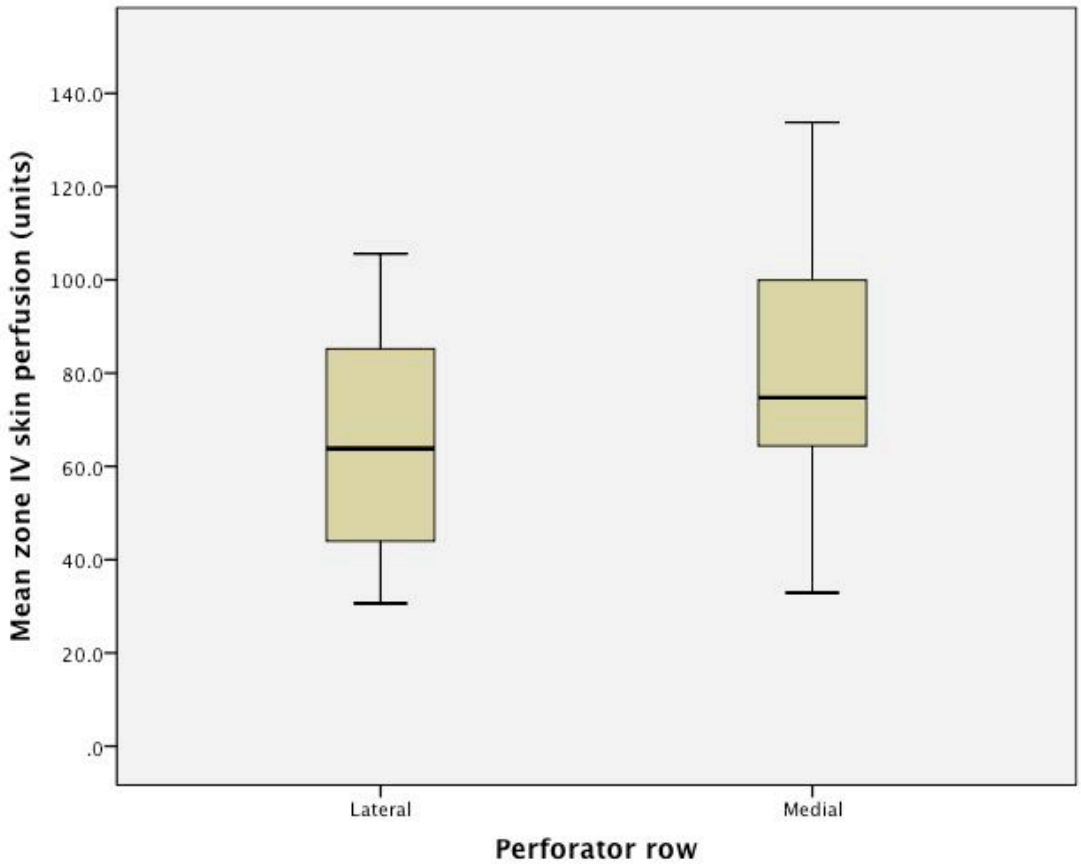
The data collected in this study was not normally distributed (confirmed by the plotting of a scattergraph) and therefore non-parametric tests were performed with SPSS® 21 for Mac® (SPSS IBM, NYC, USA) using the Kruksal-Wallis test of multiple unrelated variables and the Mann Whitney test of 2 unrelated variables. Box-plots were created in SPSS to illustrate the statistical relationships and interactions between the variables and the values obtained.

8.4.1 Medial vs. Lateral row

The statistical analysis of our row data, which as explained above examined data between patients, relied on the inter-patient variability being small, so as to be able to detect any differences in perfusion that could occur by changing the perforator row upon which the flap was supplied. As each of the 6 patients in either the medial or lateral group had multiple data points collected over a period where the variable being examined was not changed (i.e. the patient was either a medial or lateral row perforator for the entirity of the experiment) it was assumed that there was a steady state for these observations and thus values were meaned for each patient and data analysed on the fat and skin perfusion for their row. There was no significant difference in zone IV fat perfusion between medial and lateral row groups (p=0.699), and no significant difference in zone IV skin perfusion between medial and lateral perforator groups (p=0.589). Box-plots of these relationships are shown in Graphs 8.4 and 8.5.



Graph 8.4: Box-plot of median SPY fat perfusion of flap zone IV with 95% confidence intervals for different perforator rows (lateral; medial)



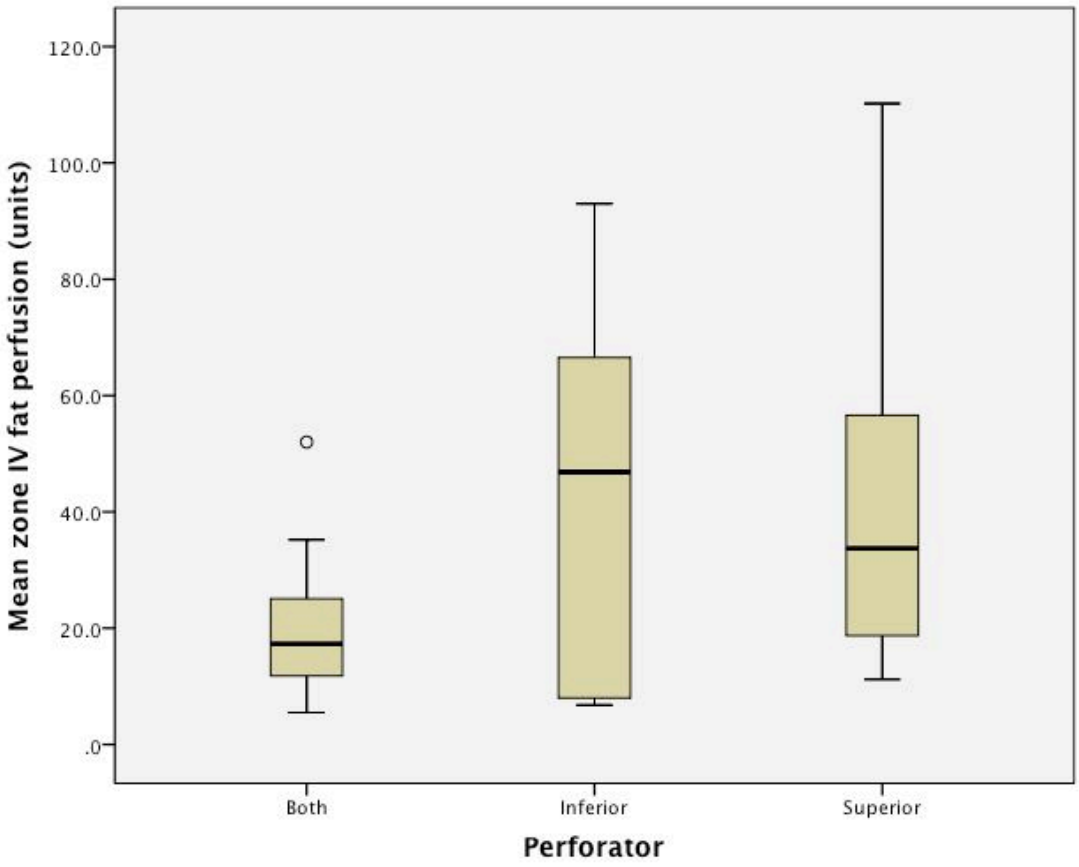
Graph 8.5: Box-plot of median SPY skin perfusion of flap zone IV with 95% confidence intervals for different perforator rows (lateral; medial)

It is clear from the box-plots 8.4 and 8.5 that both medial and lateral rows have wide ranges and are overlapping.

