The transient hyperaemic response in skin: investigations related to its application in critical illness

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The transient hyperaemic response (THR) is a novel way of assessing vascular reactivity that measures vasodilatory responses after 10-20 second periods of ischaemia. Previous studies have combined the THR test with measurements of microcirculatory perfusion using laser Doppler flowmetry in the of forearm skin of healthy volunteers.

A database of over 1000 measurements in over 100 subjects was analysed to further establish the inter- and intra-individual variability of THR measurements.

THR measurements of skin vascular reactivity were performed in laboratory experiments designed to explore the practicality of the test when investigating vascular reactivity in critical illness. Positive end-expiratory pressure caused fluctuations in skin perfusion but did not alter the THR. Both perfusion and THR could be manipulated by the iontophoresis of several drugs used in critical care, but the possibility of carrier solutions causing vasodilatory effects could not be ruled out.

Norepinephrine was successfully iontophoresed into forearm skin and caused vasoconstriction which partially reversed the decreased THR caused by localised heating.

A pilot study of cutaneous THR measurements in patients with evidence of systemic inflammation demonstrated decreases in vascular reactivity compared with the database of healthy volunteers, but the test was found to be less robust within intensive care than was anticipated.

A retrospective analysis was performed of patients admitted to intensive care following a planned operation, or presumed to have sepsis, in order to evaluate the effects of prior administration of statins and other potentially vasoactive drugs. Previous research

Abstract

demonstrated associations between statin usage and improved outcome in bacteraemic patients. This analysis identified no benefit from prior statin usage. One hypothesis generated by this research was that the pleiotropic effects of different statins were not class effects. Further research designed to evaluate the effects of different statins on skin microvascular reactivity using the transient hyperaemic response is planned.

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Publications and presentations

Aspects of the work presented within this thesis have been published in various forms.

Presentations:

The effect of iontophoresed norepinephrine and heat on skin vascular reactivity as assessed by the transient hyperaemic response in human volunteers *Anaesthetic Research Society,* Leeds, 3/12/2004

Effect of prior statin use on survival following admission to an intensive care unit with a presumed diagnosis of sepsis: a pilot study *Anaesthetic Research Society,* Sheffield, 7/7/2006

Prior use of statins and patient outcome after planned admission to ICU after major elective surgery *Anaesthetic Research Society,* Plymouth, 6/7/2007

Abstracts:

Beed M, Kaur J, Moppett IK, Mahajan RP. The effect of iontophoresed norepinephrine and heat on skin vascular reactivity as assessed by the transient hyperaemic response in human volunteers *British Journal of Anaesthesia* 2005; **94** (3): 409p

Beed M, Campion-Smith J, Hoffman A, Wilson V, Mahajan RP. Effect of prior statin use on survival following admission to an intensive care unit with a presumed diagnosis of sepsis: a pilot study *British Journal of Anaesthesia* 2005; **95** (4): 566p

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Publications and presentations

Beed M, Juttner I, Leung C, Wilson V, Mahajan RP Prior use of statins and patient outcome after planned admission to ICU after major elective surgery *British Journal of Anaesthesia* 2007; **99** (5): 765p

Peer reviewed papers:

Beed M, O'Connor MB, Kaur J, Mahajan RP, Moppett IK. Transient hyperaemic response to assess skin vascular reactivity: effects of heat and iontophoresed norepinephrine *British Journal of Anaesthesia* 2009; **102** (2): 205-9

Statement of orginiality

The work within this thesis is original work which has not been previously submitted.

All research was performed by me, although assistance was provided in data collection in some studies after prior approval with my supervisor, and this assistance is credited in the thesis.

No material within this thesis has been previously written or published by another person, except where due acknowledgement is specifically made in the text.

Medical School Ethics Committee approval, or Local Ethics Committee approval where appropriate, was sought and obtained for all volunteer studies both clinical or laboratory based.

CHAPTER ONE

Introduction and aims

The transient hyperaemic response investigated within this thesis is a relatively novel method of assessing vascular reactivity. In health control of vascular blood flow is dynamic, increasing blood flow to areas of need in order to maintain: delivery of oxygen and nutrients; removal of carbon dioxide and metabolites; balance in tissue ion concentrations; delivery hormones and other mediators; and other specialist functions, for instance urine production within the kidney. Changes in tissue blood flow can be achieved by increasing blood flow throughout the large arteries and veins (the macrocirculation), or by directing it towards a specific vascular bed using neurohumoral control mechanisms. Alternatively local control mechanisms within smaller blood vessels (the microcirculation) can direct blood flow to specific areas of need within tissues. Local control may be maintained by increases in blood flow occurring when there is a build up of vasodilatory metabolites, a decrease in tissue oxygen tension, or a myogenic response whereby changes in vessel wall tension trigger reflex contraction or relaxation of smooth muscle within the microcirculation. These two mechanisms clearly overlap, and even where they do not the effects of one may impact on the other (Guyton AC, 2005).

In the broadest sense the term “vascular reactivity” refers to the ability of the body to achieve dynamic changes in tissue blood flow through acute changes (as opposed to

chronic adaptive mechanisms such as an increase in the number of overall blood vessels).

Vascular reactivity of the cutaneous microcirculation in health and illness

The microcirculation regulates blood flow to organs and is responsible for supplying them with oxygen and nutrients, as well as removing the waste products of metabolism. It is also involved in the delivery of inflammatory mediators to tissues, the maintenance of interstitial fluid balance, and body temperature regulation. The microcirculation is the largest contributor to systemic vascular resistance, and thus is involved in blood-pressure homeostasis. Its endothelium represents a large surface area of cells which are involved in processes ranging from inflammatory responses to coagulation homeostasis. It consists of capillaries, arterioles and venules, with blood flowing from the arterial macrocirculation to the capillaries, via arterioles and metarterioles, and exiting into the venous system via venules. The properties of the microcirculation differ according to which organ they belong to, but in general the different aspects have the characteristics found in table 1.1.

Skin blood flow is highly variable due to the skin's role in temperature regulation, varying between extremes of 50ml/min and greater than 2500 ml/min depending on the environmental temperature. At room temperature average skin blood flow is typically of the order of 400 ml/min, or 250ml/m^2/min when body surface area is taken into account. Such a wide range of blood flow is possible because of the large venous plexus which is present within the subcutaneous tissue, and also because the cutaneous microcirculation is capable of shunting blood from the arterioles to venules through sections of larger arterioles and capillaries (figure 1.1). In some areas of skin which are more exposed to extremely cold contact, such as the hands or the lips, arterio-venous anastomoses also exist which can increase this shunting effect. The very small metabolic demands of skin also mean that in most circumstances blood perfusion is higher than, and thus independent of, nutritional demand (Guyton AC, 2005; Bliss M, 1998).

It is well known that changes in the vascular reactivity of the cutaneous microcirculation are associated with a variety of diseases known to affect blood vessels, including diabetes, smoking, Raynaud's disease and systemic sclerosis (Morris SJ et al, 1995; Stansberry KB et al, 1996; Hilz MJ et al, 2000; Aso Y et al, 1997; Celermajor DS et al, 1996 ; Noble M et al, 2003; Khan F et al, 1994; La Civita L et al, 1998; Goodfield MA et al, 1989). Changes in cutaneous vascular reactivity have also been identified with systemic diseases not traditionally associated with skin pathology, including Alzheimer's disease (Algotsson A, Almkvist O et al, 1995), pre-eclampsia (Eneroth-Grimfors E, 1993), and sepsis (Young JD et al, 1995), raising the possibility that skin vascular reactivity might mirror changes occurring in other, less accessible, microcirculations.

It is not clear what pathophysiological processes are at work within the microcirculation which might affect the vascular reactivity. Such processes are likely to be different for different disease states, resulting in changes such as the down-regulation of endothelial controlled vasodilatation, alteration of sympathetic tone or capillary oedema and disruption of cell-to-cell signalling (Caballero A et al, 1999; Consentino F et al, 1998; Oliviera RP et al, 2002).

Changes in vascular reactivity within peripheral blood vessels and the cutaneous microcirculation have also been noted in subjects at risk of diseases such as non-insulin-dependent diabetes, pre-eclampsia and atherosclerosis, suggesting that vascular reactivity may be altered before clinically apparent features develop (Caballero A et al, 1999; Savvidou MD et al, 2003; Celemajor DS et al, 1992).

The ability to measure change in vascular reactivity has prognostic and diagnostic implications, both in terms of progression of the disease itself and measuring its response to treatment.

Arterioles	Surrounded by smooth muscle Well innervated Size = 10-100μm
Subtypes	
Metarterioles	A short vessel with muscle cells which act as a pre-capillary sphincter between the arteriole and the capillary bed
Capillaries	No smooth muscle No innervations Size = 5-10μm
Subtypes:	
Continuous endothelium	This may have many tight junctions and transport vesicles (for example in skin and muscle), or very few (representing the blood-brain-barrier of the cerebral capillary bed)
Fenestrated	Where there are pores in the endothelium to allow fluid or small molecules to pass (found in the renal glomerulus, and also in endocrine glands)
Sinusoidal	Found in liver, lymph glands and bone marrow allowing erythrocytes and white cells to pass through large pores
Venules	Limited smooth muscle Limited innervations Size = 10-200μm
Subtypes:	
High endothelial venules	Lined with cuboidal cells allowing lymphocytes to enter

Table 1.1 The characteristics of blood vessels within the microcirculation

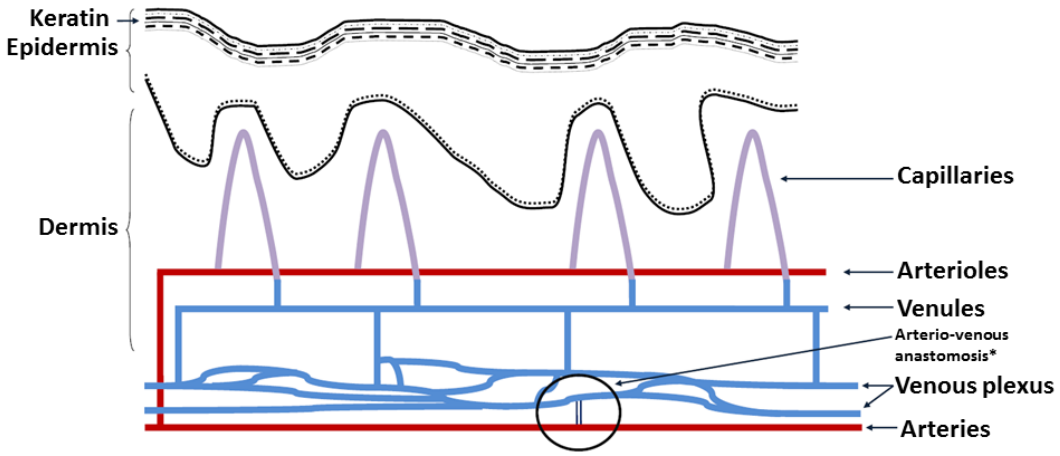

Figure 1.1 The circulation of the skin. Arterio-venous anastomoses are not found in all areas of skin (see text).

Investigating vascular reactivity

Vascular reactivity within the microcirculation may be assessed in a number of ways. In order to assess vascular reactivity a means of imaging changes within the microcirculation is required. Alternatively surrogate markers of changes within the microcirculation may be measured. In both cases the microcirculation must also be stressed in order to trigger the changes which occur as a result of intact vascular reflexes.

Several methods are available which can "image" in-vivo vascular changes. Ultrasound imaging (either 2D or Doppler) of larger blood vessels such as the brachial, superficial-femoral, and middle meningeal arteries has previously been investigated as a means of assessing vascular reactivity (Celermajor DS, 1992; Celermajor DS, 1996; Cavill G et al, 1998). These measurements are not specifically of the microvasculature, and it is unknown how well any changes are mirrored between the two sites. Measurements of oxygen saturation, using near-infra red spectroscopy (NIRS), and carbon dioxide tension, using transcutaneous electrodes, have also been investigated in situations where the microcirculation might be altered (Crookes B et al, 2006; Haisjackl M et al, 1990). Compared with transcutaneous electrodes, NIRS is a much less invasive method and can be used to target muscle vasculature; work is underway to identify which muscle areas yield the most clinical useful information. Plethysmography has been used to measure changes in blood flow, but this also is not specific to tissue beds (Bird AD et al, 1967)

Direct imaging of the microcirculation using orthogonal polarized spectral imaging has been used in conditions such as sepsis (De Backer D et al, 2002). This technique has the advantage that blood vessels within the microcirculation can actually be visualized and qualitatively assessed (delayed semi-quantitative imaging is also possible). One of the disadvantages is that it can only target the microvasculature near the surface of mucosal membranes, for example in the sublingual blood vessels.

Laser Doppler flowmetry (LDF) was first used in the 1970s and '80s as an experimental technique for measuring tissue perfusion. It has most commonly been used to monitor skin perfusion, although it can also been used to monitor the perfusion of mucosal surfaces (such as the intra-oral cavity), organ surfaces during "open" operations (via both surface and needle probes), and the choroidal surface of the eye. LDF is a non-invasive and readily portable method of measuring skin microcirculation perfusion, and for these reasons it was chosen as the technique of choice for the experiments within this thesis and will be covered in greater detail later in this chapter.

In some cases simply monitoring the microvascular blood flow over time will deliver information about vascular reactivity, for example in conditions where the vasculature becomes stressed, such as developing illness or exercise. In other cases this may be as simple as instituting limited exercise (for example hand-grip exercises), localized heating, or pressure loading the skin (Kennedy WL et al, 1991; Kellogg DL et al, 1993; Agewall S et al, 1999; Hassan AA et al, 1988; Noble M, 2003).

An alternative approach to measuring skin vascular reactivity is to evaluate the maximal dilatation the skin microcirculation can achieve in response to a stimulus. Such stimuli include the introduction of vasodilatory substances, such as acetylcholine and sodium nitroprusside, or a prolonged period of ischaemia (typically 3-10 minutes) – the post-occlusion hyperaemic response (Morris SJ et al, 1995; La Civita L et al, 1998; Goodfield M et al, 1989; Kvernebo K et al, 1989).

Laser Doppler flowmetry

Laser Doppler flowmetry uses light generated by light-emitting diodes or by helium/neon lasers of wavelengths between 540 and 850 nm (i.e. within the visible-red and near-infra-red parts of the electromagnetic spectrum); the choice of wavelength being dictated by the absorption characteristics of skin and muscle, as well as that of erythrocytes. Using a

low power laser (typically less than 1mW at source) tissue penetration of 1 to 2 mm depth can be achieved without damaging or substantially heating the intervening structures. Upon entering the tissue light scattering occurs. The scattering effect prevents laser Dopplers from focusing upon either a specific depth or upon a specific point within any two-dimensional planes represented along that depth (i.e. they have neither focus nor resolution). Instead laser Dopplers measure the average Doppler-effect within a small volume of tissue. Since light will scatter multiple times whilst passing through tissue a range of Doppler shifts will be recorded according to the formula:

$$\beta_D = -\frac{4\pi\nu\,(\sin\frac{\theta}{2}.\cos\varphi.\cos\,\alpha)}{\lambda}$$

Where β_D is the angular frequency shift (in rad/s); ν is the velocity of the scattering particle; λ is the light wavelength within the propagating medium; and θ, φ, and α represent the various scattering angles (θ is the angle between the incoming and scattered light , φ is the angle between incoming light and the direction of the scattering particle *within the plane of scattering*, and α is the angle between the direction of the scattering particle and plane of scattering).

Where tissue is not moving, erythrocytes will be the largest contributor to Doppler shifts, and when compared with ultrasound Doppler the effect of scattering will result in two important differences: firstly because most erythrocyte scattering is *forward* scattering with small scattering angles (θ is close to zero) the largest Doppler shifts will occur when particles are moving *across* the path of laser light (rather than parallel to it as with ultrasound waves); and secondly because light will be scattered multiple times before encountering an erythrocyte each scattering event can be considered to be a random change in direction when all the events are summated. Thus laser Doppler is relatively insensitive to the direction of travel of erythrocytes and can represent an average picture of their velocities in all direction. Backscattered light can be analysed by a light-sensitive

sensor resulting in an aggregate measurement of frequency distributions and phase changes (due to the varying distances travelled by individual photons at any given frequency) known as a Doppler power spectrum. The Doppler power spectrum is proportional to both the concentration of *moving* erythrocytes and the average velocity of erythrocytes. This in turns is proportional to tissue blood perfusion as:

$$Tissue\ Perfusion \propto C_{RBC}(v_{RBC})$$

Where C_{RBC} is the concentration of moving erythrocytes (Red Blood Cells), and v_{RBC} is the average velocity of erythrocytes.

The scattering effect also allows laser Doppler techniques to measure much slower objects, such as erythrocytes within the microcirculation of the skin and other organs (where average velocity can be of the order of 100-400 μm/s).

The greatest single limitation of laser Doppler flowmetry is its inability to provide an absolute measurement of blood flow. Like most methods employed to investigate the microcirculation it may be affected by regional variations in blood flow. It is also susceptible to temperature changes, movement artefact, and external light interference (Vongsavan N et al, 1993; Obeid et al, 1990; Carpentier, 1999; Cooke et al, 1990). The practical application and limitations of LDF are discussed in greater detail in chapter 2.

Hyperaemia and the transient hyperaemic response

Hyperaemia is the vasodilatation of blood vessels occurring in response to local demand. It may be active, triggered by extra activity, or reactive, provoked by tissues deprived of blood supply and oxygen for any reason. Sustained ischaemia can be used to stimulate reactive hyperaemia to occur; this is typically achieved by inflating a pneumatic limb tourniquet to suprasystolic pressures, and is often referred to as post-occlusive reactive hyperaemia. Periods of occlusion as short as 5 seconds can provoke hyperaemia, but

prolonged periods (approximately 5 minutes) are often used as they result in a sustained, maximal response. Hyperaemic vasodilatation occurs in advance of the return of blood flow and is thought involve both vasodilator metabolites and myogenic reflexes. There may also be a sympathetic element, although hyperaemia is known to occur in denervated skin. Hypoxia, decreased pH, prostanoids and endothelial nitric oxide production have all been implicated to a greater or lesser degree in the metabolic triggers of reactive hyperaemia, but it is likely that there is more one than chemical responsible (Bliss M, 1998; Crawford DG et al, 1959;Khan F et al, 1991; Guyton AC et al, 1964; Larkin SW et al, 1993; Carlsson I et al, 1987; Engelke KA, 1996; Tagawa T et al, 1994; Nugent AG et al, 1999).

The transient hyperaemic response (THR) occurs when arterial occlusion is only briefly applied. In this instance the vasodilatation which results is not sustained and the hyperaemic response is transitory in nature. It is thought that brief periods of arterial occlusion (typically between 5 and 30 seconds) do not allow for the build up of vasodilatory metabolites, and so any hyperaemic response is likely to be dominated by the myogenic response (Moppett IK, Davies JA, et al 2003).

Using only a brief stimulus for hyperaemia offers certain advantages compared to classical means of inducing hyperaemia. Firstly it is unlikely that such a brief period of decreased blood flow is likely to damage “down-stream” tissues and organs. This is particularly important where the “down-stream” organ is likely to be particularly sensitive to periods of decreased perfusion, for example when the artery occluded is the common carotid artery the organ affected is the brain (and in clinical scenarios this may well be an already damaged brain with areas which are highly perfusion-dependent). Secondly THR can be used to target examination of the myogenic response, rather than the rate of build up of anoxic metabolites. And lastly tests involving THR can be repeated at short intervals, as they are unaffected by intervening periods of prolonged hyperaemia, which

allows for dynamic testing to be performed before and after discrete events (Moppett IK, Davies JA, et al 2003; Brown H et al, 2003).

The THR test involves inducing a transient hyperaemic response with some form of arterial compression and measuring the perfusion, or flow, immediately before and after. The transient hyperaemic response of the cerebral circulation has been investigated using transcranial Doppler ultrasound measurements of the middle cerebral artery (Cavill G et al, 1998; Sherman R, Bowie R, 2002; Sherman R, Armory P, 2003). Blood velocity within the middle cerebral artery can be estimated using a trans-cranial Doppler ultrasound probe which is placed against the temporal bone (figures 1.2 and 1.3). Although, as previously stated, trans-cranial Doppler differs from laser Doppler flowmetry, resulting in measurement of peaks of blood velocity as compared to a continuous measurement of tissue perfusion. Despite this the principles of the THR test remain the same, with the transient hyperaemic response ratio (THRR) being the ratio of perfusion/flow-flux (or flow velocity) immediately after release of arterial compression to the perfusion/flow-flux immediately before compression (figure 1.4).

The cutaneous THR test can be performed on any area of skin where arterial occlusion is possible and a laser Doppler probe can be fixed to the surface of the skin via an adhesive dressing. In practice this means that limbs are favoured, particularly the forearm. Arterial occlusion and release must both be rapid, and there must be no collateral circulation, in order for a true hyperaemic response to be induced. A compressed gas pneumatic tourniquet can be used to achieve this in limbs (Marval PD et al, 2004), as can digital compression of the brachial artery for forearm measurements (Brown H et al, 2003). Arterial occlusion is applied for 20-30 seconds (as compared with typical occlusion periods of 5-10 seconds for trans-cranial Doppler THR measurements), with a “recovery” period after each THR test to allow time for the dispersal of hypoxic metabolites which

might otherwise accumulate with repeated testing prolonging or increasing any hyperaemic response.

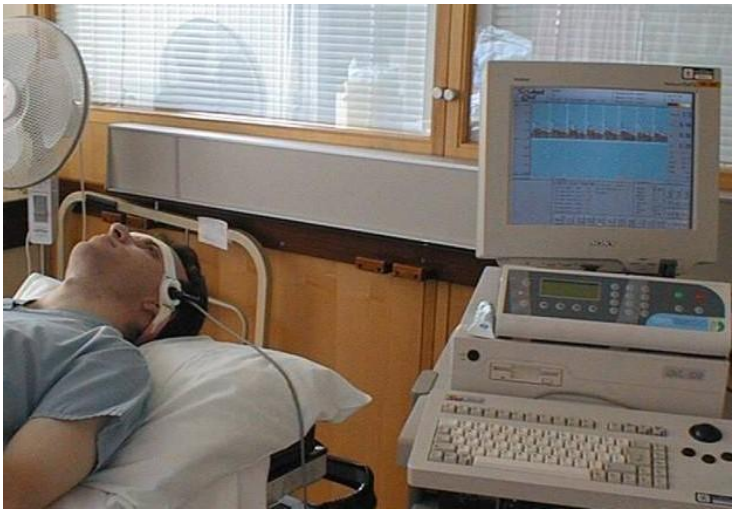

Figure 1.2 Trans-cranial Doppler ultrasound measurements on a human volunteer. Reproduced with the permission of Professor R Mahajan.

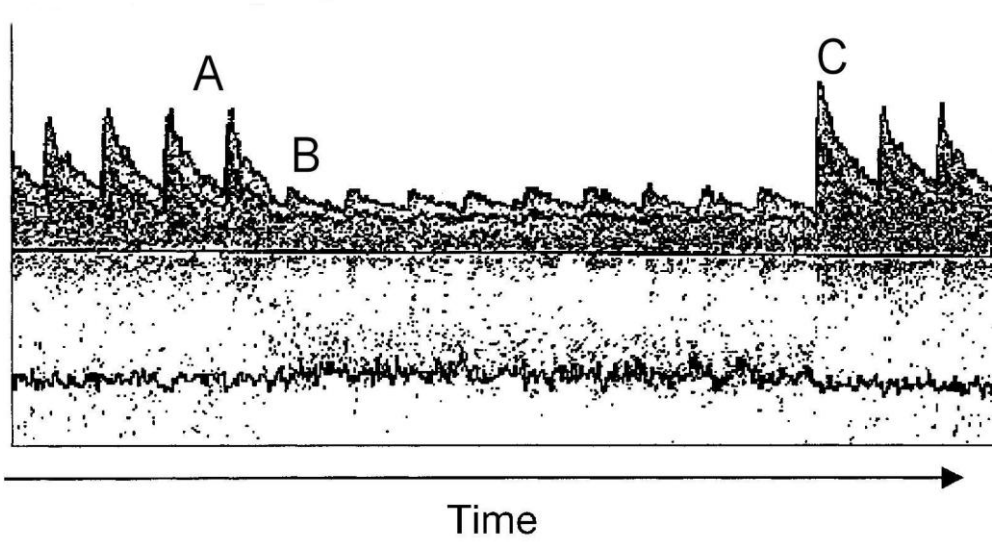

Figure 1.3 A sample trans-cranial Doppler trace of a transient hyperaemic response test, where A is the peak middle cerebral artery blood velocity prior to ipsilateral carotid artery occlusion, B is the peak blood velocity during arterial occlusion, and C is the increased peak blood velocity occurring after the occlusion is released, representing a hyperaemic response. The transient hyperaemic response ratio = C/A. Reproduced with permission from Dr R Sherman.

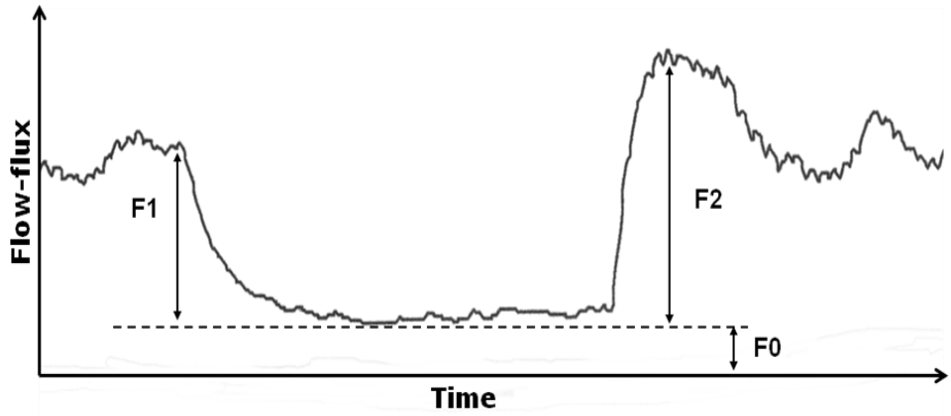

Figure 1.4 A sample trace of the transient hyperaemic response test using laser Doppler flowmetry, where F0 is the biological zero which occurs during arterial compression, F1 is the baseline flow-flux representing perfusion prior to arterial compression and F2 is the increased flow-flux occurring after the occlusion is released. The transient hyperaemic response ratio can be calculated as F2 / F1.

What is known about the transient hyperaemic response

Several small studies involving healthy volunteers have been performed using LDF measurement of the transient hyperaemic response in forearm skin in order to examine various aspects of the response. The THR test in volunteers has been demonstrated to be reproducible, well tolerated and simple to perform (Hancock et al, 2001). The effects of age (Abaza et al, 2004), and gender (not published) have been investigated, as have the effects of various physiological stresses on the THR, including: prolonged ischaemia, decreased skin perfusion, hypoxia, and hyper- and hypocapnia (Moppett IK, Jones LN et al, 2003; Hardman JG, et al 1997; Marval PD et al, 2004; Richardson JR et al, 2004). The results of these studies will be examined in greater detail later in this thesis, but taken as a whole they add support to this method of investigating vascular reactivity as being robust and reproducible, and minimally affected by minor physiological changes.

THR has also been investigated in forearm skin which has been manipulated by external heating, and by exposure to exogenous vasodilators and vasoconstrictors delivered by iontophoresis (O'Connor MB et al, 2003; Brown H et al, 2003; Webster VL et al, 2002). Iontophoresis is a method of transdermal drug delivery which utilizes electrical charge to promote the movement of chemicals across the skin (explained in greater detail in chapter 2). Iontophoresis is a method of drug delivery which has been extensively investigated and allows localized manipulation of the cutaneous microcirculation whilst avoiding systemic changes (Coston AF et al, 2001; Korula M, 2004). Iontophoresis is easily combined with LDF and THR measurements. The results of the manipulation of THR by this method are discussed in more detail later in the thesis.

The effects on cutaneous THR of topical and orally administered medications which are used clinically have also been studied. The medications involved include non-steroidal anti-inflammatory drugs, NSAIDs, (ibuprofen and rofecoxib) and local anaesthetic creams (EMLA, Rapyda, and Ametop) (Moppett IK, Davies JA et al, 2003; Wiles MD et al, 2010).

The study involving NSAIDs demonstrated that LDF measured flowflux increases can be detected after the oral administration of medications predicted to have a vasoactive effect. Despite the increase in flowflux there was no demonstrable change in the THRR after administration of NSAIDs, suggesting that THRR and flowflux may to some extent be considered independently of each other.

Vascular reactivity in critical illness

The studies described above were performed in healthy volunteers. Although THR measurements of the cerebral circulation performed using transcranial Doppler ultrasound have been performed in patients, to date no studies have been published which have investigated the cutaneous transient hyperaemic response in patients with concurrent disease.

One of the diseases known to have an extreme effect on vasculature reactivity is the inflammatory response to infection, sepsis. Sepsis is a multi-system inflammatory response associated with profound cardiovascular alterations which can result in shock and a loss of tissue perfusion. The exact mechanisms by which these occur remain unclear, and may even depend upon the specific cause. What is clear is that sepsis and septic shock can alter endothelial and local tissue responses, and that current methods of optimising tissue blood perfusion rely on global, systemic measurements of cardiovascular status, such as blood pressure, central venous pressure and cardiac output, or non-specific markers of organ perfusion such as urine output, serum lactate measurement, and level of consciousness (De Backer, Vincent J-L et al, 2002; Snowden C et al, 2002; Crowley SR et al, 1996; Sheehan M et al, 2002).

Ideally critically ill and septic patients should be evaluated and resuscitated in response to non-invasive measurements of organ perfusion, but as yet there are no reliable methods of performing this, and in any case perfusion is likely to be different in different organs,

necessitating multiple different modes of measurement. Attempts have been made to measure the perfusion of specific organs and monitor their response to resuscitation, assuming it to be a surrogate marker of organ perfusion in other organs, or, if not that, then at least a useful goal in its own right. The most notable example of this is gastric tonometry where the hydrogen ion concentration within the stomach is used as an indicator of regional oxygenation and perfusion within the stomach wall (Russell JA, 1996). To date no organ-specific perfusion monitors exist that have gained wide-spread clinical acceptance.

Although the skin can occasionally be the originating site of inflammation and sepsis in critically ill patients, for example in cellulitis or toxic-epidermal-necrolysis, for the most part it can be considered a peripheral end-organ affected by inflammation originating elsewhere. And due to its low nutritive requirements it does not usually fail (i.e. become ischaemic to the point of dying), even in profoundly shocked individuals in whom the perfusion of other organs has failed to such an extent that multiple organ failure has occurred. The skin, however, is a very large organ, with a great deal of vascular endothelium and microcirculatory reserve. Given that the endothelium is known to be intimately involved in the processes which occur in sepsis (Vallet B, 2002; Reinhart K et al, 2002), it is reasonable to assume that the vascular reactivity of the skin microcirculation might be affected in sepsis, even when there are no outward signs of this occurring. Thus measurement of vascular reactivity in skin, which is readily accessible in all but the most extreme cases, might act as a surrogate marker for impaired vascular reactivity, and hence impaired perfusion, elsewhere in the body. Evidence that skin vascular reactivity is altered in septic patients already exists, though its clinical significance has yet to be proven (Hartl WH et al, 1988; Haisjackl M et al, 1990). The transient hyperaemic response, being non-invasive, readily repeatable and primarily being affected by the myogenic response offers the opportunity of exploring a new aspect of the changes affecting the vascular reactivity in the cutaneous microcirculation in critically ill or

septic patients. It also offers the possibility, when combined with iontophoresis of different agents, of exploring the dynamic changes which may occur in response to different agents associated with critical illness, both in patients and healthy volunteers. With the exception of vasopressors (epinephrine, phenylephrine, and vasopressin) and NSAIDs, very few agents which have been used as treatments for critical illness, or as part of the general management of critical illness, have been investigated in this way before (Brown H et al, 2003; Moppett IK, Jones LN et al, 2003; Kubli S et al, 2003; Kienbaum P et al, 2008).

Aims

The aim of the research within this thesis was to further explore the cutaneous transient hyperaemic response with a view to investigating the changes which occur in skin vascular reactivity in critically ill patients. Many of the investigations presented here are pilot or proof-of-concept studies aimed at answering the following questions:

1. Can a large enough dataset of THR measurements be obtained such that variability can be quantified and a “normal” range of THR ratios be made available for comparison?
2. Is it possible to model some of the effects on THR that might occur in critical illness by, for example, artificially causing microcirculatory vasodilataion and attempting to modify it with various treatments?
3. Can cutaneous THR be successfully measured in critically ill patients; and can it be manipulated in this same group?

The effects of statins on critically ill patients

There are many immuno-modulatory and vaso-active agents that have been investigated in order to ascertain whether or not they can improve the outcome of critically ill patients. Of those agents investigated many have poorly understood mechanisms of action and appear to have actions which overlap many different areas of physiological function, for

example they might appear to downgrade the immune response and/or alter micro-vascular blood flow, capillary permeability or interfere with the blood coagulation pathway. Examples of drugs which are thought to have multiple mechanisms of action include steroids, anti-thrombin III, activated protein-C, and L-NAME (Briegel J, 2000; Ilias W et al. 2000; Bernard GR et al, 2001; Avontuur JAM et al, 1998).

In the time period over which research into the transient hyperaemic response in skin was being carried out for inclusion in this thesis, new evidence was published which suggested a link between HMG CoA reductase inhibitors (statins) and improved outcome in patients with bacteraemia (Liappis AP et al, 2001; Almog Y et al, 2004). These improved outcomes included a lower incidence of severe sepsis, with patients being less likely to require ICU admission. There was also a trend towards improved survival in bacteraemic patients. Although these results were retrospective and observational in nature, they resulted in speculation that statins might have effects that were separate in nature to their well-known cholesterol-lowering properties – pleiotropic effects. It was hypothesised the pleiotropic effects might be immuno-modulatory and/or vaso-active in nature (Almog Y, 2003).

Further aims

In response to this new evidence concerning statins two further questions were proposed:

4. Is it possible to evaluate the effects of factors known to be associated with critical illness, in particular the prior usage of vaso-active or immuno-modulating agents, such as statins?
5. Can the micro-circulatory effects of potentially vaso-active or immuno-modulating agents, such as statins, be investigated in volunteers using the cutaneous measurement of THR?

In order to achieve these aims it was thought necessary to first confirm the finding of an association between improved outcome and the premorbid prescribing of statins. For the most part previous retrospective studies have investigated the outcomes of patients who were not critically ill. For these reasons a retrospective study was designed to examine the effects of the prior administration of statins and cardiovascular medications in critically ill patients, the results of this study can be found in chapter six of this thesis.

Following on from this, a study involving healthy volunteers has been designed with the aim of investigating whether or not statins have any effect on the vascular reactivity of the cutaneous microcirculation as measured by the transient hyperaemic response. At the time of writing this thesis, the aforementioned study has yet to be started. As a result the data from the retrospective study alone is included in chapter six, in the expectation that data from complementary volunteer studies will be available at some point in the future.