8.4.2 One vs. Two perforators and Superior vs. Inferior perforators

This analysis did not need to rely on low inter-patient variability, as the variables analysed were intra-patient and studies were performed with each patient as their own control. Again, each of the 12 patients had multiple data points collected over a period where the variable being examined was not changed (i.e. during the scan period the patient's flap was supplied by either superior, inferior or both perforators) thus it was assumed that a steady state existed for these observations and values were meaned for each patient on each perforator. The data was analysed on the fat and skin perfusion for each perforator combination .

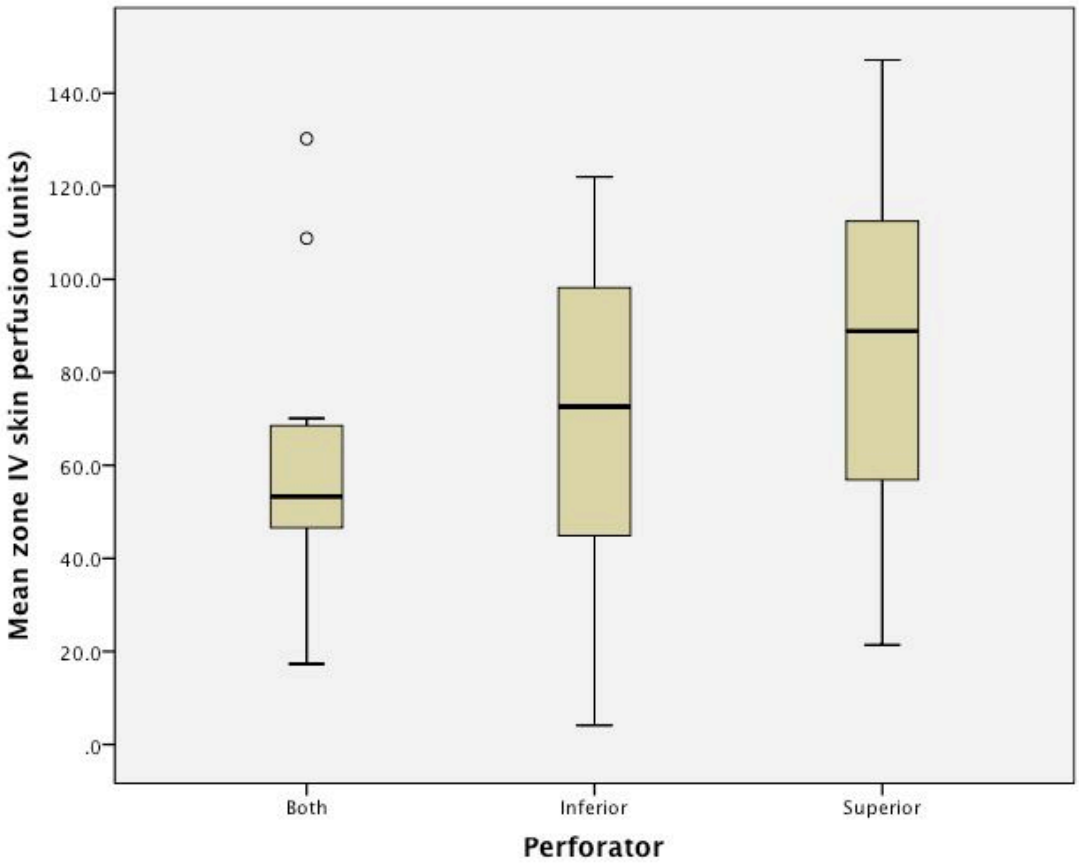
A single superior perforator supplied the fat of zone IV significantly better (p=0.039) than both perforators. Though a similar trend existed for higher zone IV fat perfusion on the single inferior perforator compared to both perforators there was no statistically significant difference in zone IV fat perfusion identified between the single inferior perforator and both perforators (p=0.198). There was no significant difference in zone IV fat perfusion identified between the superior and inferior perforators (p=0.977). Box-plots of these relationships are shown in Graph 8.6.



Graph 8.6: Box-plot of median SPY zone IV fat perfusion with 95% confidence intervals for all perforator combinations (superior; inferior; both)

The lower median value on both perforators is clear to see, along with the narrower confidence intervals and range. Though both the superior and inferior perforators have wide ranges, the very wide confidence intervals of the inferior perforator overlap with both perforators.

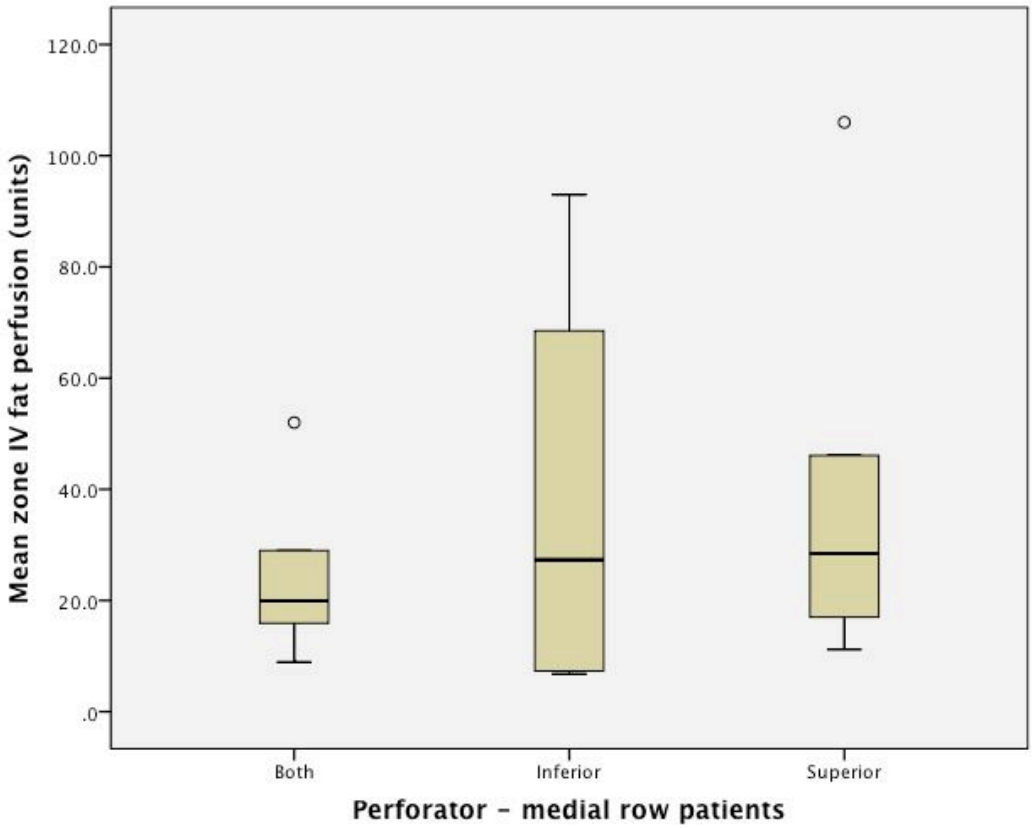
In perfusion of zone IV skin, there was no significant difference between the superior perforator compared to both perforators (p=0.08), no significant difference between the inferior and both perforators (p=0.291) and no significant difference was identified between the superor and inferior perforators (p=0.347). A box-plot of these relationships are shown in Graphs 8.7.