CHAPTER TWO

Materials and methods

In this chapter the materials and methods common to the studies performed will be outlined and justified. The refinement and adjustment of techniques used in individual experimental protocols will be further explained in the relevant chapters. The relevant volunteer information leaflet and consent forms for each study (or patient / relative information and consent / assent forms where appropriate) can be found within the appendices of the thesis.

Laser Doppler flowmetry

Two distinct techniques of laser Doppler flowmetry exist, laser Doppler perfusion monitoring (LDPM) and laser Doppler perfusion imaging (LDPI). LDPM utilises fibreoptic technology to sample a small volume of tissue and measure changes in perfusion over time. By contrast LDPI uses a scanning laser light generated at a distance from the subject tissue and can be used to build up a map, or image, of tissue perfusion over a broad area of tissue, but with an inevitable time lag from one end of the imaged area to the other, which is proportional to the area scanned.

LDPM laser light can be delivered to, and measured from, the surface of tissue via a surface probe. All of the investigations within this thesis which involve laser Doppler

flowmetry are of the LDPM type using surface probes. Both the probes and the laser Doppler flowmeter used were designed by Moor instruments (Moor Instruments, Axminster, Devon). This equipment was used because it is designed to monitor the Doppler signal of the cutaneous microcirculation and communicate acquired data directly to a computer; it is also non-invasive and is compatible with equipment designed to alter the microcirculation by iontophoresis and cutaneous heating.

The DRT4 is a microprocessor-controlled laser Doppler monitor which generates laser light at a wavelength of 785nm (±10nm) and an operating power of 0.5-1.5mW (figure 2.1). This typically allows tissue penetration of 1-2mm without damaging or substantially heating the intervening structures. Laser light is transmitted from the DRT4 module via 2m fibre-optic cables to skin probes. Two different laser Doppler probes were used in the experiments within this thesis. The DP1T/7 laser Doppler probe (figure 2.2) consists of a central delivery fibre surrounded by 8 collecting fibres in a 2mm ring. The DP12-V2 needle laser Doppler probe (figure 2.3) is a 2 fibre delivery and collecting system with 0.5mm fibre separation which can be inserted through the middle of a heating probe (figure 2.6). Flux values as measured by the DRT4 module are recorded in arbitrary units (AU) and have an accuracy of ±10% and a precision of ±3%, the coefficients of variation being lower for the DP1T/7 probe because of the larger “collecting” area (DRT4 user manual, 2006). All of the experiments performed were done in ambient conditions which were within the operating temperature range of the DRT4 module (15-30°C).

Laser probes were calibrated according to the manufacturer’s instructions (DRT4 user manual, 2006). Since the DP1T/7 probe is capable of laser Doppler flowmetry and temperature measurement, both optical and temperature calibration were required. Probes were cleaned between subjects with running water and allowed to air dry, as per manufacturer’s instructions, except where the probe was involved in iontophoresis where

cleaning was similar to that used for iontophoresis chambers as described later in this chapter.

Laser probes were fixed to skin using adhesive dressings and good skin contact was ensured, although this was more important for temperature measurement than for flowmetry measurements. The forearm was chosen as the site for flowmetry because it had been previously used for measurements (Brown et al, 2003; Webster et al, 2002, Moppett IK & Davies JA et al, 2003, Moppett IK & Jones LN et al, 2003) and because it has advantages when performing the THR test.

As discussed in the introduction laser Doppler flowmetry is qualitatively different from other familiar forms of medical Doppler. The greatest single limitation of laser Doppler flowmetry is its inability to provide an absolute measurement of blood flow. Any movement of interstitial tissues, vascular smooth muscle, and even the residual Brownian motion of intracellular particles leads to a background reading of Doppler flux. This background reading is often referred to as a “biological zero”, and it is compared with this biological zero that changes in flux which represent perfusion are measured. Such measurements often have arbitrary units assigned to them, but are in reality merely a ratio of change compared with biological zero. Ideally the area of tissue being sampled should remain as stationary as possible during the period of sampling. In practice the biological zero is taken to mean the baseline flux which will be a combination of both true biological zero and baseline perfusion.

It is not only movement that can increase the background level of flux that is measured. Relatively minor increases in temperature can cause an increase in the scattering due to increased residual Brownian motion. This results in an increase in the true biological zero which is proportional to temperature between the ranges of 15°C to 37°C. Also because

back-scattered light is used to calculate flowflux it is susceptible to interference from extraneous light sources.

Laser Doppler flowmetry (as LDPM) has excellent temporal resolution but only limited potential for spatial resolution, and so the sampled area may be unrepresentative of overall tissue perfusion, and areas of regional variation may be missed. An extreme example of this can occur when the area of tissue being sampled involves all or part of a major blood vessel. In this case the much greater velocities of the erythrocytes within the major vessel will dominate the Doppler power spectrum analysis of perfusion, and the motion of the blood vessel wall will also lead to increased fluctuations in the biological zero.

In order to avoid artefacts in blood flowflux measurement the following precautions were taken*:

- Where possible the surroundings where LDF measurements were taken were kept at 19-23°C, and high levels of ambient light were avoided.
- Subjects were allowed to acclimatize to ambient conditions for a period of 20 minutes prior to any measurements being taken. During this time subjects were allowed to rest in a semi-reclining supine position with the forearm supported.
- Prior to probe placement skin was prepared by cleaning with an alcohol wipe and allowing to air dry. Where excessive hair was present which may have obstructed the fibreoptic channels it was shaved off beforehand.
- The probe was placed at 90° to the skin, at least 8cm from the ante-cubital fossa to decrease the likelihood of measurements involving flowflux from major vessels. After placement the baseline was examined for evidence of marked baseline pulsatile blood flow, and in cases where this was found to occur then the probe was re-sited in a different position.
- The flowflux cable was secured to the forearm to prevent artefact within the cable

- Subjects with damaged or broken skin, or who had a known allergy to adhesive dressings were excluded from taking part in any studies.
- Subjects were discouraged from moving during measurements, were encouraged to breathe as normally as possible, and were discouraged from talking
- Although the DRT4 module is capable of sampling at 40Hz measurements of flowflux variation were done using a time constant of 0.5seconds to avoid excessive artefact interference

*Where subjects were intensive care unit (ICU) patients it was not always possible to tightly control environmental conditions or avoid patient movement

These aspects of the protocol are consistent with existing recommendations (DRT4 User Manual, 2006; Nilsson et al, 1980).

All recordings of measurements were made and analysed using the software Moorsoft for Windows/DRT4 v1.2 (Moor Instruments, Axminster, Devon, UK).

Cutaneous temperature measurement

Where a heating probe was used temperature measurement was done via that probe and is described later in this chapter. Where there was no heating probe skin temperature was measured using the DP1T/7 probe in conjunction with the DRT4 module which has a measurement range of 5-50°C, a resolution of 0.1°C and an accuracy of ±0.3°C (DRT4 user manual, 2006).

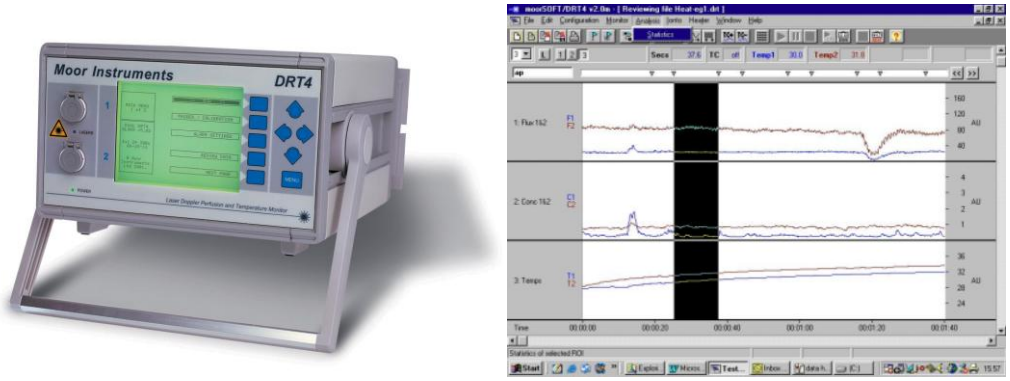

Figure 2.1 DRT4 laser Doppler flowmeter and screenshot of Moorsoft DRT4 computer program (Moor Instruments)

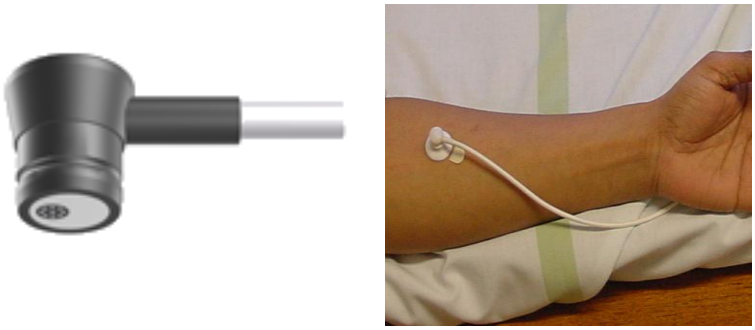

Figure 2.2 DP1T/7 laser Doppler probe and temperature monitor (Moor Instruments)

Figure 2.3 DP12-V2 blunt needle end laser Doppler probe (Moor Instruments)

Flowflux measurements and the transient hyperaemic response

Baseline flowflux (F_1) was measured only after a 60 second period of recording in order to ensure that a steady state had been arrived at.

The choice of forearm skin for measuring flowflux allowed the measurement of the hyperaemic response after digital compression of the brachial artery at the ACF (ante-cubital fossa) – an end artery with no collateral flow. Positioning the LDF probes at least 8cm distal to the ACF minimized the movement artefact caused by digital compression. In previous studies adequate digital compression was confirmed by the presence of a sudden drop in flowflux to biological zero (F_0). As an added measure digital pulse oximetry was used in the experiments presented within this thesis as a means of confirming that digital brachial artery pressure was sufficient to abolish distal pulsatile blood flow.

An acceptable THR manoeuvre was defined as one in which a sudden drop in flowflux from baseline to biological zero which was maintained for 20 seconds with no demonstrable distal pulsatile flow; and was followed by a sudden rise after the release of digital compression back to baseline or higher levels of flowflux. Peak flowflux after release was defined as the hyperaemic peak (F_2). As demonstrated previously (in figure 1.4) THRR was defined as $(F_2- F_0)/(F_1 – F_0)$. All of the above is consistent with the manner in which cutaneous THRR has previously been measured in other similar studies (Brown et al, 2003; Webster et al, 2002, Moppett IK & Davies JA et al, 2003, Moppett IK & Jones LN et al, 2003).

Apart from the choice of a compression period of 20 seconds which has been previously discussed, there is no clear-cut consensus on how F_0, F_1 and F_2 are defined. For instance should F_0 be the lowest biological zero point? Should F_1 be the point measurement immediately before digital compression (and therefore more susceptible to movement

artefact from the application of digital pressure)? And at what point after the release of digital pressure should a rise in flowflux be no longer attributable to the THR, and therefore not be defined as F_2? In all of the THR measurements made in the following experiments the following definitions were used:

- F_1 was a 20 second average of flowflux recording immediately prior to the THR manoeuvre
- F_0 was a 15 second average of biological zero during the period of arterial compression during which no distal pulsatile blood flow was demonstrable using a digital oxygen saturation probe
- F_2 was the peak hyperaemic response recorded using a 0.5 second time constant occurring within 20 seconds of the release of brachial artery compression
- A minimum of 90 seconds was required between completion of a THR and the start of the F_1 20 second averaging time of the next THR

Where the subjects of experiments were intended to be healthy volunteers the following exclusion criteria were applied:

- Under 18 (there was no upper age limit)
- Pregnancy
- Smokers
- Evidence of pre-existing circulating disorders, including, but not limited to: peripheral and central vascular disease (e.g. known claudication, cerebrovascular disease, ischaemic heart disease); vasculitis (e.g. systemic sclerosis, systemic lupus erythematosus, arteritis, Wegener's disease and Behçet's disease); Raynaud's disease; diabetes; chronic kidney disease; hypertension; hypercholesterolaemia
- The use of any regular prescribed medication, including aspirin
- The recent use of any over-the-counter remedies including paracetamol, non-steroidal anti-inflammatory drugs, and cold remedies

- The recent use (within the past 24 hours) of any herbal remedies

With the exception of the lower age limit (which was done to facilitate consent issues) all of the above criteria were consistent with other studies involving THR measurements (Brown et al, 2003; Webster et al, 2002, Moppett IK & Davies JA et al, 2003, Moppett IK & Jones LN et al, 2003). In each case this was because underlying conditions or medication use might reasonably be expected to interfere with the hyperaemic response (Celermajor DS et al, 1996; Noble M et al, 2003; Morris SJ et al, 1995; Stansberry KB et al, 1996; La Civita et al, 1998; Khan et al, 1994; Celermajor DS et al, 1992). Other issues suspected of affecting vascular reactivity, including age and gender, are addressed with the appropriate experiments discussed later in this thesis.

All subjects were advised to avoid strenuous exercise, and avoid consuming food and alcohol or caffeine-containing substances for at least 6 hours before the duration of the study. Although it is standard practice in the field of microvascular research to recommend abstinence from exercise, food and caffeine in the preceding period there is very little evidence concerning how much of an effect may occur in healthy volunteers, and how long the period of abstinence should be in order to avoid any confounding effects (Duffy et al, 2001; Williams MJ et al, 1999; Perrin ME et al, 2004; Kvernmo HD et al 1998).

Within the clinical study involving ICU patients similar exclusion criteria were applied, except that the only circulatory disorders within the exclusion criteria were peripheral vascular disease, Raynaud's disease, insulin-dependent diabetes and vasculitis. Smokers and patients with cardiac or renal disease were not excluded. Concurrent medication use was also allowed.

In all subjects heart rate and arterial oxygen saturations were recorded using a pulse oximeter before and during THR measurements, as was non-invasive blood pressure (using a blood pressure cuff attached to the contralateral arm).

Transcutaneous iontophoresis

Iontophoresis is a method of transdermal chemical delivery which utilizes a small electric current to actively transport chemicals into subcutaneous tissues via the processes of electromigration and electroosmosis.

The epidermis provides the greatest barrier to transcutaneous chemical movement, and is traversed using a number of routes, which include paracellular gaps, transcellular movement, and via appendageal pathways (e.g. sweat ducts and hair follicles). Appendageal pathways are thought to account for the majority of chemical movement of water soluble chemicals, whilst lipophilic chemicals may involve proportionally more trans- and paracellular transport.

The quantity of chemical displaced transdermally depends upon a number of different factors (Rawat et al 2008, Coston et al 2001):

- Electric field intensity, current amplitude, current duration
- Chemical solution pH and concentration
- Molecular size and degree of ionization (and to a lesser extent polarization)
- Epidermal (and in particular keratin) thickness and continuity; epidermal charge and pH

One of the major drawbacks of transdermal iontophoresis is that there is only limited control over the quantity of chemical delivered, and no simple way of confirming that quantity. This drawback, and the fact that current duration is proportionally more important

than current amplitude (i.e. prolonged iontophoresis may be required to deliver relatively small chemical concentrations), has resulted in there being only a limited number of clinical applications for iontophoretic drug-delivery. These include iontophoretic fentanyl delivery for analgesia, and the iotnophoresis of tap water delivery as a treatment for hyperhidrosis (Power I 2007; Midtgaard 1985). Reverse iontophoresis, the use of electrical current to attract chemicals through the skin, has been used to measure glucose concentrations in diabetes, and chloride concentrations in cystic fibrosis (Seig et al 2003).

Compared with its clinical applications iontophoretic drug delivery has been widely used as a research tool for a wide variety of reasons: it can provide needle-free transdermal drug introduction and a large enough area of skin is normally readily accessible in both volunteers and patients; where systemic effects are desired it can avoid first-pass hepatic metabolism; and although the exact amount delivered cannot be calculated, where local effects are required it can be tailored to deliver just enough drug to provoke a local response, whilst avoiding a systemic response.

In the context of both volunteer and patient studies within this thesis iontophoresis was considered the most appropriate way to alter the vascular reactivity of the cutaneous micro-circulation in a relatively non-invasive way.

A custom-designed Perspex iontophoresis chamber (the ION1 iontophoresis chamber, Moor Instruments) was used which allowed both iontophoresis and LDF, temperature measurement and cutaneous heating to take place simultaneously (figure 2.4). The chamber was attached to the volar aspect of the forearm using an adhesive dressing similar to that used to attach standard LDF probes. As with LDF probes skin was cleaned with alcohol wipes and allowed to dry prior to chamber placement. Excess hair at the site was lightly shaved off where necessary.

Iontophoresis current was applied between the inert platinum wire within the iontophoresis chamber and a 4x4cm dispersive return electrode (MIC1-CP, Moor Instruments) placed approximately 10-15cm from the chamber (figure 2.5). Good electrical contact was maintained by placing a conductive gel hydro pad (MIC1-GP, Moor Instruments) between the return electrode and skin. Iontophoresis was controlled using a MIC1-e iontophoresis controller (Moor Instruments) and involved a pulsed pattern of current with fourteen 45 second periods alternating between 75μA and no current. THR measurements were not recorded until at least 150 seconds had passed since the final period of iontophoresis. Although a "tingling" sensation can be observed by subjects a current of 75μA is not uncomfortable or painful. During prolonged periods of applied charge the skin surface may become polarized resulting in decreased efficiency of iontophoresis. In order to avoid this a pulsed pattern may be used (Rawat et al, 2008).

As has been previously mentioned the main adjustable determinants of iontophoresis are the current amplitude and duration. The current flux of the ION1 chamber can deliver a current flux of 1mC/cm^2 when a 71μA current is delivered over 10 seconds.