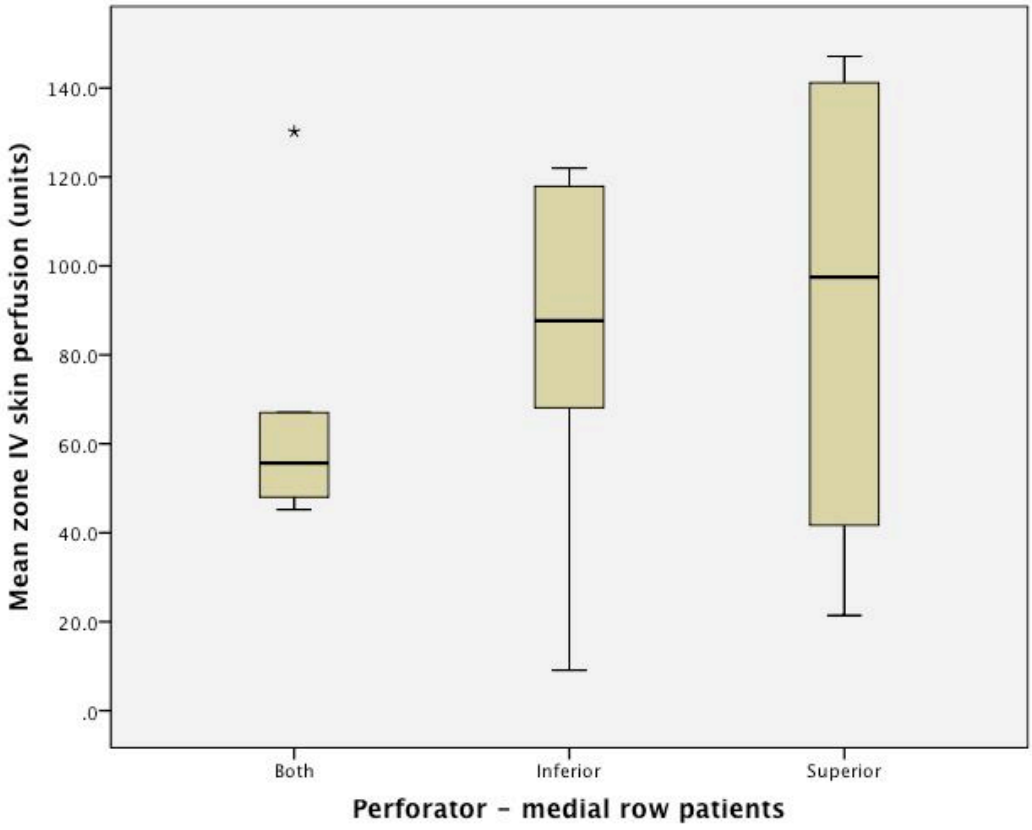
Graph 8.7: Box-plot of median SPY zone IV skin perfusion with 95% confidence intervals for all perforator combinations (superior; inferior; both)

Again the wide overlapping confidence intervals for all three perforator combinations are clear to see. As there were 6 medial and 6 lateral row patients, data was split into these groups to analyse for the same pattern within the different row groups.

In the medial row patients, there was no significant difference in the perfusion of zone IV fat between the superior perforator and both perforators (p=0.589) or between the inferior and both perforators (p=0.937) or between the superior and inferior perforators (p=0.699). This same pattern was observed for zone IV skin perfusion, with no significant difference between the superior perforator and both perforators (p=0.589), between the inferior and both perforators (p=0.310) or between the superior and inferior perforators (p=0.699). These relationships can be seen more clearly in box-plot Graphs 8.8 and 8.9.



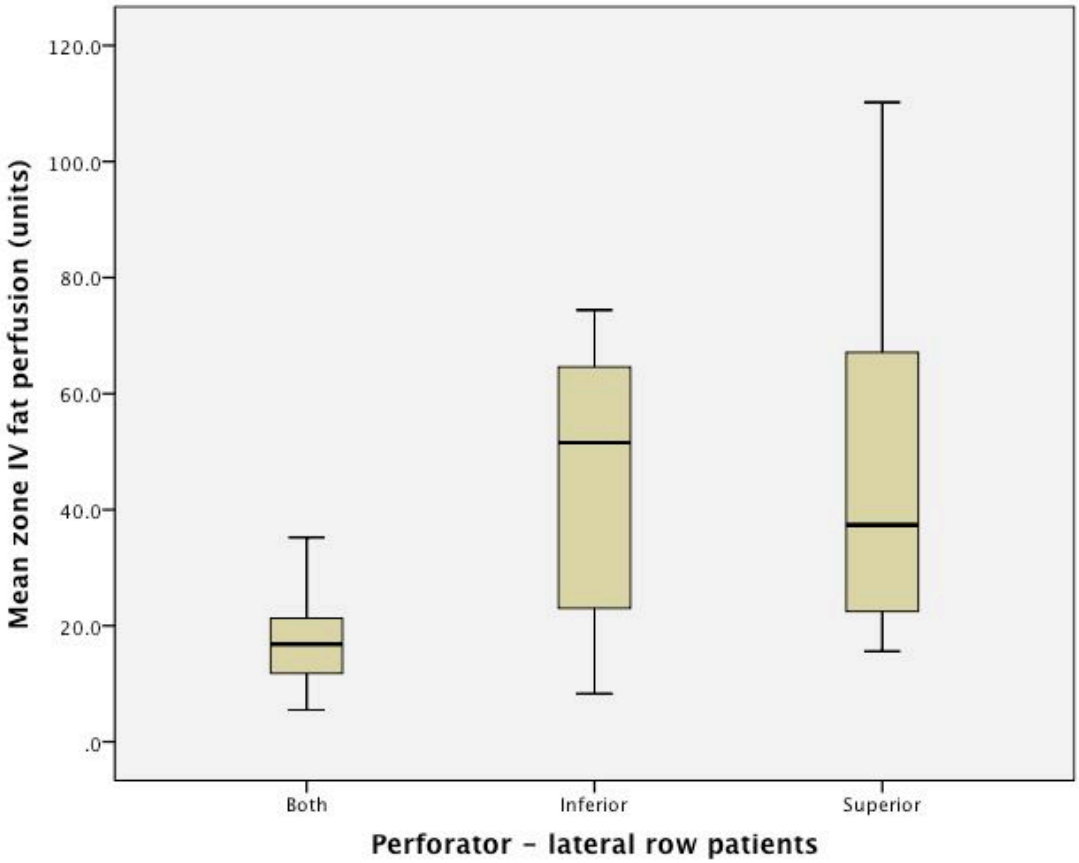
Graph 8.8: Box-plot of median SPY zone IV fat perfusion with 95% confidence intervals for all perforator combinations (superior; inferior; both) on Medial row patients



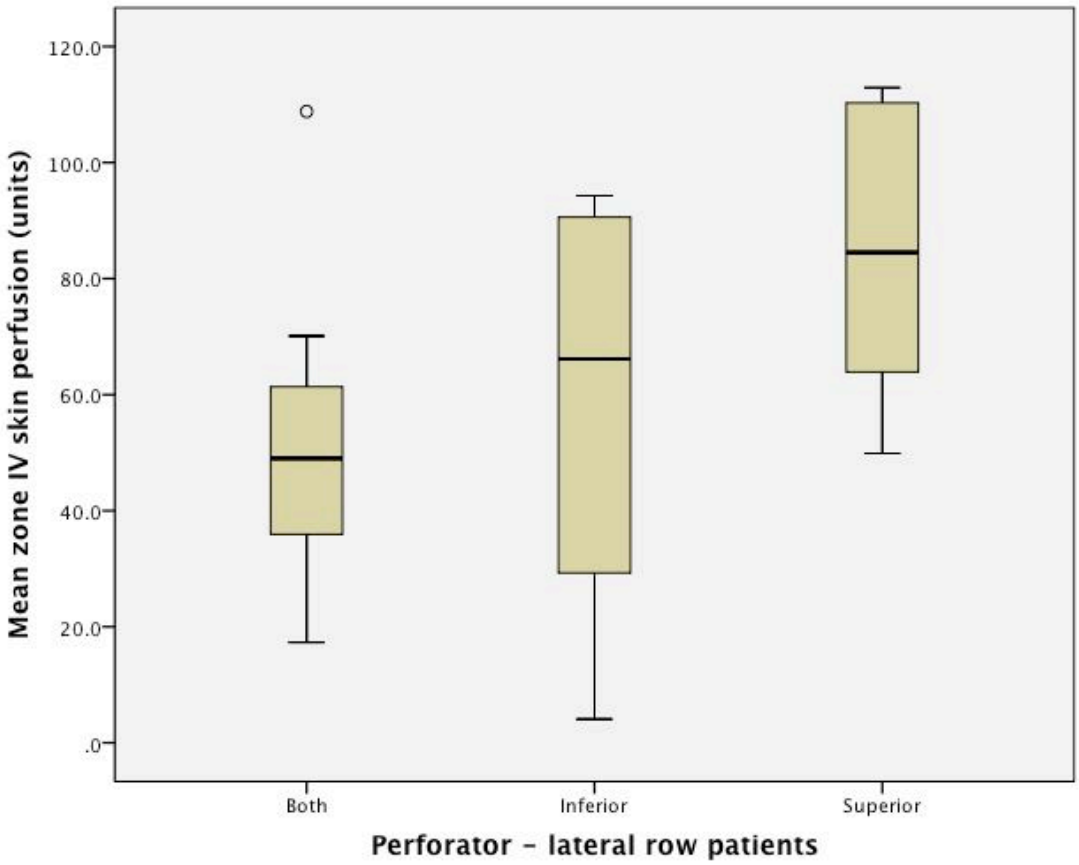
Graph 8.9: Box-plot of median SPY zone IV skin perfusion with 95% confidence intervals for all perforator combinations (superior; inferior; both) on Medial row patients

Again, wide confidence intervals and ranges of the inferior and particularly superior perforators are clearly overlapping with each other and both perforators.

In the lateral row patients, there was a significant difference in the perfusion of zone IV fat between the superior perforator and both perforators (p=0.035). The difference between the inferior and both perforators just failed to reach statistical significance (p=0.051). There was no significant difference between the superior and inferior perforators (p=0.937). In zone IV skin, there was no significant difference in perfusion of zone IV skin between the superior perforator and both perforators (p=0.061), no significant difference between the inferior and both perforators (p=0.628) or between the superior and inferior perforators (p=0.180). These relationships can be seen more clearly in box-plots 8.8 and 8.9.



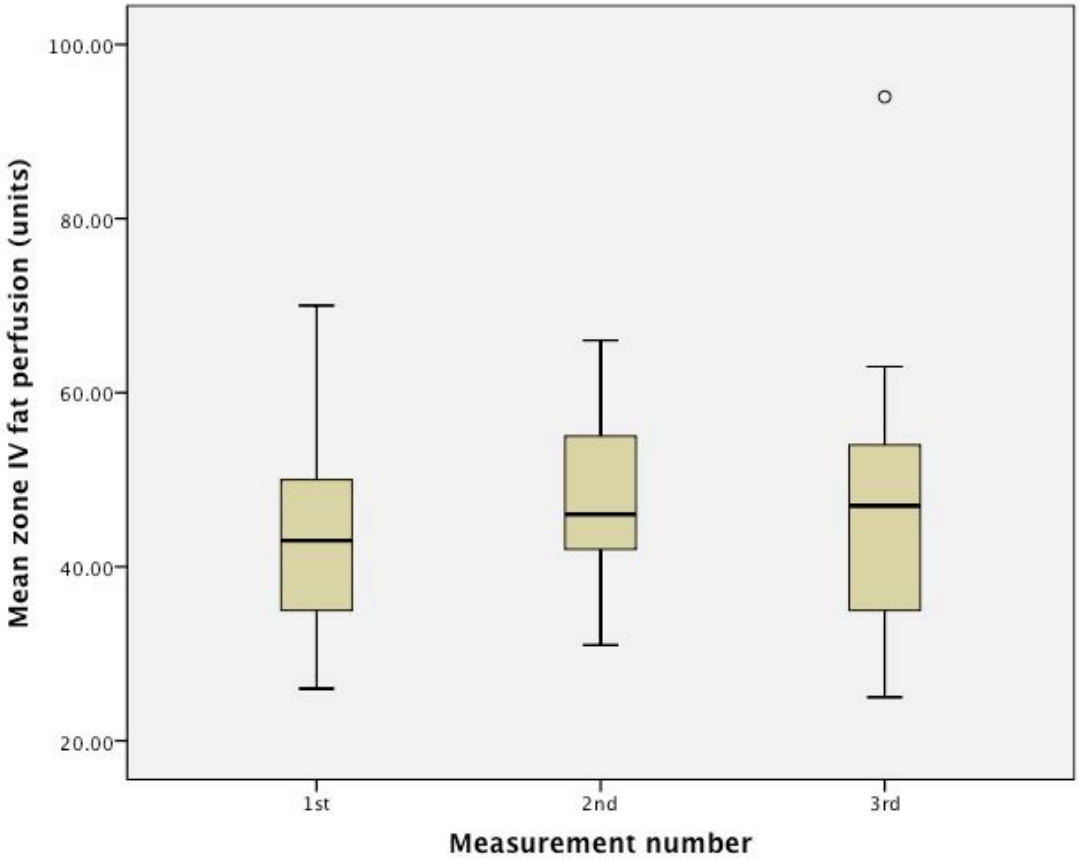
Graph 8.10: Box-plot of median SPY zone IV fat perfusion with 95% confidence intervals for all perforator combinations (superior; inferior; both) on Lateral row patients