Cumulative dose response curves have been demonstrated with various chemicals using a series of low currents (i.e. less than 100μA) of increasing duration (up to 40 seconds) separated by increasingly longer response periods (MIC1-e user manual, 1998). Although in the drugs being studied the dose response to iontophoresis had not previously been tested, it seemed likely that, should they behave in a manner similar to other chemicals the protocol chosen here would result in a maximal local effect and avoid electrical or thermal induction of the axon reflex, a neurological vasodilatory effect which can occur with currents exceeding 250μA (Westerman et al, 1988). The Mic1-e iontophoresis controller delivers current with an accuracy of ± 1μA when the controller is kept within an ambient temperature range of 15-30°C, as was the case in all experiments. The MIC1-e controller was in turn controlled by the DRT4 module described earlier.

The choice of polarity for the iontophoresis chamber depended upon the charge of the active ion. For example where the active ion was positive the electrode within the iontophoresis chamber was anodal and the return electrode cathodal.

When performing iontophoresis the choice of drug concentration was dependent upon several factors which included stability in solution, ease of dilution and efficacy of iontophoresis. As previously stated the total dose of drug delivered by iontophoresis is predominantly dependent upon current amplitude and duration. Low drug concentrations may affect this by limiting the amount of active drug available for iontophoresis. Conversely high drug concentrations may result in decreased electrical conductivity and reduce iontophoresis (Rawat et al, 2008; MIC1-e user manual, 1998).

Since there is no way of knowing the absolute drug concentration at the site of iontophoresis, each study involving iontophoresis, where there was no previous experimental work to suggest a dose, was designed to evaluate the maximal effect on local skin vascular reactivity at drug concentrations approximating standard drug concentrations used in clinical settings.

Non-specific vasodilatation associated with iontophoresis has previously been reported in several studies. Suggested mechanisms by which this can be minimized have included: minimizing maximal voltage and current settings, pre-treatment with aspirin, avoidance of local skin heating, and the use of hypertonic saline (5 moles/L) as a dilutional agent (Westerman et al, 1988; Droog et al, 2003; Åsberg et al, 1999; Durand et al, 2002). A study by Brandrivskyy et al (2004) using a pulsed current protocol, similar to the one described above, provided little evidence of vasodilatation when deionised water or 5 molar saline were iontophoresed into skin, which is in keeping with previously unpublished work from our centre using the above protocol to iontophorese 0.9% saline (0.308 molar saline). The only study which previously has examined the relationship

between 0.9% saline and THR (Brown et al, 2003) demonstrated that 0.9% saline did not cause a significant effect on baseline flowflux, but did decrease the THR ratio. Because the effects of 0.9% saline on flowflux and THRR were already established, it was chosen over 5 molar saline as the carrier diluent where one was necessary (for the drugs midazolam and norepinephrine).

When filling the iontophoresis chamber with fluid, care was taken to avoid trapping air bubbles within the chamber and thus ensure contact between the chamber fluid and skin. Fluid was continuously monitored and topped up as required using the secondary filling well present on the chamber (figure 2.5). The presence of fluid between the LDF probe and skin was predicted to reduce the strength of the flux signal, and so where required, control probes were sited within inactive iontophoresis chambers containing 0.9% saline.

Following each period of iontophoresis the iontophoresis chamber was cleaned as per the manufacturer's instructions using de-ionised water for 2 minutes to prevent contamination between patients and avoid the build up of accretions on the electrode. After passive drying the chamber was cleaned with an alcohol wipe and allowed to passively dry a second time

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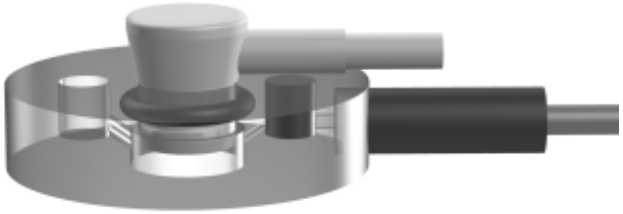

Figure 2.4 Custom built Perspex iontophoresis chamber allowing simultaneous laser Doppler flowmetry

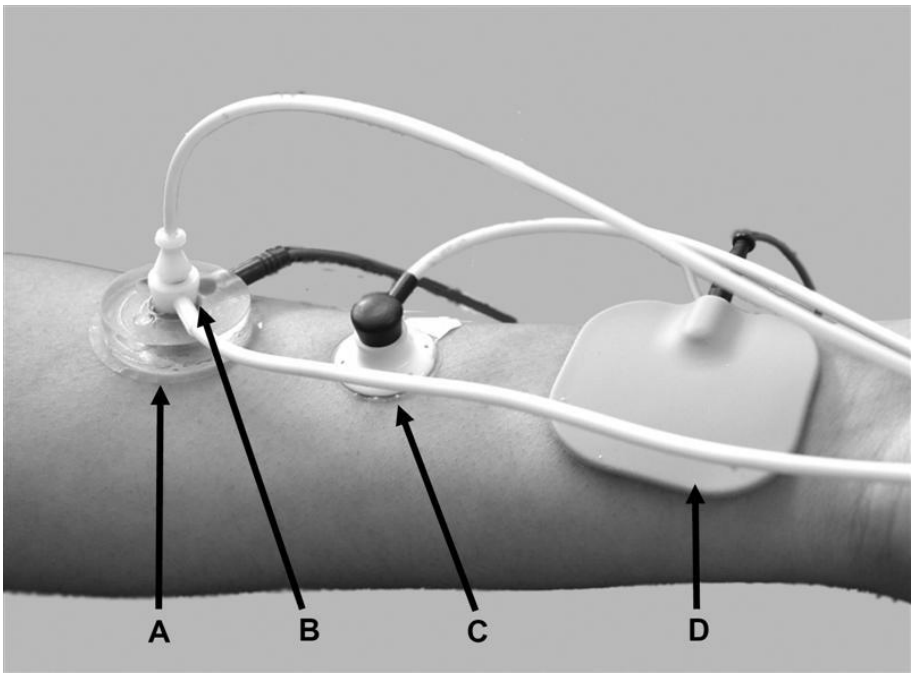

Figure 2.5 Iontophoresis, cutaneous heating and laser Doppler flowmetry apparatus in situ on a volunteer's forearm. A – Perspex iontophoresis chamber; B – cutaneous heating probe with needle LDF probe; C – standard LDF probe; D – return iontophoresis electrode. In this example there are two LDF probes with the standard probe being a control probe used to confirm that a transient hyperaemic response had taken place.

Cutaneous heating

Localized cutaneous heating was performed using an SHP2 heating probe controlled by an SH02 heating controller unit (both by Moor Instruments). The SHP2 probe has a 6mm heating element which is placed in contact with skin. The probe can be placed within the iontophoresis chamber or attached directly to skin. Unlike the laser Doppler probes no user calibration is required. The SH02 controller is a dual channel heater unit which has a heating range of 20-45°C, a resolution of 0.1°C, and an accuracy of ± 0.3°C. The environmental operating temperature of the controller unit is 15-30°C which encompassed the operating conditions in which all studies were performed (SH02 user manual, 1998).

Where localized cutaneous heating was required the temperature of skin was monitored to ensure a constant temperature for at least 30 seconds before LDF measurements were performed. The choice of temperatures is discussed in the relevant chapters.

The techniques for decontamination and cleaning of temperature probes which were used in combined heating and iontophoresis experiments were similar to those described earlier for iontophoresis chambers.

Epidemiological studies

In those studies which required the collection of epidemiological data the principles of data collection and reporting advocated by the STROBE guidelines (Strengthening the Reporting of Observational Studies in Epidemiology) were adhered to as far as was possible (von Elm et al, 2008). The exact details surrounding data collection and analysis can be found within the appropriate chapters.

Data analysis

All data collection and analysis was performed using SPSS statistics package version 17 (SPSS incorporated, Chicago, Illinois, US).

Figure 2.6 SHP2 cutaneous heating probe and SH02 heating module (Moor Instruments)

CHAPTER THREE

Characterisation of the THR test

Introduction

All of the previous volunteer studies which have been undertaken in order to estimate the variability of THRR within individual subgroups have been small in nature, typically involving 8-15 volunteers, each providing 3-5 measurements. One of the earliest studies, by Hancock et al, 2001, demonstrated the THR to be reproducible with a moderate degree of within- and between-subject variability. Other studies have reported differing degrees of variability using laser Doppler flowflux measurements which can be improved by the application of extreme measures of vasodilatation such as the iontophoresis of acetylcholine (Kubli S et al, 2000).

Attempts have been made to classify the individual-related variables of cutaneous blood flow measured by laser Doppler flowmetry using larger volunteer samples or by undertaking confirmatory measurements in separate groups. A review by Bircher et al, 1994, identified variables considered important in assessing the variability associated with laser Doppler flowmetry; these included: age, sex, race, anatomical sites, intra- and inter-individual variation, temporal variation, physical and mental activity, food, drugs and

nicotine, and menstrual cycle. Crucially these studies have not involved the transient hyperaemic response.

Variables which might affect the THRR and have been studied in small volunteer groups include:

- Age:

  Flowflux was decreased; THRR was unaffected (Abaza et al, 2004)

- Gender:

  No differences in flowflux or THRR (not published, personal communication)

- Inter-, intra-individual variation:

  33% within-and 66% between- subject variation, overall coefficient of variation 0.38 (Hancock et al, 2001)

- Menstrual cycle:

  Equivocal results (not published, personal communication)

- Exercise:

  No differences in flowflux or THRR (Perrin ME et al, 2004)

In order to confirm the findings of these earlier studies it was decided that a large dataset of THR responses from healthy volunteers be collated, using both new data and previously collected data which was reanalysed including over 1000 individual measurements in over 100 subjects. This is over ten times the numbers and measurements used in the original experiments designed to evaluate the variability of the THR test. It was decided to focus on age, gender and individual variation, and data from previous studies which specifically attempted to evaluate these areas was not included within the new dataset. It was hoped that by combining the data in this way variability could be better quantified and a “normal” range of THR ratios be made available for comparison in situations where the subject could not act as their own control.

In addition, since all of the previous data relates to standard LDF probes, a dataset was compiled of flowflux and THRR measurements from “needle” probes.

Lastly, since some studies have suggested that the transient hyperaemic response might be better measured by examining the “area under the curve” (i.e. taking into to account both the magnitude and the length of the THR – Marval et al, 2004), the time taken for the hyperaemic response to return to baseline values was also measured and analysed.

Methods

All previously performed THR studies which allowed specific consent for the anonymous use of recorded THR traces were identified and permission sought from the lead-investigator to re-analyse data for suitability for inclusion in an aggregate database. Only those studies which involved control recordings were considered (i.e. those in which the study protocol specified flowflux and THR measurements made in the absence of any intervention). In total 5 studies were identified as being suitable for re-analysis; this included studies in which the subjects, whilst remaining anonymous, were known not to have been involved in any of the other 4 studies (i.e. there was no dupliction of subjects between studies). Other than confirming that reference was made to it in the study protocol associated with an individual recording, no attempt was made to confirm the ambient temperature conditions or that a pre-recording acclimatization period was enforced. The same is true of the pre-recording abstinence requirements in chapter 2. The results of 4 of these 5 studies have since been published (Moppett IK & Davies JA et al, 2003, Moppett IK & Jones LN et al, 2003; Perrin ME et al 2004; O'Connor MB et al, 2003).

The control recordings from each of the studies included within the volunteer experiments documented within chapter four of this thesis were also re-analysed.

In addition a further 14 volunteers were recruited who underwent a series of 9 THR manoeuvres each which were recorded and analysed specifically for this research. Despite the use of old data within this investigation, the majority of data was acquired by the author of this thesis.

In total flowflux and THR recordings from 126 subjects involving 6 observers were included. All of the data recordings, whether from new or old studies, were individually re-analysed by me. Where traces were not adequately clear, were not accompanied by skin

temperature measurements, or did not have sufficient gaps between THR manoeuvres or a sufficient lead-in time to allow for steady state (as defined in chapter 2) they were excluded.

Results

1175 separate THR traces were analysed and THRRs calculated. Five THR traces were found not to demonstrate transient hyperaemic responses and were not included in further analysis. The dataset was made up of THR traces taken from 101 volunteers and with THR tests being performed by six different observers. A summary of the THRR data can be found in table 3.1. The number of measurements from a single subject made by a single observer varied from 3 to 50. The majority of single-subject, single-observer subgroups contained 10 or more measurements, with 5 groups containing 30 or more measurements (figure 3.1).

Distribution and normality

THRR data frequency histograms were compiled in order to assess the distribution (figure 3.2) and Q-Q plots were calculated which demonstrated marked deviation from an expected normal distribution (figure 3.3). THRR data is a ratio of two numbers, and in common with other similar data its distribution is substantially right-skewed. This is true for both the large aggregate dataset and also of the measurement from single-subject, single-observer subgroups comprising 10 or more measurements.

Evidence with similar ratio data suggests that a normal distribution may be achieved using a logarithmic transformation (Bland M, 1987; Altman DG, 1991). Box-Cox analysis was performed to confirm this and demonstrated a root-mean-square-error nadir corresponding to a lambda value of near zero (figure 3.4), which identifies a natural logarithmic transformation as being most likely to approximate normal distribution (Box GEP et al, 1964). Even after applying a natural logarithmic (Ln) transformation a Kolmogorov-Smirnov test for normality identified that complete normal distribution had not been achieved for the aggregate dataset ($p=0.002$), but had been for all subgroups with $n\geq30$ ($p=0.20$; $p=0.20$; $p=0.19$; $p=0.54$; $p=0.20$)

Data frequency histograms and Q-Q plots for the entire dataset indicated a near-normal distribution had been achieved (figures 3.5 and 3.6) for the majority of the range. Approximation with normal distribution improved still further after the aggregate dataset was corrected for extremes of skin temperature (see next section), with a Kolmogorov-Smirnov test significance of p=0.040.

Variation with skin temperature

Skin temperature ranged from a minimum of 26.4°C to a maximum 37.1°C (mean 30.6°C, standard deviation 1.36). The correlation between resting skin temperature and LnTHRR was confirmed using Pearson correlation testing (r= -0.096, p=0.001); and, since homoscedasticity could not be assumed, also by Spearman rank correlation (r= -0.084, p=0.004). As r was less than 0.1 the correlation can be considered to be small (Cohen J, 1988) but significant (figure 3.7)

By limiting the dataset to avoid extremes of temperature it was hoped to minimise still further any correlation. After arbitrarily excluding all THR traces measured on skin with a temperature of >< 1.5 standard deviations of the mean temperature (i.e. 28.5°C to 32.6°C) no statistically significant correlation was identifiable (Pearson: r= -0.013, p=0.682; Spearman: r= -0.015, p=0.638). Limiting the aggregate dataset in this way decreased the number of measurements to 1010 and improved the approximation of normal distribution as previously stated.

Variable	THRR, (arbitrary units) n=1175
Mean	3.490
Standard deviation	1.812
Standard error of the mean	0.053
Variance	0.193
Minimum	1.141
Maximum	17.440

Table 3.1 Summary of the raw THRR data (n=1170) from the aggregate dataset

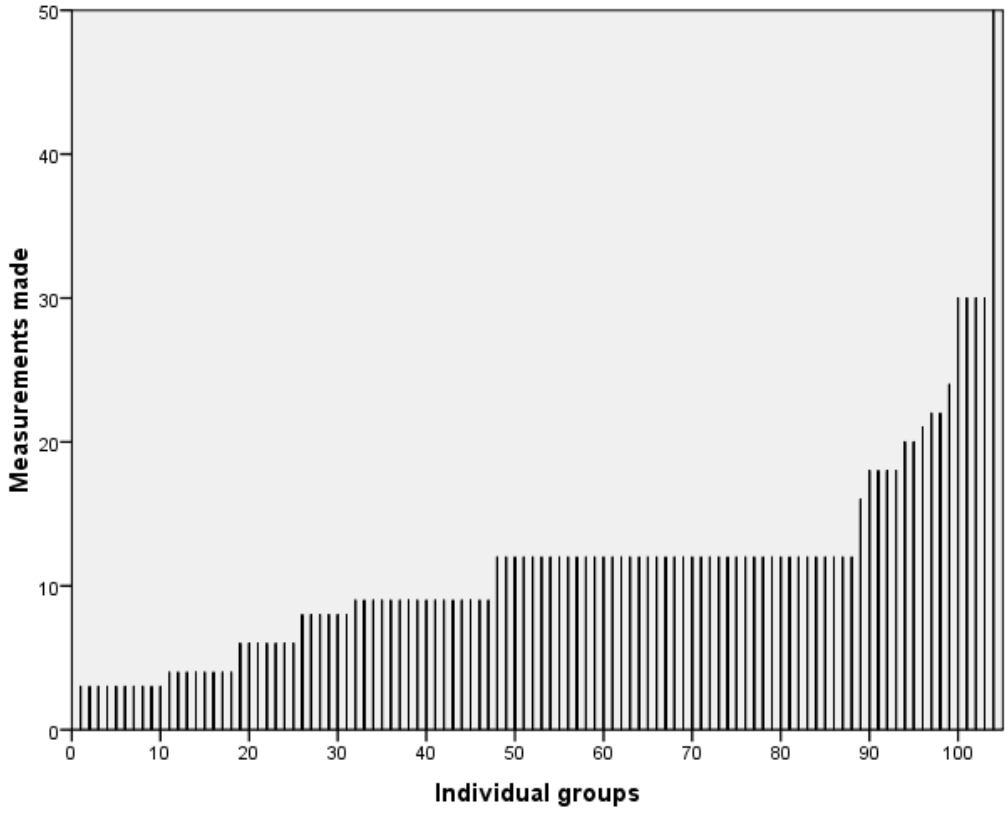

Figure 3.1 The number of THRR measurements made in each single-subject, single-observer subgroup making up the aggregate dataset.