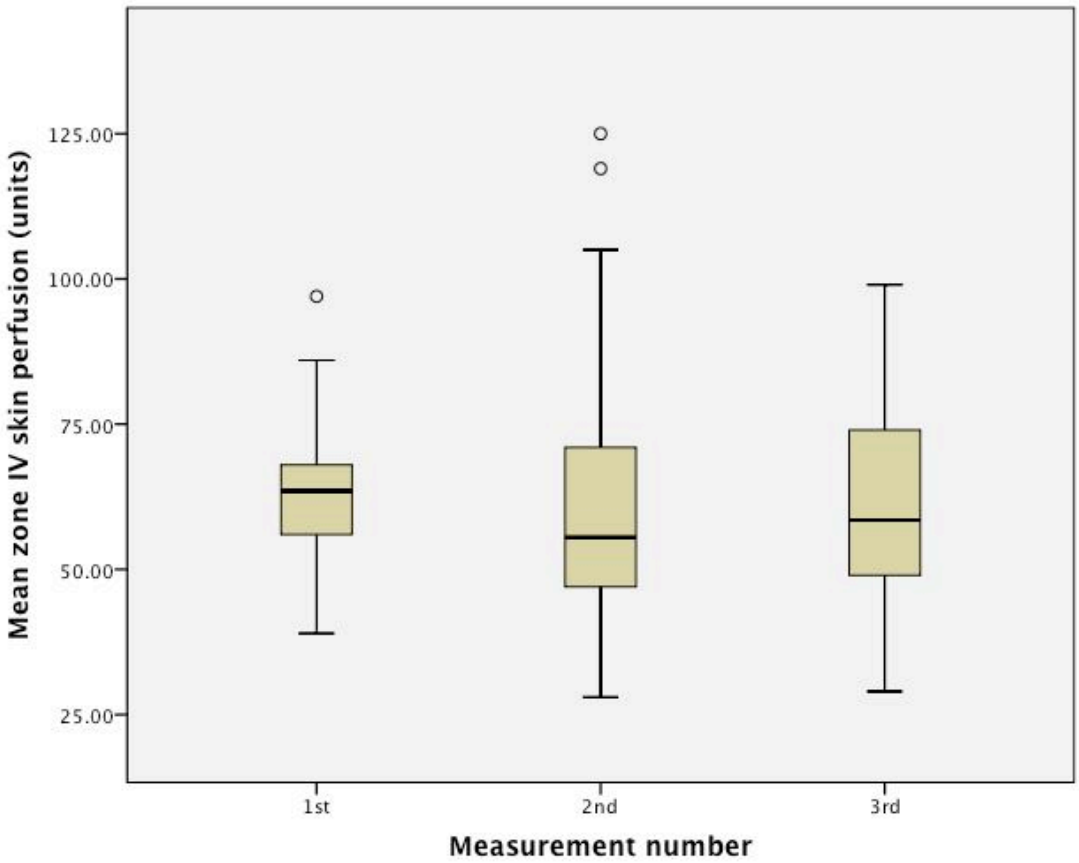
Graph 8.11: Box-plot of median SPY zone IV skin perfusion with 95% confidence intervals for all perforator combinations (superior; inferior; both) on Lateral row patients

Control patient

Analysis of the data obtained from the repeated measures testing of the control patient revealed no significant differences observed for fat perfusion (p=0.511) or skin perfusion (p=0.434). This can be more clearly demonstrated by the box-plots below in Graph 8.12 and 8.13. The median values, confidence intervals and ranges are all very similar.



Graph 8.12: Box-plot of median SPY zone IV fat perfusion with 95% confidence intervals for control patient repeated measures on both perforators



Graph 8.13: Box-plot of median SPY zone IV skin perfusion with 95% confidence intervals for control patient repeated measures on both perforators

8.5 Discussion and Conclusions

8.5.1 Model validation and potential confounders

Zone IV

Zone IV was selected to study in this experiment because as discussed in the Introduction, it is commonly discarded and thus anything which could enhance the reliability of this area would make the flap more clinically augmentable and useful. Zones I-III of the flap are routinely harvested with low rates of flap necrosis. In addition, the two zones of the flap about which there is no debate regarding perfusion (accepting the anatomical delineation of the zones as they stand) are zone I and zone IV. Although this is a physiological study, there would be little clinical benefit in trying to enhance the perfusion in the area of the flap most reliably perfused and thus zone IV was selected.

SPY validation

Many studies reporting clinical data on the use of ICG-NIR-VA use image fluorescence comparison alone without the quantitative software, which does not lend itself to research comparison of values for further studies(Newman and Samson. 2009). Little quantative data regarding measured perfusion values of DIEP flaps in-vivo has been reported in the literature, and as different systems and software give different values and ranges for perfusion units, comparing different ICG-NIR-VA system is not applicable. However, the data values collected in this study using the equipment and software outlined in Chapter 5 is comparable to data previously collected in clinical studies assessing the perfusion of DIEP flaps using the exact same SPY ICG-NIR-VA system, particularly when using a reference point as outlined in the materials and methods of this research(Bank et al. 2012, Losken et al. 2012). This is of course a crude comparison, but as few studies reporting this data exist, it serves to lend some validity to our model and measurements. Further evidence for model validity was presented by comparing the values obtained for LDI and SPY data, as the LDI system is well-validated in assessment of skin perfusion and relatively good correlation was seen in the graphs plotting the mean values. As expected, this correlation was closer when the scans were performed in quick succession, however the same trends were seen in both the LDI and the SPY scans for the perfusion of zone IV skin in different patients.

Temperature/Arterial inflow

Over the 30 minute period of data collection, the blood pressures and thus mean arterial pressures, heart rates and core temperatures of the patients remained stable, though the loss of data for Patient 9 is accepted as a limitation and potential confounder. It can be seen clearly in the graphical representation of the mean arterial pressures in the Results section (Graph 8.1) that whilst small variation existed, there was less than 10mmHg variation in any one patient and often much less. The heart rates exhibited a similar steady pattern (see Graph 8.2), with no

patient's heart rate altering more than 10bpm during the data collection period. This supports the assumption of constant flow into the flap resulting in a steady driving pressure. One patient whose MAP showed more variation than most was the Control patient, and repeated measures testing of the data collected for the flap perfusion with no alteration in this patient gives weight to the lack of effect of these small variations. The measurements of core temperature at 15-minute intervals remained stable, but it is accepted that more frequent measurements during the data collection period would have helped to support this and thus it is accepted as a design limitation and potential confounder.

Data sampling

As mentioned in the Methods section, when sampling data points on the flap software image, a piece of tracing paper was used for each different flap with 50 regularly-spaced crosses, to try to ensure that the same points on the flap were selected at each measurement of the different perforator combinations. The sampling protocol selection of 50 points across the flap to compare to the reference point was initially performed using a pilot examination of 7 points across the fat perfusion scans of flaps of patients 1-6. Initially this was performed as a pilot study to observe if any difference in fat perfusion appeared to exist between the perforator combinations. The choice of 50 point selection data was decided upon to ensure accurate full-flap sampling was undertaken, but the comparison of the results from the 7-point and 50-point data revealed the same pattern and when analysed using non-parametric Kruksal-Wallis and Mann Whitney tests it revealed that a single superior perforator provided significantly ($p=0.01$) better perfusion than both perforators, as did a single inferior perforator ($p=0.025$). No significant difference was observed between the single perforators ($p=0.897$). These results echo the main 50-point data findings of all patients and a box-plot of this pilot method can be seen in Appendix 1.

The repetition of the same data pattern for the first 6 patient's fat perfusion gives some weight to the validity of the sampling method used in the flap perfusion analysis, though it is conceded that this data does not represent every patient or skin perfusion. Testing of the reliability of the sampling protocol could have been improved by repeated assessment of the same scan using the tracing paper method to ensure the same data was captured and this is conceded as a limitation.

Data Analysis

The use of the reference point on the SPY-Q analysis software was performed as stated, to compare the perfusion of the flap to a reference point on un-dissected skin of the patient's own body so as to allow them to act as their own control. The size of the scan (including the flap and a small amount of surrounding normal un-dissected tissue) limited the choice of the location of the '100' marker, therefore this point was always chosen in the middle of the un-dissected skin on the scan. Tracing paper was used to select the same point for the '100' marker each time for each consecutive scan of fat or skin on the different perforator combinations to try to be consistent in the comparison. It is accepted however, that this choice of point is rather arbitrary

and governed by the visible un-dissected tissue within the scan area. Additionally, a repeated measures analysis (similar to the repeated measures data collection control patient) repeatedly analysing the same scan with the '100' marker placed anew each time would have increased reliability of the analysis of the perfusion data and provided evidence for constancy across interventions. The location and repeatability of the '100' marker placement is therefore accepted as a potential confounder.

Venous drainage/Contralateral anatomy

As discussed in the animal study, the role of venous drainage on the perfusion of the flap could have an effect, However, unlike the animal model, the clinical study included a control patient, which had repeated analysis measures taken over a 30-minute time period after the flap had been isolated on the pedicle. This was performed, as stated, to gain a measure of reliability and reproducibility of the data collection and sampling method. Another reason was to ensure that the intra-vascular dye was not being accumulated within tissues which were retaining dye due to venous congestion, and thus making the second and third scans of any randomised patient appear better 'perfused' when in actual fact the dye was simply being retained from the previous scans creating a false positive result. This was not observed, and the repeated measurements showed no significant difference and indeed were almost identical. This provides some support to the data collection, sampling and analysis methods and also allays concern regarding dye retention.

As with the animal study, a potential confounder which could not be addressed is the unknown effect of variation in the divided anatomy of the opposite side of the flap, which could have an effect due to differences in anatomy and perforasomes. This is therefore acknowledged as a potential confounder.

8.5.2 Summary of results and Discussion

Perforator Row

There was no overall statistical difference in zone IV fat and skin perfusion identified between the medial and lateral perforator rows. Since there existed very large inter-patient variability this could be in fact the truth (i.e. there is no difference in zone IV fat and skin perfusion between medial and lateral perforators) or that any effect was masked by the huge variability, which could only be overcome by using much larger numbers of patients, beyond the scope of this project. Therefore no conclusions can be drawn from this study regarding the difference in zone IV fat and skin perfusion on medial and lateral row perforators. However, this work does provide a measure of data standard deviation for any researcher wishing to use the SPY system ICG-NIR-VA technique to compare perfusion in patients and could be used to help perform future power calculations in that respect.

Perforator number and location

In our study, a single superior perforator provided significantly higher perfusion of zone IV fat compared to both perforators, though no significant difference was observed between the single inferior perforator and both perforators or between the two single perforators. There was no significant difference in zone IV skin perfusion between any perforator combination.

When patients were seperated into their different rows (medial and lateral) to observe if the same pattern existed it was observed that in the lateral perforator group a single perforator, whether superior or inferior provided significantly higher zone IV fat perfusion than both perforators but no significant effect on zone IV skin perfusion was observed on any perforator combination. The medial perforator group patients had no significant differences in zone IV fat or skin perfusion across any perforator combination.

The numbers in this study are too small to allow formal subgroup analysis. It is possible that the trend observed for higher fat perfusion on a single perforator (superior or inferior) exists, but was masked by higher variability in the medial row patient group. It is also possible that the lateral perforator row supplies zone IV fat better than the medial row, though as discussed this is not a conclusion which can be drawn from this study due to the large inter-patient variability. It is also possible that no difference exists in the perfusion of zone IV fat and skin on the different perforator combinations, as the numbers are small and the p-values tend toward the lower side of significance.

If a trend does exist for higher zone IV fat perfusion in a single perforator, this could link with findings from Chapter 7. In the animal study it was observed that significantly higher flow and lower pulsatility indices existed with a single perforator as opposed to both perforators, indicating lower resistance. However, there was no significant effect on the perfusion of flap skin in that model, concurring with the lack of any effect on zone IV skin perfusion seen in our clinical study in any of the analyses. As mentioned in Chapter 7, if single perforator use creates shunting through arterio-venous channels in the subcutis, this would result in increased perfusion of the subcutaneous tissue and fat, but not the skin. This is a possible explanation for the higher zone IV fat perfusion seen on a single perforator in the human study, but no effect on the skin perfusion.

As mentioned, though increased flow would seem logically a desirable component to achieve in a flap, very few papers in the literature support any clinical benefit from having a higher measured flow rate in the flap pedicle. In addition, if the proposed explanation for these observations is correct i.e. a physiological decrease in resistance with a single perforator due to arterio-venous shunting in the subdermal plexus, then this may or may not be beneficial clinically. Shunting at the subcutis could potentially result in decreased nutrative flow to the

skin, which could also mean that although the measured flow is higher, the viability is worse. The converse to that argument would be to propose that if the fat receives a higher proportion of the flow then this could be beneficial for fat viability and potentially reduce fat necrosis as skin necrosis is less common. The fact remains that the effect of flow upon perfusion is complex and the resulting effect on tissue viability is not known.