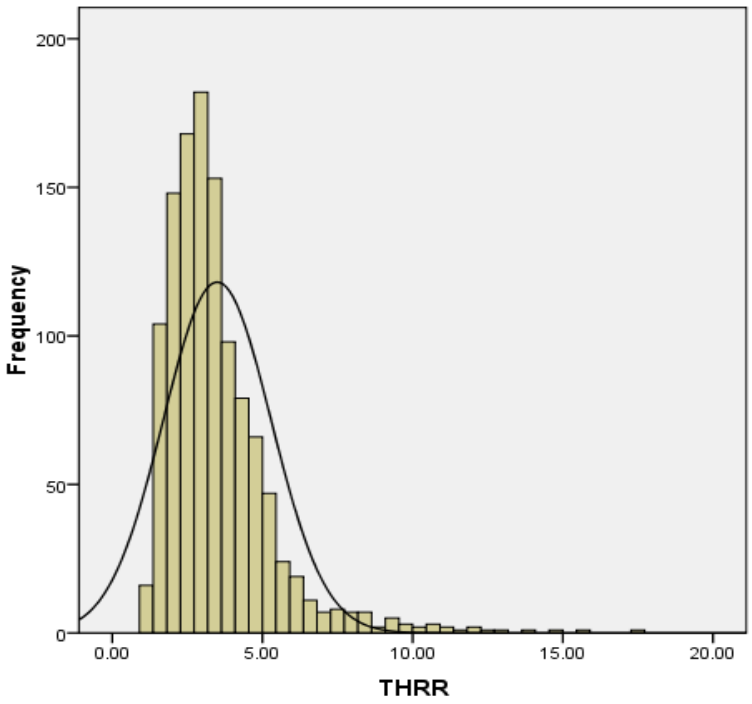

Figure 3.2 Histogram of the frequency distribution of THRR data

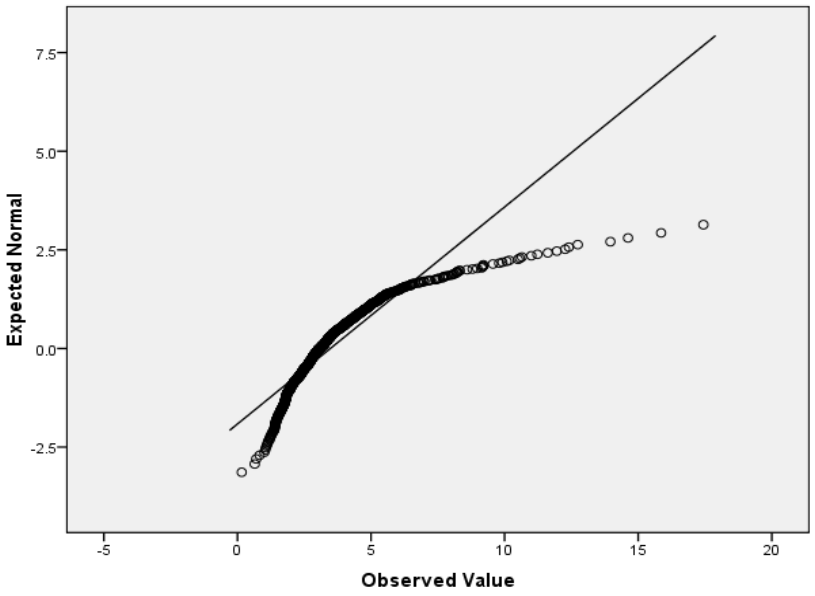

Figure 3.3 Q-Q plot demonstrating deviation from normal distribution for THRR data

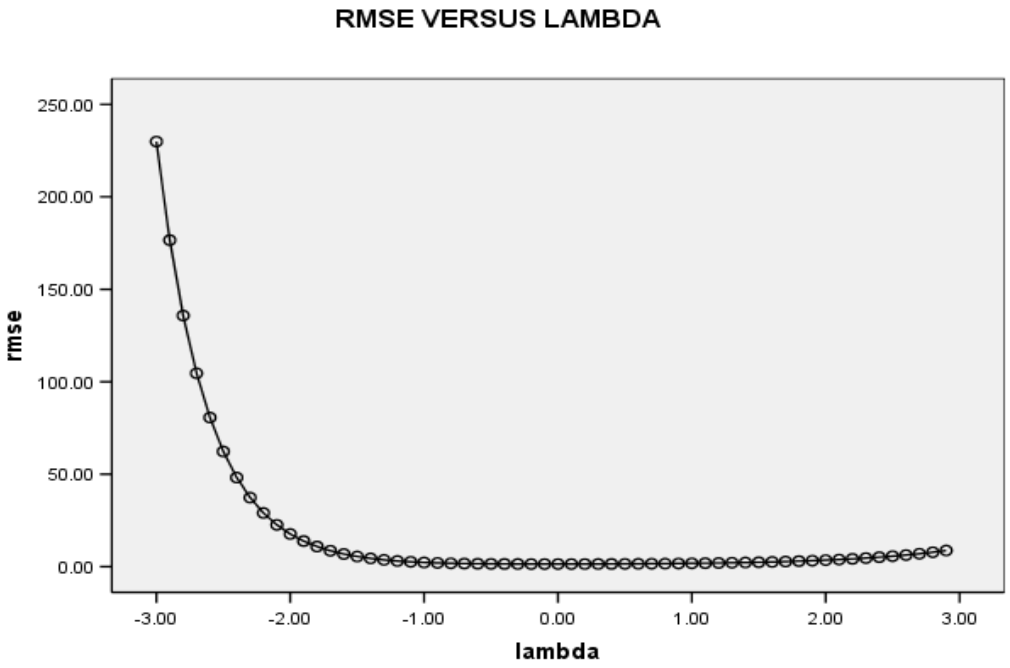

Figure 3.4 Box-Cox transformation equation graph of root-mean-square-error (RMSE) versus lambda of THRR measurements. The RMSE nadir occurs at a lambda of approximately 0.000

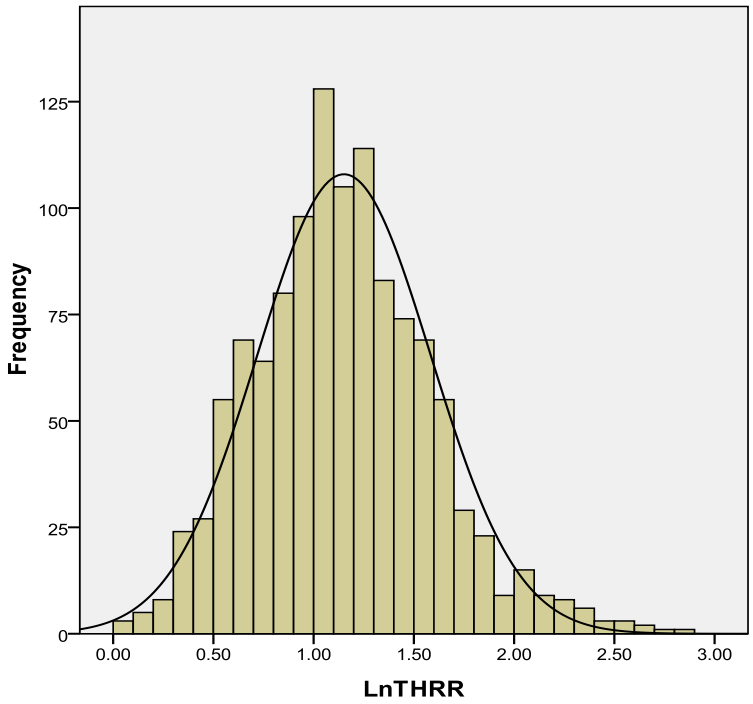

Figure 3.5 Histogram of the frequency distribution of natural logarithm transformed THRR (LnTHRR) measurements

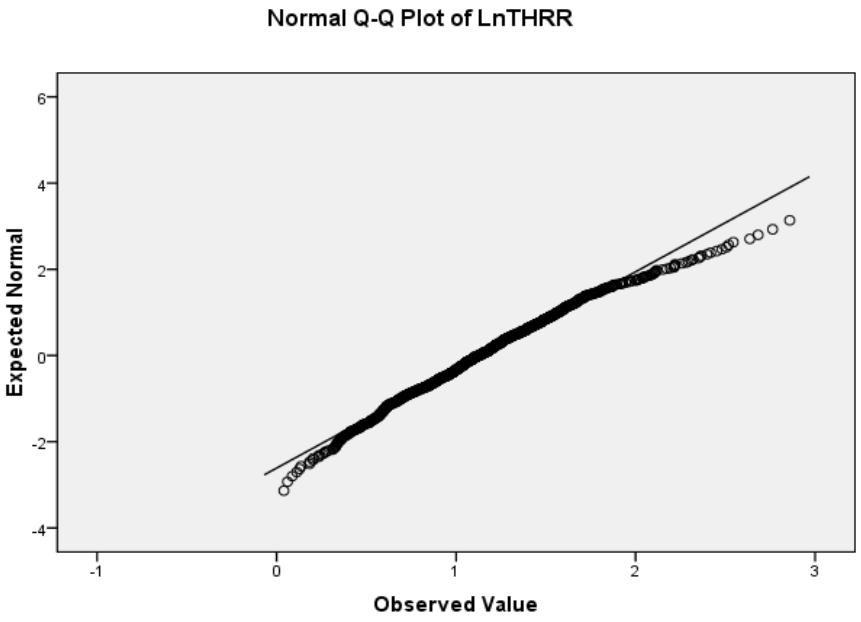

Figure 3.6 Q-Q plot demonstrating approximation of normal distribution of LnTHRR measurements

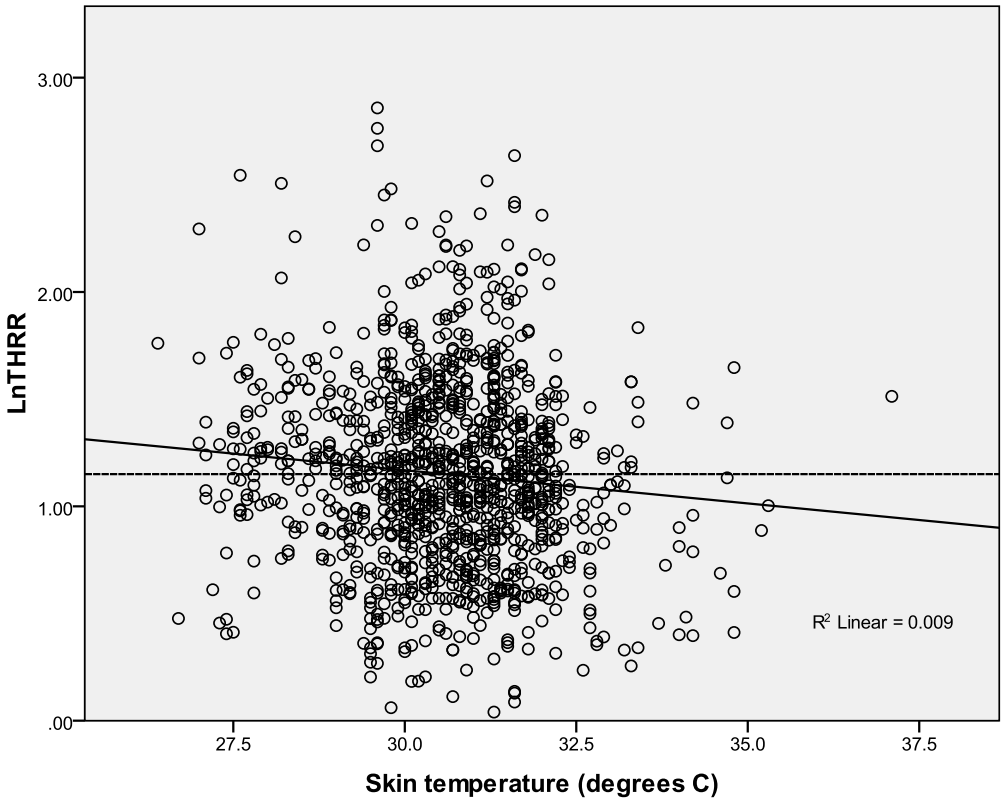

Figure 3.7 Scatter plot graph of LnTHRR variation with skin temperature. The dotted line represents the overall mean whilst the unbroken line represents an interpolated line assuming a linear relationship between temperature and LnTHRR.

Variation within and between groups

Heteroskedasticity was demonstrated between all groups of measurements within the temperature-limited aggregate dataset when examined using the Levene statistic. Because small or uneven sample sizes are predicted to affect variance the Levene statistic was also separately applied to comparisons limited to subgroups with equal measurement numbers; and also to comparisons between the largest 10 subgroups (with ≥ 20 measurement per subgroup). Variance was unequal between all subgroups, even where comparisons were limited to the same observer, subject, or both (in all cases $p<0.001$). This was true of both untransformed THRR measurements and log transformed data.

The differences in variances made it difficult to identify the range of within-subject and between-subject variation. The same differences also interfered with attempts to measure point at which the mean of repeated measures might be expected to approximate the mean.

The co-efficients of variation were calculated for THRR measurements from the largest 10 subgroups revealing a range of co-efficients from 0.23 to 0.43.

Using the entire temperature-limited aggregate dataset (i.e. combining the total within and between subject variability) the coefficient of variation for LnTHRR was 0.38. Error appears to be dependent upon the absolute value of the measurement with a correlation coefficient of 0.296 ($p=<0.004$), as demonstrated by the graph of absolute differences plotted against mean values for each subgroup (figure 3.8).

Where the mean LnTHRR values from each individual were compared the overall mean did not change but the standard deviation was 0.31.

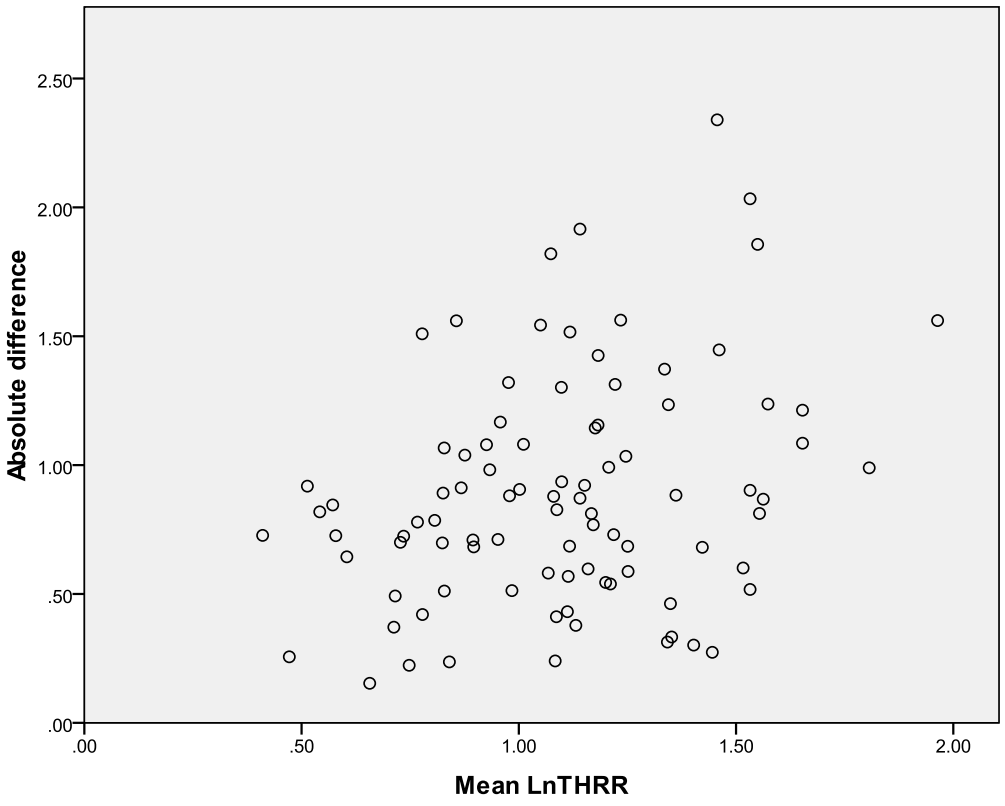

Figure 3.8 Scatter plot graph of the absolute differences in LnTHRR measurements plotted against mean LnTHRR values for each subgroup

THRR measurements made using needle-probes

A smaller dataset was collated of THRR measurements made using laser Doppler needle probes (see chapter two). 215 measurements were made, involving 50 volunteers. There were far fewer needle-probe readings per volunteer, with only 18 volunteers contributing more than 5 measurements. Limiting the skin temperature range of samples eligible for inclusion and log-transformation of the resulting THRR measurements resulted in a dataset of 192 measurements, which were normally distributed (Kolgorov-Smirnov test, $p=0.200$).

The average skin temperature measured by needle probes was 29.5°C (SD 0.61) which consistently lower than that recorded by normal laser Doppler probes (mean 30.4°C, SD 0.82). This finding was confirmed by taking paired samples from the same subject, at the same time with the same observer, using a paired t-test ($p<0.001$).

The overall mean of LnTHRR was smaller than was measured using the larger probes (mean 1.052, SD 0.471). Using single-volunteer subgroups where more than 5 measurements had been taken coefficients of variation ranged from 0.16 to 0.54 (table 3.2).

Using paired samples (as described above) revealed no significant differences between LnTHRR measurements taken using needle probes when compared with standard probes (standard probe mean: 1.14, SD 0.33; needle probe mean 1.09, SD 0.41; unequal variance t-test $p=0.363$).