Part IV: Discussion and Conclusions

Chapter 9: Factors affecting choice of perforator vessel selection

9.1 Null hypotheses revisited

1. There is no difference in the total pedicle flow of an epigastric flap rat model whether one or two perforators are used

The null hypothesis is rejected, as a single perforator, whether superior or inferior, significantly increased the total pedicle flow compared to both perforators.

2. There is no difference in the total pedicle flow of an epigastric flap rat model whether a superior or inferior perforator is used

The null hypothesis is accepted, as there was no significant change in total pedicle flow between the superior and inferior pedicle.

3. There is no difference in the skin perfusion of an epigastric flap rat model whether a one or two perforators are used

The null hypothesis is accepted, as no significant difference could be detected in flap skin perfusion whether one or two perforators were used.

4. There is no difference in the skin perfusion of an epigastric flap rat model whether a superior or inferior perforator is used

The null hypothesis is accepted, as no significant difference could be detected in flap skin perfusion whether a single superior or single inferior perforator was used.

5. A single brief period of ischaemic preconditioning makes no difference to the total pedicle flow of an epigastric flap rat model

The null hypothesis is rejected, as the period of ischaemic preconditioning significantly increased the total pedicle flow.

6. A single brief period of ischaemic preconditioning makes no difference to the skin perfusion of an epigastric flap rat model

The null hypothesis is accepted, as the period of ischaemic preconditioning had no significant effect on flap skin perfusion.

7. There is no difference in the perfusion of zone IV fat of DIEP flaps whether a medial or lateral row perforator is used

The null hypothesis is accepted, as no significant difference was identified in zone IV fat perfusion between lateral and medial row perforators.

8. There is no difference in the perfusion of zone IV skin of DIEP flaps whether a medial or lateral row perforator is used

The null hypothesis is accepted, as no significant difference was identified in zone IV skin perfusion between lateral and medial row perforators.

9. There is no difference in the perfusion of zone IV fat of DIEP flaps whether one or two perforators from a single row is used

The null hypothesis is rejected, as a single superior perforator provided significantly increased perfusion to zone IV fat compared to both perforators.

10. There is no difference in the perfusion of zone IV skin of DIEP flaps whether one or two perforators from a single row is used

The null hypothesis is accepted, as no significant difference was identified in zone IV skin perfusion between one or two perforators.

11. There is no difference in the perfusion of zone IV fat of DIEP flaps whether a superior or inferior perforator from a single row is used

The null hypothesis is accepted, as no significant difference was identified in zone IV fat perfusion between a single superior and a single inferior perforator

12. There is no difference in the perfusion of zone IV skin of DIEP flaps whether a superior or inferior perforator from a single row is used

The null hypothesis is accepted, as no significant difference was identified in zone IV skin perfusion between a single superior and a single inferior perforator

9.2 Summary and discussion

The studies performed in this research have found that significantly higher total pedicle flow and significantly lower pulsatility indices were observed when a single perforator was used compared to both perforators in our animal model, and a brief period of ischaemic preconditioning appears to increase flow. Zone IV fat was significantly better perfused with a single superior perforator compared to both perforators but no effect was observed the the single inferior perforator or any difference in zone IV skin perfusion. No significant differences in zone IV fat or skin perfusion were observed between medial or lateral row.

9.2.1 Confounders

To be clear about any conclusions we draw from this data the potential confounders for which evidence was presented to reduce and those which were accepted are summarised and listed below:

Animal study – confounders reduced or accepted

- Number reduction – the reduction in numbers due to exclusion of the first 4 animals is accepted as a limitation of this study. The resource equation has given support to the numbers still being adequate to detect differences but the posibility of bias in the exclusion of these 4 animals is acknowledged.

- Flow model – evidence presented on similar data and models used in the literature

- Perfusion model – no direct literature support to data, but flap zone differences correctly identified using the model, similar to that in the literature giving weight to data collection accuracy.

- Temperature/Arterial inflow – tight maintenace of animal temperatures was performed and data presented. Arterial inflow not measured and accepted as a potential confounder

- Venous drainage/Contralateral anatomy – the effect of venous drainage on the flap is unknown and accepted as a potential confounder as is variation in divided antomy of the contralateral side.

- Observer error – reduced inter-observer with use of a single surgeon/investigator however intra-observer error accepted as a potential confounder

Human study – confounders reduced or accepted

- SPY validation – subjective comparison with similar data collected by similar study using the SPY system in DIEP flaps. Reasonable SPY and LDI correlation for skin perfusion.

- Temperature/Arterial inflow – stable MAP and heart rate data presented for patients. Core temperature stable but measured only at 15 minute intervals and thus accepted as a potential counfounder.

- Data sampling – Sampling concordance between 7-point and 50-point data supporting valid method of sampling. Reliability could have been improved by repreated measures testing af a single scan and this is accepted as a potential confounder

- Data analysis – Repeated measures testing with a single scan with '100' marker re=placed would have improved relaibility and this is accepted as a potential confounder.

- Venous drainage/Contralateral anatomy – Repeated measures assessment with control patient did not retain dye, supporting the lack of any dye retention effect of venous congestion. Variation in divided antomy of the contralateral side is an unknown variable and accepted as a potential confounder.

As discussed in Chapter 7, the increased flow observed on a single perforator in the animal model was combined with a reduced pulsatility index (measure of vascular resistance). Revisiting Ohms law:

FLOW = DRIVING PRESSURE / RESISTANCE

In a vascular system of constant arterial pressure, if resistance decreases, flow will increase. As desribed by Rubino (2006) and Patel and Keller (2008), in a model of perforators, vascular resistance and flow, it would be logical to expect a decrease in resistance if two perforators are used, as this would create resistances would in parallel, rather than in series for a single perforator(Rubino et al. 2006, Patel and Keller. 2008). In the findings of our animal study, this was not the case as a single perforator produced a higher flow and lower measure of vascular resistance. Combined with the lack of any significant perfusion of flap skin in the animal model and our human study which suggests that zone IV fat is better perfused with a single superior perforator, it is possible that whatever is affecting the resistance of the tissues and therefore increasing flow or perfusion on the single perforator, is related to the skin and subcutis. As mentioned, if the single perforator use means that arterio-venous channels are opening in the subdermal plexus, this would cause a physiological decrease in resistance and an increase in flow bypassing the skin. As mentioned, this change in flow could mean a decrease in nutrative flow to the skin or better perfusion of subcutaneous tissue.

The increase in flow after a brief period of IP did not translate to any difference in skin flap perfusion, and as previously discussed, may indicate no resultant significant change in perfusion or vascular channels opening subdermally. Any clinical implications are of course, unknown though as previous studies have used IP in a single long period or multiple shorter cycles, this shorted single period of IP would be far more conducive to perform during a flap operation, where it could be timed during a natural break.