	Laser Doppler	
	Standard probe (n=1010)	**Needle probe (n=192)**
Mean LnTHRR	1.152	1.052
Standard deviation	0.439	0.471
Standard error of the mean	0.138	0.331
Coefficient of variation	0.381	0.432

Table 3.2 Comparison of mean LnTHRR readings, and associated variation, using both standard laser Doppler probes and needle laser Doppler probes.

Other factors measured by laser Doppler flowmetry

- Baseline flowflux variability

The mean baseline flow-flux for standard probes was 20.40 (minimum 7.5, maximum 126.7, SD 11.84); and for needle probes was 10.25 (minimum 3.7, maximum 46.9, SD 6.60). The difference in flow-flux between standard and needle probes was significantly different (independent t-test assuming unequal variances, $p<0.001$). Baseline flowflux also exhibited heteroskedasticity between volunteers.

There was no clear correlation between skin temperature and baseline flowflux, although there was a suggestion of increased flowflux variability as skin temperature increased (figure 3.9). Increasing flow-flux was also associated with a reduction in range and absolute value of the THRR response (figure 3.10).

- THR shape

Four differently shaped THR responses were identified. The most common response (figure 3.11 Trace A) involved a sudden increase in flow-flux in less than 5 seconds with a gradual decline to baseline values over 15-20 seconds. Occasionally a more gradual increase in flowflux was seen where both the increase and decline in flow-flux occurred over a number of seconds (figure 3.11B). The time taken for flow-flux values to return to baseline values also varied, and in some cases there appeared to be second, smaller peak (figure 3.11C) or inflection point (figure 3.11D).

The average time taken to return to baseline flow-flux levels following a THR test (THR time) was 16.1 seconds (minimum 4, maximum 70, SD 7.2). Although the time taken to return to baseline appeared to be related to the size of the THRR, relatively small hyperaemic responses could also be prolonged.

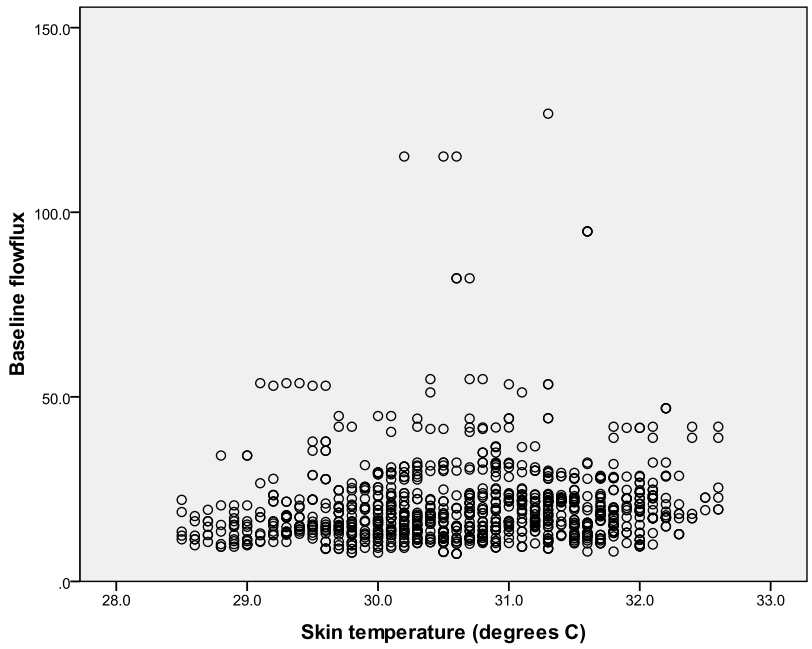

Figure 3.9 Scatter plot graph of baseline flowflux versus skin temperature within the temperature-limited dataset

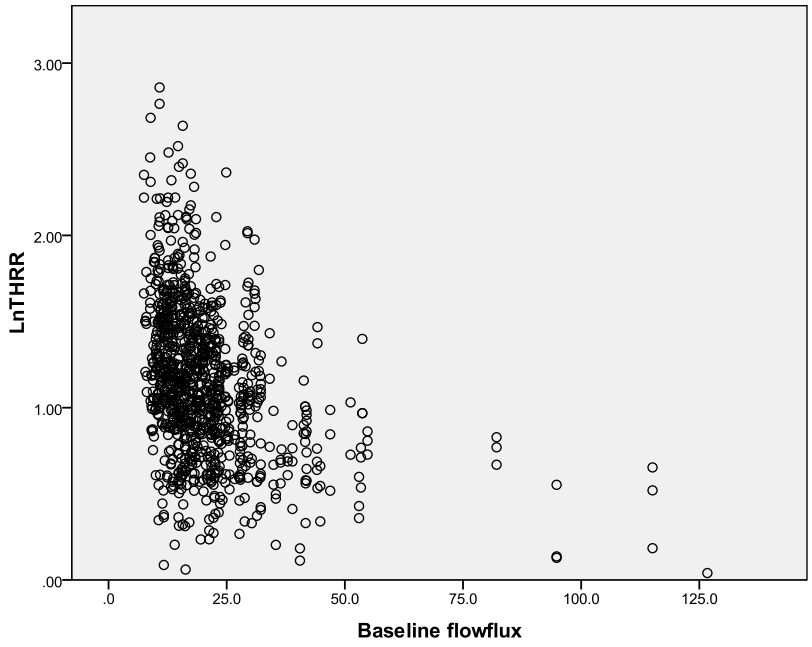

Figure 3.10 Scatter plot graph of LnTHRR versus baseline flowflux within the temperature-limited dataset

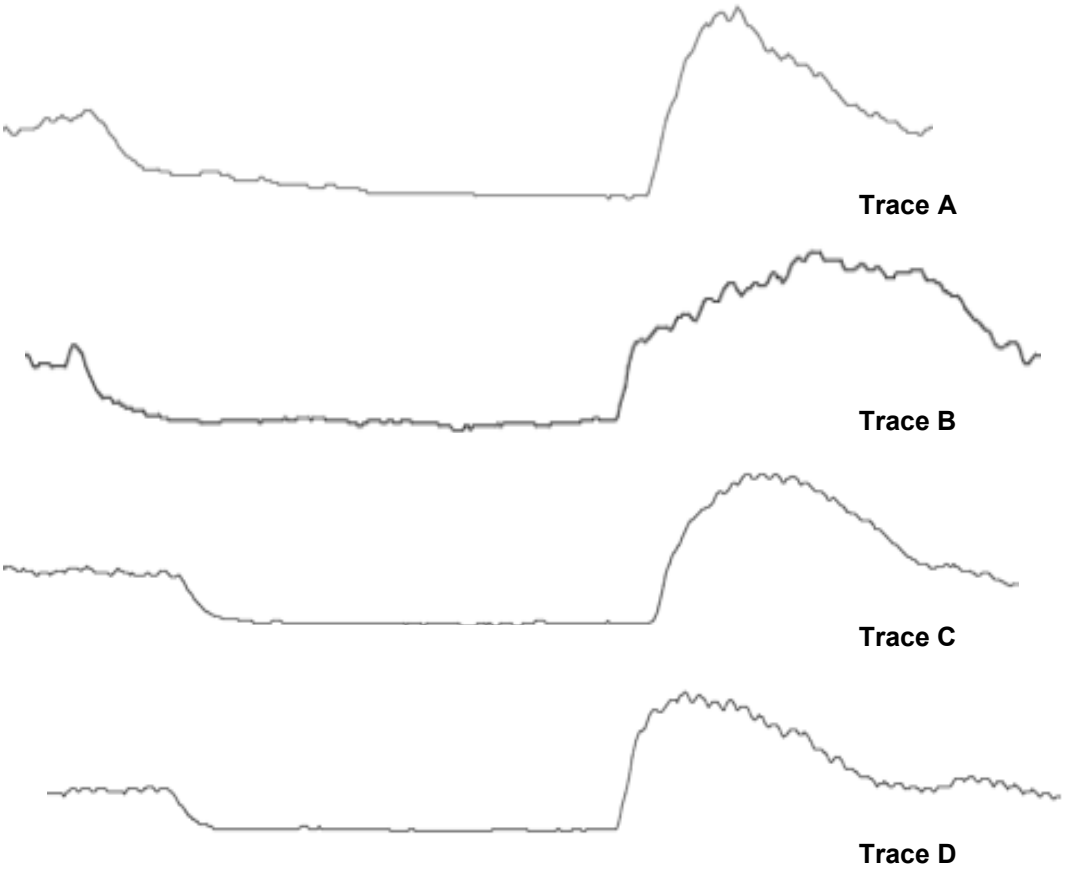

Figure 3.11 Sample THR traces. Trace A is the most commonly seen THR response. Trace B demonstrates a slowly rising hyperaemic response to transient ischaemia. Traces C and D demonstrate slow resolution of hyperaemia with a longer time taken to return to baseline flow-flux levels, and with a possible inflection point or secondary, lesser hyperaemic response.

Age and gender

Using average values from the temperature-limited dataset gender did not appear to make any difference in terms of recorded skin temperature, baseline flow-flux, LnTHRR, or THR time (table 3.3). Subjects' ages ranged between 18 and 51 years, and over this range there was a correlation between increasing age and increased skin temperature using Spearman rank correlation test ($r= -0.370$, $p<0.001$); and an increase of half a degree was found in older subjects (35 years and over) compared with younger subjects 25 years and under (unequal variance independent t-test, $p=0.006$). There were no statistically significant correlations between age and baseline flow-flux, LnTHRR, or THR time (figure 3.12).

	All (n=88)	Male (n=58)	Female (n=30)	*P values*
Mean skin temperature, °C (SD)	30.66 (0.82)	30.67 (0.79)	30.64 (0.89)	0.885
Mean baseline flowflux (SD)	20.40 (11.84)	22.23 (9.10)	22.46 (19.11)	0.951
Mean LnTHRR (SD)	1.15 (0.44)	1.11 (0.34)	1.03 (0.48)	0.216
Mean THR time, seconds (SD)	16.1 (0.72)	15.8 (0.42)	15.4 (0.38)	0.656

Table 3.3 Mean skin temperature, flowflux, LnTHRR, and THR time in male and female subjects taken from the temperature-limited dataset

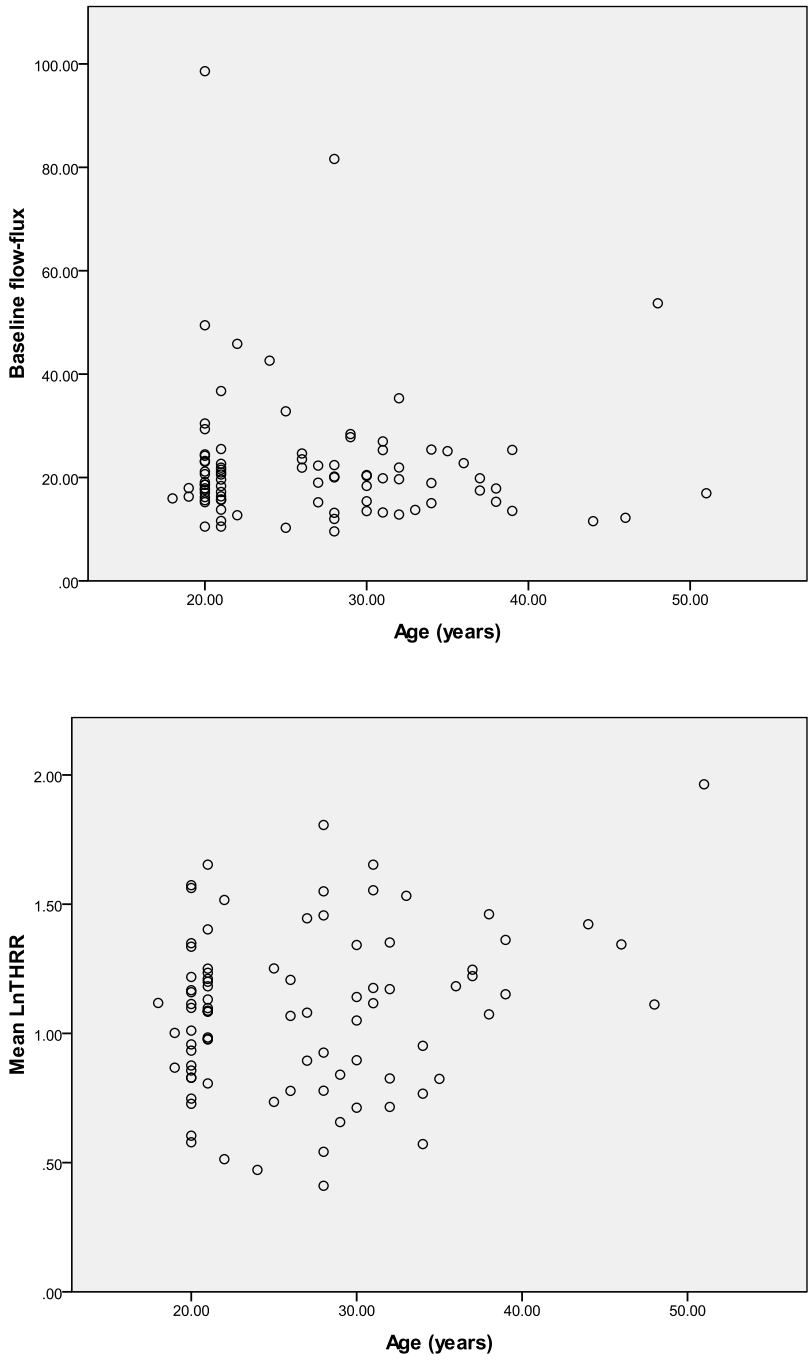

Figure 3.12 Scatter plot graphs of average flow flux and LnTHRR versus age of subjects

Discussion

These results confirm that THRR data is not normally distributed. Given the nature of the data this is not unexpected. Previous studies involving THRR data have compared results using both parametric and non-parametric statistical tests (Webster VL et al, 2002; Brown H et al, 2003; Moppett IK and Davies J et al, 2003; Moppett IK and Jones LN et al, 2003). Tests such as ANOVA and Mann-Whitney *U* are often considered to be relatively robust and able to cope with deviations from normality distribution (Bland M, 1987); unfortunately the finding of heteroskedasticity may be more of a problem as it may undermine some of the basic assumptions of such tests. Heteroskedasticity commonly occurs when combining data from groups which are small or have unequal sample sizes, but in this analysis it persisted even when data was limited to groups of the same size. Differences in variance occurred between data from different subjects, and to a lesser extent also between data taken from the same subject at different time points (with no change in observer or change of measurement site).

It was hoped that a natural logarithmic transformation could convert the data to near-normal distribution, as was the case; and in doing so might decrease the variance between groups of measurements. This did not happen and it seems likely that heteroskedasticity is an intrinsic property of the THR test, and these results would suggest that it is, at least in part, related to an increase in variability with larger measurements (figure 3.8).

Given that THR measurements are ratios of change in flowflux, which is measured in arbitrary units, a logarithmic scale is an appropriate method of representation (Bland M, 1987). Although a further variance-stabilising data-transformation (for example taking the square-route of the natural logarithm of the THRR data) could also be applied, standard deviations calculated from the transformed data could not be back-transformed (Bland M, Altman DG, 1996).

It is unclear how much the finding of heteroskedasticity could have interfered with the statistical analysis of results from previous studies, although it could be predicted that where ANOVA is used the power of the F-test would decrease (Weerhandi S, 1995).

A previous study by Hancock et al, 2001, using a single observer and 10 subjects on whom 10 THR measurements revealed a cumulate mean THRR of 1.86 (standard deviation 0.7) with the proportion of within-subject variance being 33% (total mean square 3.707). This would mean the overall coefficient of variation was 0.38 and the within subjects coefficient of variation was 0.23.

In contrast the overall coefficient of variation of THRR measurements within the temperature limited aggregate dataset was 0.55. ANOVA testing was not performed, but of the 10 largest single-subject, single-observer groups, all but two groups had an individual coefficient of variation of greater than 0.3. This would suggest that the data by Hancock et al might have underestimated the between- and within-subject variability.

It is unclear how this finding might affect previous studies with negative results, and it is possible these may need to be interpreted with caution.

LnTHRR measurements and power calculations

Previous studies used a measurement sample size of 3 in order to approximate the mean that would be achieved with larger repetitions of measurement. Using LnTHRR measurements derived from large single-subject, single-observer subgroups within the temperature-corrected aggregate dataset a worst-case-scenario can be estimated (i.e. applying the highest coefficient of variation to the smallest mean – a scenario which would be unlikely to occur if variability increases with larger measurement averages). In such a scenario the lowest mean is 0.90 and the largest standard error of the mean was 0.30 delivering a 95% probability that the mean of three measurements sample from an

individual will be with within ± 33% of the "true" mean. In practice the largest deviation that could be generated using real data, rather than worst-case scenario, was ± 20%.

Previous studies have involved between ten and twenty volunteers (Webster VL et al, 2002; Brown H et al, 2003; Moppett IK and Davies J et al, 2003; Moppett IK and Jones LN et al, 2003). Assuming that the mean LnTHRR values from single-subjects within the aggregate dataset did approximate their "true" means, a power calculation done using the standard deviation between subjects (0.31) suggests that using 15 subjects would identify a >28% change in LnTHRR (α 0.05; β 0.8).

Decreasing variability

There are many reasons why the THR test may have such a degree of variability, from subtle variations in skin blood flow or ambient light interference, to the difficulty in ensuring a true biological zero during arterial compression.

One of the advantages of the THR test is that, compared with other measurements of post-ischaemic reactive hyperaemia, it does not require prolonged periods of ischaemia (Kvernebo K, 1989; Patterson GC, 1955). It is possible that using digital compression to perform the THR test failure to achieve rapid arterial occlusion and release, or failure to maintain complete occlusion might increase the variability of results. In a previous study by Marval et al, 2004, digital compression was replaced by a pneumatic tourniquet driven by carbon dioxide, of the type used to facilitate orthopaedic procedures. This enabled very rapid inflation / deflation but had the disadvantage of requiring extra equipment and being very uncomfortable. In this study the overall coefficient of variation remained >0.3. For these reasons it was considered that digital arterial compression was likely to remain the least invasive and potentially more clinically useful method.

The relationship between temperature and THRR is not obvious. Biological zero is known to be related to skin temperature, in part due to the increase in residual Brownian motion (DRT4 manual, 2006; Fredriksson I et al, 2007), and flowflux itself has previously been shown to increase with skin temperatures over 37°C (O'Connor MB et al, 2003). O'Connor's research also revealed that THRR decreased as skin temperatures increased with active heating from 33-43°C. In our data only a very weak correlation between temperature and THRR was found at ambient skin temperatures.

In order to minimize or mitigate the effects of these differences in variability it would seem appropriate to design studies which use paired measurements from one subject and ensure that minimal skin temperature changes occur where paired data is used. Where this is not possible measurements could be compared with the aggregate dataset represented here, in which case it may be appropriate to limit LnTHRR readings to subjects with initial skin temperatures within the ranges of 28.5°C to 32.6°C. For the research involved in this thesis the above suggestions were observed where possible and only statistical tests that do not assume equal variance were used.

The use of needle probes

Although measurements made using needle probes appear to correlate well with measurements made using standard probes when paired readings are taken at the same time, when this is not possible it would seem prudent to limit comparisons to measurements with other needle probes where possible.

The relationship between flowflux and THR

One would expect that higher flowflux values might result in lower THR measurements resulting from there being less latent vasodilatation available in an already vasodilated system. This was found to be the case in this study (figure 3.10), but the relationship was not linear in nature and transient periods of ischaemia provoked a range of hyperaemic

responses for a given range of flowflux values. Low levels of skin perfusion have also been reported as lowering the THR (Hardman JG et al, 1997).

The vascular tree is known to be in a constant state of vasomotion (Bliss M, 1998) and factors which promote or inhibit the myogenic response to ischaemia may be present at all levels of baseline flow. It can be hypothesized that the duration of the THR response might be altered even where high levels of baseline flowflux diminish the magnitude of the hyperaemic response. Whilst this supports the idea that the THR test is a measure of autoregulation it means that it cannot be considered in isolation of skin perfusion.

The presence of factors promoting or inhibiting the hyperaemic response does not fully explain the different types of THR traces which can be identified. The pulsatile nature of capillary blood flow can be identified in flowflux traces but contributes only a very small component of baseline flowflux (in this study of the order of 5% of variation above and below baseline mean). Overlaid on this is the fluctuation caused by regular contractions which occur in metarterioles and pre-capillary sphincters at a rate of 5-10 contractions/minute (Bliss M, 1998). Within these studies fluctuations could sometimes be identified overlying the baseline flow, but they were not very marked. It can be hypothesized that fluctuations became more pronounced after ischaemia and became superimposed on the THR trace. If this is the case it might further add to the inherent variability of the THRR.

Age and gender

Previous studies have suggested that resting skin flowflux, and resting vasomotor tone are not substantially different between young and older healthy volunteers, but that responsiveness to cutaneous heating (at some sites), α-adrenergic vasoconstrictor stimulus, and vasodilatory stimulus (using nitroprusside or acetylcholine) may be blunted (Evans E et al, 1993; Algottson A et al, 1995; Dinenno FA et al, 2002).

Within the aggregate dataset there was no clear relationship between age and the flowflux or the THR response. A previous study by Abaza KT et al, 2004, comparing 33 young (21-25 years old) and 33 older (65-85 years old) volunteers, identified a significant decrease in flowflux in the older subjects (10.6 versus 15.2) but also identified no difference in THRR.

The data relating to the effect of gender on skin perfusion and vascular reactivity are mixed. Algotsson et al, 1995, found that resting skin perfusion measured by LDPI was lower in women than in men and exhibited a greater response to vasodilators. Other research suggests that the hyperaemic response to prolonged ischaemia was affected by the phase of the menstrual cycle, being reduced in the luteal phase (Bungum L et al, 1996) or increased in the follicular and luteal phases (Hashimoto M, et al, 1995). Another study found no gender difference or menstrual phase difference, but did identify a difference between post-menopausal women and older men as well as younger women and men (Gooding KM et al, 2001).

Within the aggregate dataset presented here there were no gender differences in flowflux or THRR.

CHAPTER FOUR

Manipulation of the THR test in volunteers

Introduction

A series of exploratory studies were designed in order to better understand the response of the THR test to aspects of therapy associated with the management of critical illness.

In the first experiment healthy volunteers were asked to breathe using a respiratory circuit in which there was expiratory resistance (positive-end-expiratory pressure, PEEP). The effects of external respiratory support on the transient hyperaemic response have not previously been investigated, although the effects of hyperventilation, hyper- and hypocapnia and hypoxia have. Previous studies have demonstrated that moderate decreases in expired air carbon dioxide tension (1 kPa below baseline values) result in decreased flowflux and THRR measurements, but only compared with moderate hypercapnia (1 kPa above baseline values). Hyperventilation without hypo- or hypercapnia did not affect the response (Richardson JR et al, 2004). Moderate hypoxia (oxygen saturations of 80-85%) has also been shown not to affect either flowflux or THRR measurements (Marval PD et al, 2004).

Respiratory support is commonly required in the management of critically ill patients either via an endotracheal tube or non-invasive respiratory support using a face-mask. The mode of this support can vary but mostly consists of PEEP which can be combined with an inspiratory pressure in order to drive or assist ventilation. The addition of external pressure will raise the mean intra-thoracic pressure. Where continuous pressure is applied, for instance using continuous positive-airways pressure (CPAP), the intra-thoracic pressure is likely to remain positive even when the patient is breathing spontaneously. Where PEEP alone is applied intra-thoracic pressures are likely to oscillate between negative and higher-than-normal positive pressures.

CPAP has been shown to decrease cardiac stroke volume in healthy volunteers and deplete intra-thoracic blood volume, shifting towards intra-abdominal organs. It might be predicted that such a change in large central vascular beds might trigger changes elsewhere in the body. A study by Fournell A et al, 2003, on gastric mucosal oxygenation demonstrated that it was decreased in proportion to the level of CPAP applied, suggesting a redistribution of blood away from the gastric mucosa. Changes in limb volume, which were ventilation related and occurred after the application of PEEP, have also been identified using strain gauge plethysmography (Christ F et al, 1995). In the latter study it was shown that cyclical changes occurred within the microvasculature, termed vasomotion, which were in phase with the changes in central venous pressure (CVP) caused by ventilation.

In the study outlined below (termed protocol one) PEEP was used instead of CPAP for two reasons. Firstly this would allow for moderate average rises in intra-thoracic pressure to be evaluated; and secondly in order to reproduce the cyclical changes previously mentioned and measure the changes in flowflux they generated whilst attempting to perform THR measurements.

In the second study (protocol 2) various agents were iontophoresed into forearm skin in order to measure their effects on cutaneous vascular reactivity. All of the agents chosen are commonly used within critical care, propofol and midazolam as sedatives, and in the case of hypertonic saline (HTS) and mannitol as resuscitation fluids predominantly used in patients with traumatic brain injury.

Mannitol and hypertonic saline have previously been promoted as diluents, specifically because they are not well iontophoresed (Asberg A et al, 1999; Abou-Elenin K et al, 2002), and in theory should not cause vasodilatation even though they, and the other agents chosen, are known to have vasodilatory effects in vivo when delivered intravenously.

There is very limited data concerning the iontophoresis of lipid soluble agents such as propofol and it seemed unlikely that propofol would cause any vasodilatation, so in essence this was an exploratory study to examine whether or not these agents could be used to trigger vasodilatory responses.

The effects of the iontophoresis of vasodilatory agents, such as acetylcholine and sodium nitroprusside, on vascular reactivity have been extensively investigated and two studies have even demonstrated the ablation of THRR after their administration (Webster VL, et al, 2002; Brown H et al, 2003). The iontophoresis of constrictive agents, such as norepinephrine, epinephrine and phenylephrine is less well investigated with regard to changes in vascular reactivity. The study by Brown H et al, 2003, showed a decrease in flowflux with epinephrine, but not phenylephrine, and did not demonstrate a change in THRR with either agent. It was hypothesised that vasoconstriction would be unlikely to manifest changes in the vascular reactivity of healthy skin because low nutritive requirements resulted in an already low resting flowflux. In contrast studies measuring

changes in forearm vascular plethysmography have demonstrated a response to phenylephrine in healthy volunteers and, to a lesser extent, in patients with septic shock.

In the third experiment, protocol 3, iontophoresis of a different vasoconstrictor, norepinephrine, took place in healthy volunteers. In case norepinephrine was ineffective at vasoconstricting unstressed skin, a non-pharmacological method of vasodilating skin, localized heating, took place concurrently. Localised heating has previously been shown to increase skin flowflux and decrease THRR. A peak temperature of 42°C was chosen because it has been shown to be well tolerated and is the temperature at which the vasodilatory response plateaus, at least in volunteers in whom core body temperature was within the normal range (O'Connor MB et al, 2002; Taylor WF et al, 1984).

Methods

Three separate but linked protocols were used to manipulate the cutaneous THR in healthy volunteers in an attempt to replicate some of the conditions and circumstances to which critically ill patients might be subjected. Protocol one involved the application of positive-end-expiratory pressure (PEEP) to spontaneously breathing volunteers; protocol two involved the iontophoresis of pharmacological agents commonly administered to critically ill patients; and protocol three used localized cutaneous heating probes to mimic the vasodilatation seen in sepsis and systemic inflammatory states, combined with the co-administration of iontophoresed vasopressor agents. Optimal experimental conditions were obtained as previously described in chapter 2. The equipment used, along with the techniques for flowflux, THR measurement, iontophoresis and localised skin heating have also been previously described in chapter 2.

Protocol one: the effect of PEEP on THR

Baseline flowflux and THR measurements were performed on fifteen healthy volunteers. Each volunteer was then asked to breathe through a tight-fitting face mask which was attached via a T-piece to a breathing circuit. The breathing circuit had fresh gas (medical air) flowing into a reservoir bag attached to the inspiratory limb at 15 l/min and a PEEP valve attached to the expiratory limb (figure 4.1). The PEEP valve was set to apply 10 cmH_2O of resistance to expiratory flow. After the volunteer had been breathing through the circuit for five minutes flow flux and THR were once again measured. During all periods of flow-flux and THR measurement cutaneous pulse oximetry (SpO_2) and end-tidal capnography ($ETCO_2$) were used to measured blood oxygen saturation and the end-expiratory partial pressure of exhaled carbon dioxide.

Protocol two: the effect of iontophoresed chemicals on THR

Two separate groups of 15 healthy volunteers were recruited. Each individual underwent two separate episodes of baseline flowflux and THR measurements followed by

measurements taken after cutaneous iontophoresis of one of four chemicals, such that there were 15 sets of paired before and after data for each chemical. The chemicals used were: hypertonic (2.7%) saline solution, mannitol 10% solution, propofol 1% solution (emulsified in 10% soybean oil, 1.2% purified egg phospholipid, and 2.25% glycerol, marketed as Diprivan®), and midazolam diluted in 0.9% saline solution to a concentration of 1mg/ml. Flowflux and THR measurements were simultaneously made from both the active (iontophoresed) sites and control sites on the same forearm.

Midazolam was iontophoresed using a cathodal current due to the degree of positive ionization its water soluble state. Propofol, 2.7% saline and mannitol were all iontophoresed using an anodal current. In the case of 2.7% saline this was based upon the fact that although previous studies using hypertonic saline demonstrated only minimal hyperaemic effects after iontophoresis, these effects appeared to be larger when using anodal currents (Asberg et al, 1999; Brandrivskyy et al, 2004). Mannitol, a sugar alcohol, does not readily ionise in solution, but has previously been iontophoresed using anodal currents (Kirjavainen MH et al, Kim et al, 1993). Propofol iontophoresis was performed using an anodal current, even though it is not water-soluble and only ionizes at very high pH. Although attempts have been made to encourage the transdermal transmission of propofol, these have not utilized iontophoresis (Yamato K et al, 2009).

As propofol is an opaque white solution of oil droplets it would be impossible to use LDF whilst propofol was present within the iontophoresis chamber. For this reason the chamber was filled with 0.9% saline prior to baseline laser Doppler flowmetry, after which the chamber was washed out and then filled with propofol prior to iontophoresis. After iontophoresis the chamber was again washed out with saline and LDF measurements recorded. propofol

Protocol three: the effect heat of localized cutaneous heating and the co-administration of iontophoresed vasopressors on THR

Serial measurements of forearm flowflux and THR were taken before and after localised cutaneous heating (i.e. heating of skin directly under the LDF probe) using three groups of healthy volunteers. In first group 0.1% norepinephrine in 0.9% saline solution was iontophoresed prior to skin being heated to 35°C and then 45°C. Details of the study design for each group can be found in table 4.1. Simultaneous flowflux and THR measurements were also taken from control sites. In the other two groups skin was heated to either 35°C or 45°C prior to the iontophoresis of norepinephrine. The time taken to heat skin to the predetermined temperature varied between individuals, but was always less than two minutes.

Norepinephrine was iontophoresed using a cathodal current (i.e. the iontophoresis chamber was the cathode), and followed the iontophoresis protocol outlined in chapter two.

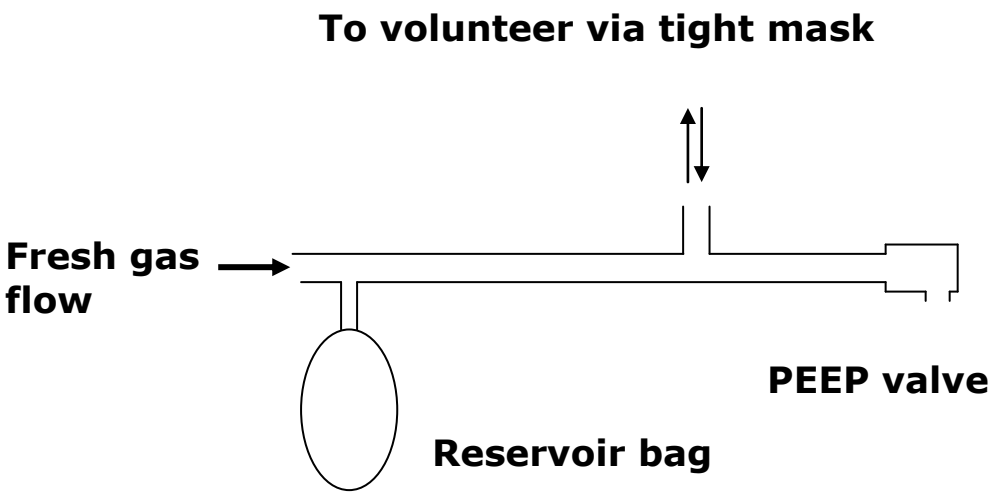

Figure 4.1 Breathing circuit used to provide PEEP to volunteers in **protocol one**

Group	Phase 1	Phase 2	Phase 3	Phase 4
1	Unheated skin	Iontophoresis	Heated to 35°C	Heated to 42°C
2	Unheated skin	Heated to 35°C	Iontophoresis at 35°C	
3	Unheated skin	Heated to 42°C	Iontophoresis at 42°C	

Table 4.1 The order in which localized cutaneous heating and norepinephrine iontophoresis occurred in each group within **Protocol 3**. Flowflux and THR measurements of both active and control sites occurred at each phase. There was no fourth phase in groups 2 and 3.

Results

Protocol one: the effect of PEEP on THR

Of the 15 volunteers, 11 were male and 4 were female. The median age was 34 years old (range 25 to 48). Resting heart rate, mean-arterial pressure (MAP), end-tidal CO_2, SpO_2 and skin temperature were not significantly altered by the application of PEEP (table 4.2).

Baseline flowflux, LnTHRR measurements and duration of THR were not significantly altered after the application of PEEP (table 4.3 and figure 4.2), even though flow-flux measurements showed marked fluctuations once PEEP was applied, which occurred in time with respiration (figure 4.3).

Variable	**Baseline, n=15**	**With PEEP, n=15**	***P*-value**
	Values are means (SD)		
Heart Rate, beats/min	71 (12.6)	74 (11.9)	0.09
Mean arterial pressure, mmHg	84 (11)	82 (8.3)	0.46
End-tidal CO_2, kPa	4.9 (0.5)	4.8 (0.5)	0.35
SpO_2, %	96 (1.4)	96 (1.5)	0.49
Skin temperature, °C	29.7 (1.5)	29.9 (1.6)	0.22
Flowflux	15.75 (11.01)	15.47 (12.43)	0.949
LnTHRR	1.30 (0.45)	1.37 (0.59)	0.687
THR time, seconds	15.0 (5.0)	12.6 (4.9)	0.196

Table 4.2 Skin temperature, cardio-respiratory variables, flowflux, LnTHRR, and THR time of volunteers before and during application of PEEP. Samples compared using paired t-test except for flowflux, LnTHRR and THR time which are compared using independent t-test with equal variance not assumed.