Flow through a vessel relates to tissue perfusion, but is a complex mechanism and shunting is just one of the examples which demonstrate the lack of proportional correlation between a change in flow and change in tissue perfusion. It is possible that the lack of any significant effect observed on the perfusion of flap skin in the human or animal model may indicate that the change in flow and resistance was of an order which did not affect tissue perfusion. In addition, the relationship of tissue perfusion and tissue viability is complex, as it is not known at what level of perfusion skin or fat necrosis would occur, or indeed what level of perfusion would affect fat or skin viability. These are clinical postulates, and this research was physiological so it is fully accepted that it is not possible in this study to demonstrate that any change in flow would have produced a clinical change in tissue perfusion or that any change in flow or perfusion would have any clinical effect on viability of tissue to create a clinical outcome.

9.3 Potential clinical implications

The findings of this research suggest that total pedicle flow and DIEP flap zone IV fat perfusion is significantly increased with the use of a single perforator as opposed to two and a single brief period of pedicle clamping increases flow. These findings are very interesting but it is reiterated that the data presented is physiological and experimental and not based on any clinical outcome measures. Therefore direct translation of these findings to the clinical scenario is obviously inappropriate, because as mentioned, it cannot be demonstrated by this research if any clinical benefit would be conferred by the increase in total pedicle flow or zone IV fat perfusion demonstrated. With this firmly in mind, the following potential benefits are discussed:

As demonstrated by Lorenzetti (Lorenzetti et al. 2001a) the flow within flap tissue is not reliant on the recipient flow to where it is transferred, instead appears to be linked to the anatomical and ohysiological constituents of the flap. If this is the case, then augmenting a flap to have increased flow pre-transfer could mean that it ‘takes’ this increased flow with it when anastamosed. Whether this would have any benefit in terms of increased flap survival or reduced fat/skin necrosis is not known.

Dissection of two perforators, as opposed to a single perforator, increases the duration of the operation and longer operating times have been shown to confer higher venous thromboembolism risk(Edmonds et al. 2004), a risk which is already increased in breast cancer patients with active malignancy(Edmonds et al. 2004). Dissection of an extra perforator also increases the risk of donor site morbidity, through increased risk of rectus muscle fibre and

motor nerve damage, linked with increased risk of abdominal herniation(Vandevoort et al. 2002, Lee et al. 2010). If a single perforator was shown to be more reliable, this could affect the way that DIEP flaps are raised and reduce these risks.

If clinical studies supported the findings of increased perfusion of zone IV fat with a single perforator translating to less fat necrosis then this could increase the size of the flap to be taken, potentially reducing need for symmetrisation procedures. This could reduce risk and inconvenience to the patient and save healthcare providers money, as symmetrisation procedures have been shown to account for significant expenditure after the initial reconstruction, decreasing the impact of the current argument that autologous reconstuction is more convenient for the patient and more cost-effective than implant-based reconstruction in the long term(Malyon et al. 2001, Kroll et al. 1996, Rusby et al. 2010)

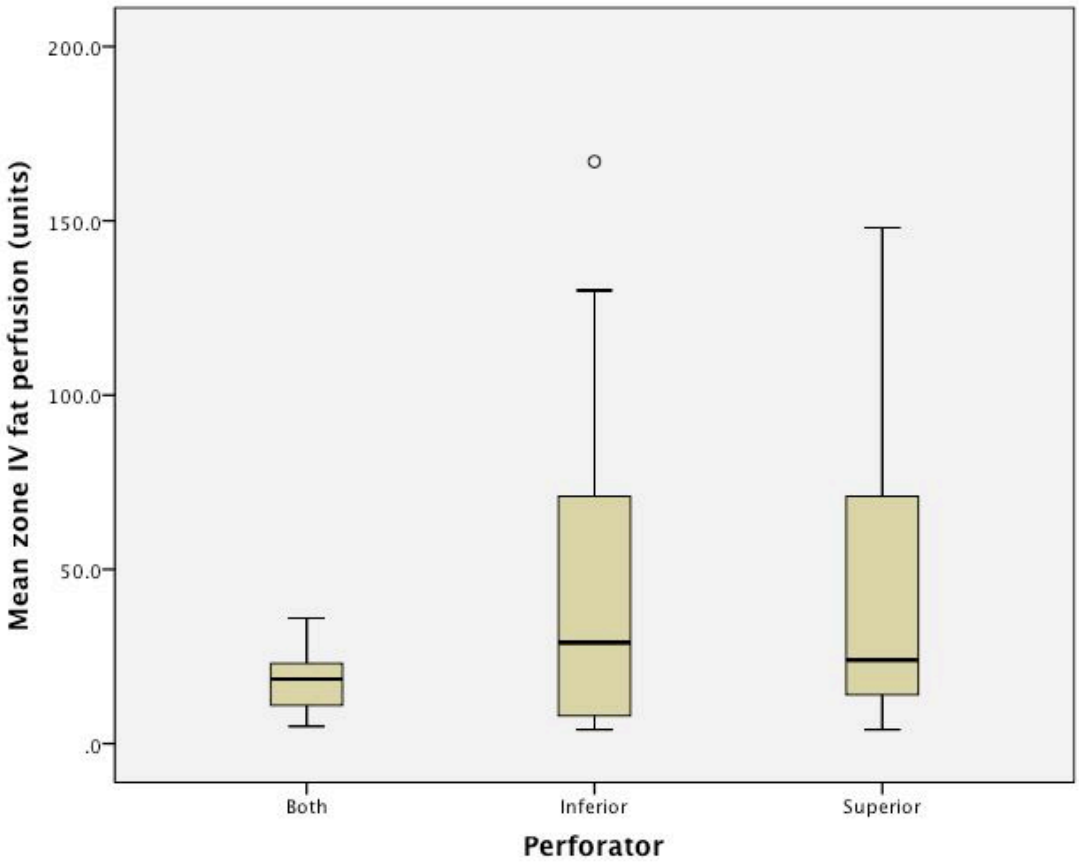
If further study and clinical correlation proved that a single 15-minute period of ischaemic preconditioning was beneficial to flap survival or reduced fat necrosis, then a short period of pedicle clamping could be performed quite easily without lengthening the operation, for example whilst raising the recipient vessels or taking a comfort break.

9.4 Future work

Future projects which could follow this experimental physiological study could include a clinical outcome study measuring fat necrosis and flap survival on one vs. two perforators, however this could not assess zone IV, because as discussed the vast majority of surgeons discard this from their flaps and thus any randomisation into this group would be away from best practice and unethical. Therefore prior to this, a study investigating the effect on perfusion of zone IV fat and skin of altering venous drainage and assessing the impact of the superficial venous system has been commenced and results are currently being analysed.

Appendices

Appendix 1



Graph A1: 7-point SPY fat perfusion data on the different perforator combinations (both; inferior; superior) for patients 1-6

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