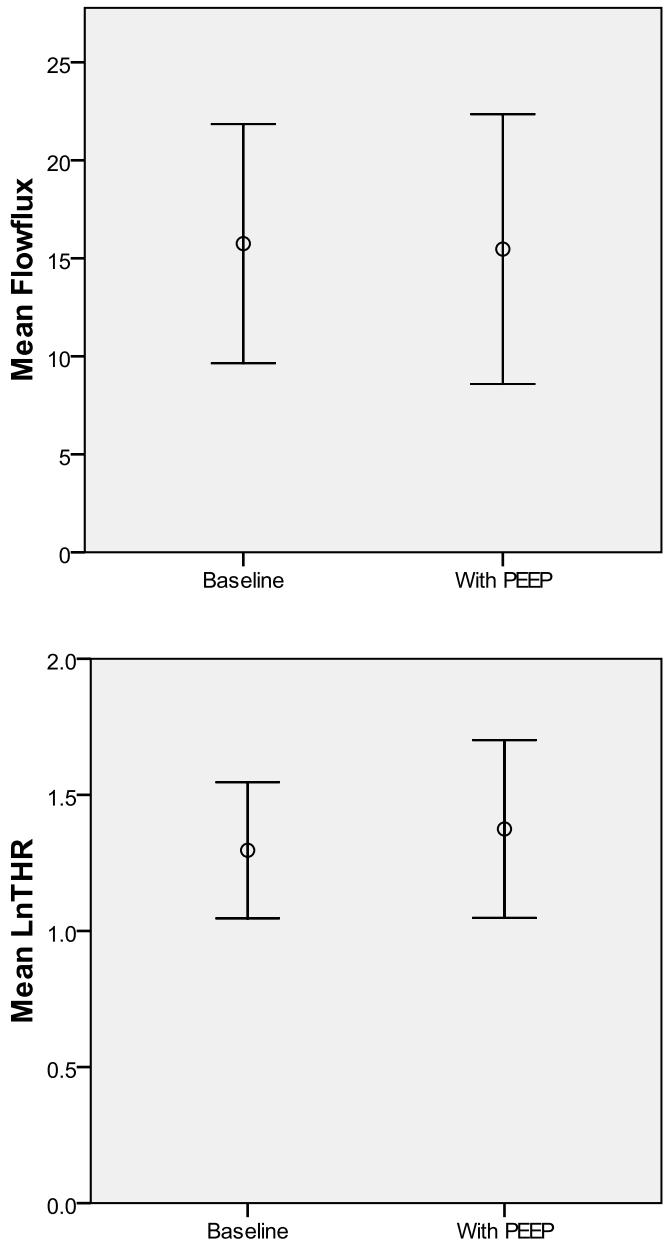

Figure 4.2 Mean flowflux and LnTHRR values in volunteers before and after the application of 10cmH_2O PEEP. Error bars represent 95% confidence intervals.

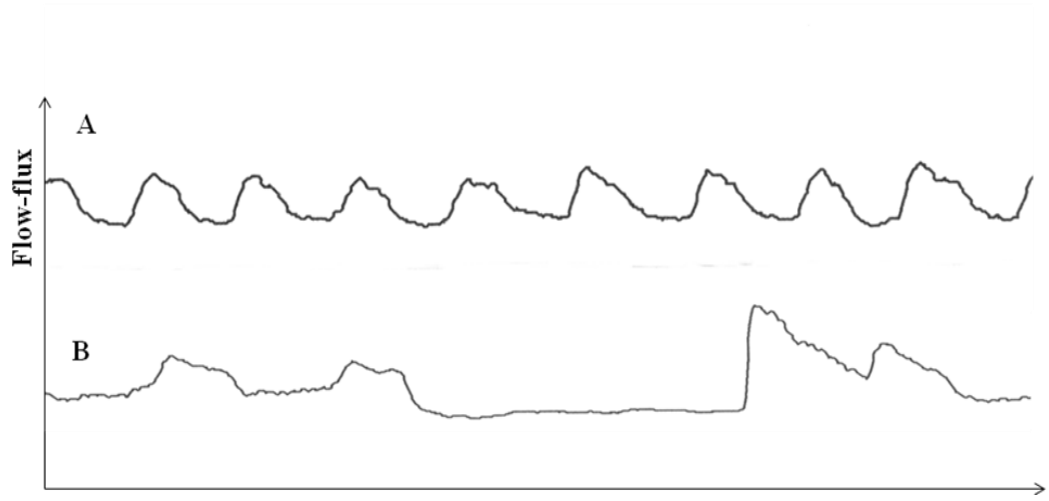

Figure 4.3 A sample flowflux and THR trace demonstrating the effect of PEEP on flow-flux in some volunteers. A: flowflux variation during baseline measurements; B: flowflux variation during THR

Protocol two: the effect of iontophoresed chemicals on THR

In each group there were 7 female and 8 male volunteers aged between 18 and 33 (median age 21 in both groups). Resting skin temperature in all subjects was within the guidelines set out in chapter 3. Only resting mean arterial pressure was measured and in each group this was within what could be considered normal acceptable limits (overall mean 81mmHg, SD 12)

Skin temperature, flowflux measurements, LnTHRR measurements and the duration of the THR response did not significantly change throughout the course of the experiments at any of the control sites (table 4.3).

Although there was no change in skin temperature after the iontophoresis of propofol or mannitol, both midazolam and 2.7% saline were associated with a statistically significant rise. In both cases the absolute rise was very small (0.7ºC and 1.1ºC respectively).

All of the drugs involved caused an increase in flowflux after iontophoresis and in each case there was an associated fall in LnTHRR (table 4.3, figure 4.4, and figure 4.5). Propofol and mannitol were associated with the highest rise in flowflux and the greatest fall in LnTHRR (in both cases the THR response was nearly ablated). Despite the decreases in LnTHRR with all drugs statistically significant decreases in THR time were only seen with midazolam and propofol.

	Chemical	Probe	Iontophoresis		*P*-value
			Before	After	
Skin temperature, °C Mean (SD)	Midazolam	Control	30.0 (1.3)	30.0 (1.3)	0.970
		Active	29.1 (0.8)	29.8 (0.6)	0.018
	Propofol	Control	30.3 (1.2)	30.1 (1.0)	0.654
		Active	29.1 (1.0)	28.5 (0.9)	0.071
	2.7% Saline	Control	29.9 (0.6)	29.9 (1.3)	0.799
		Active	29.1 (0.7)	30.2 (0.6)	<0.001
	Mannitol	Control	30.4 (1.9)	30.3 (1.8)	0.812
		Active	29.5 (1.8)	30.4 (1.6)	0.179
Flowflux Mean (SD)	Midazolam	Control	20.36 (7.82)	17.44 (7.80)	0.316
		Active	7.35 (2.89)	37.4 (29.57)	0.001
	Propofol	Control	23.11 (9.85)	18.17 (8.02)	0.144
		Active	7.90 (3.67)	57.94 (14.83)	<0.001*
	2.7% Saline	Control	20.29 (8.24)	16.94 (6.18)	0.235
		Active	9.97 (4.97)	33.72 (25.93)	0.003
	Mannitol	Control	21.01 (8.93)	20.16 (9.35)	0.799
		Active	6.07 (2.16)	50.16 (20.41)	<0.001
LnTHRR Mean (SD)	Midazolam	Control	1.12 (0.30)	1.21 (0.33)	0.428
		Active	1.21 (0.43)	0.52 (0.75)	0.005
	Propofol	Control	1.19 (0.43)	1.18 (0.35)	0.928
		Active	1.23 (0.39)	0.09 (0.10)	<0.001
	2.7% Saline	Control	1.02 (0.22)	1.13 (0.28)	0.260
		Active	1.06 (0.38)	0.36 (0.40)	<0.001
	Mannitol	Control	0.89 (0.26)	1.01 (0.28)	0.208
		Active	1.27 (0.72)	0.13 (0.13)	<0.001
THR time, seconds Mean (SD)	Midazolam	Control	16.1 (6.9)	14.8 (4.0)	0.528
		Active	16.2 (6.4)	9.8 (6.5)	0.013
	Propofol	Control	15.0 (4.7)	15.5 (4.0)	0.757
		Active	15.6 (5.4)	11.8 (4.6)	0.050
	2.7% Saline	Control	19.3 (10.7)	19.3 (10.1)	0.986
		Active	15.6 (5.5)	20.8 (7.1)	0.482
	Mannitol	Control	22.0 (7.7)	19.4 (5.7)	0.319
		Active	21.7 (8.0)	17.2 (6.5)	0.106

Table 4.3 Skin temperature, flowflux, LnTHRR and THR time before and after the iontophoresis of midazolam, propofol, 2.7% saline or mannitol (designated active), compared with a control site with no iontophoresis (designated control). *P*-values represent the statistical significance comparing before and after values using an independent samples t-test where equal variances are not assumed

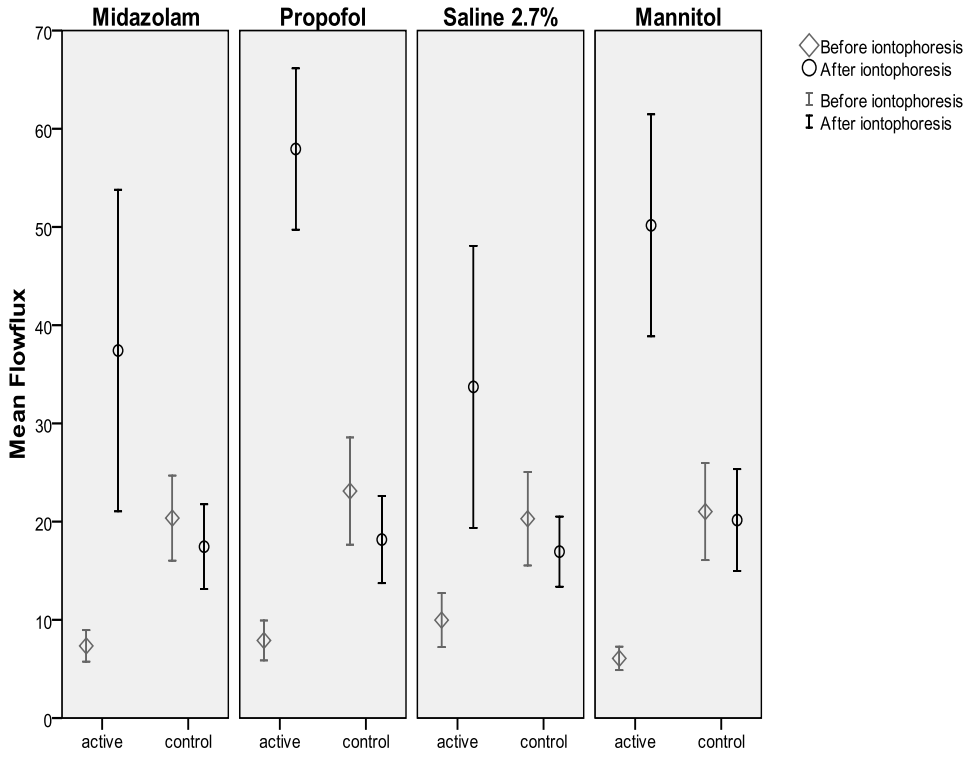

Figure 4.4 Graph of mean flowflux before and after the iontophoresis of midazolam, propofol, 2.7% saline or mannitol (designated active), compared with mean flowflux at control site where no iontophoresis took place (designated control). Error bars represent 95% confidence intervals.

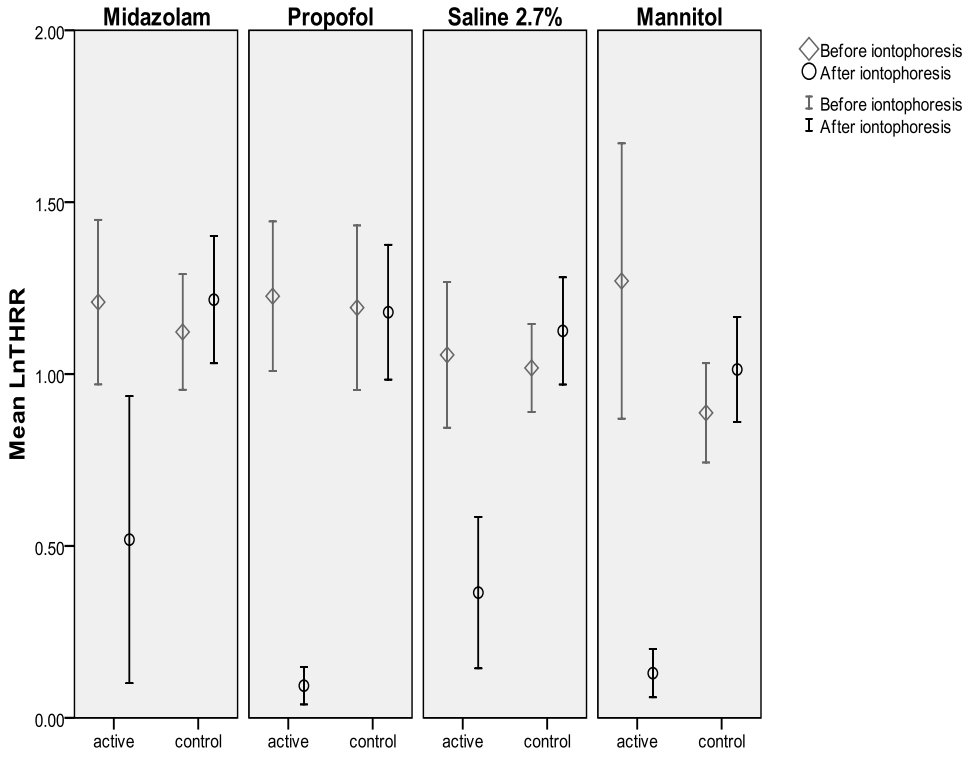

Figure 4.5 Graph of mean LnTHRR before and after the iontophoresis of midazolam, propofol, 2.7% saline or mannitol (designated active), compared with mean LnTHRR at control site where no iontophoresis took place (designated control). Error bars represent 95% confidence intervals.

Protocol three: the effect of localized cutaneous heating and the co-administration of iontophoresed vasopressors on THR

The age range and gender breakdown of volunteers within each group of the Protocol 3 can be found in table 4.4, along with the average resting mean arterial pressure and resting skin temperature.

Within the first group, where the iontophoresis of norepinephrine occurred prior to cutaneous heating to 35°C and then 42°C, skin flowflux decreased after the iontophoresis and did not increase after heating to 35°C but did increase after heating to 42°C back to levels which were statistically unchanged from levels before the introduction of norepinephrine (table 4.5). Skin temperature increased slightly after the iontophoresis of norepinephrine (mean increase 1.5°C). LnTHRR and the duration of the THR response both decreased after iontophoresis and remained at low levels even after heating to 42°C (table 4.5 and figure 4.6).

Within the second group, the iontophoresis of norepinephrine *after* cutaneous heating to 35°C, there was a rise in flowflux accompanying heating and a decrease after norepinephrine, but this was not statistically significant (table 4.6 and figure 4.7). LnTHRR decreased only after norepinephrine which was statistically significant when compared with heated skin, but not with unheated skin. The duration of THR also decreased after norepinephrine compared with both heated and unheated skin.

Within the third group, the iontophoresis of norepinephrine *after* cutaneous heating to 42°C, flowflux increased dramatically after heating, but after norepinephrine returned to values approximating baseline levels (table 4.7 and figure 4.8). LnTHRR also decreased dramatically after heating, and in many cases a “negative hyperaemic response” was seen (figure 4.9). Norepinephrine increased the magnitude of LnTHRR but not back up to baseline levels. The duration of THR decreased after heating and this decrease was not

reversed by the iontophoresis of norepinephrine, although it should be noted that the THR time after heating refers to a significant number of “negative hyperaemic” episodes, whilst the THR time after norepinephrine only refers to positive, or true, hyperaemic responses.

	Median age years (range)	**Gender**	**Blood pressure** MAP: mmHg (SD)	**Skin temperature** °C (SD)
Group 1 (iontophoresis followed by skin heating)	31 (26-44)	7 female 8 male	82 (14)	30 (1.6)
Group 2 (iontophoresis at 35°C)	33 (25–51)	7 female 8 male	87 (10)	31 (1.2)
Group 3 (iontophoresis at 42°C)	34 (20–51)	9 female 6 male	85 (14)	30 (0.9)

Table 4.4 Baseline characteristics of volunteers prior to cutaneous heating or iontophoresis in all three groups within Protocol 3, including median age, gender, resting blood pressure (recorded as mean arterial pressure, MAP, in mmHg) and resting skin temperature.

Group 1: the iontophoresis of norepinephrine followed by cutaneous heating

	Phase 1	**Phase 2**	**Phase 3**	**Phase 4**	
	Iontophoresis of norepinephrine		**Cutaneous heating**		
	Before	After	35°C	42°C	*P*-value
Temperature °C, (SD)	28.0 (1.5)	29.5 (1.6)	**35.0**	**42.0**	0.013*
Flowflux (SD)	12.83 (5.00)	6.55 (1.55)	6.21 (1.08)	9.55 (2.58)	<0.001**
LnTHRR (SD)	1.04 (0.39)	0.65 (0.32)	0.72 (0.41)	0.59 (0.37)	0.009†
THR time (SD)	18.2 (9.1)	8.3 (4.0)	8.7 (3.3)	6.1 (2.8)	<0.001‡

Table 4.5 Forearm skin temperature, flowflux, LnTHRR, and THR time values in volunteers from Group 1 where iontophoresis of norepinephrine was followed by cutaneous heating to 35°C and then 42°C

* Independent sample t-test comparing skin temperature before and after iontophoresis, but before skin heating

** ANOVA test statistic with Tamhane's T2 test applied: phase 1 vs. phase 2 (p = 0.001); phase 2 vs. phase 3 (p = 0.983); phase 3 vs. phase 4 (p = 0.001); phase 1 vs. phase 4 (p = 0.188); phase 1 vs. phase 3(p = 0.001); phase 2 vs. phase 4 (p = 0.005).

† ANOVA test statistic with Tamhane's T2 test applied: phase 1 vs. phase 2 (p = 0.036); phase 2 vs. phase 3 (p = 0.997); phase 3 vs. phase 4 (p = 0.941); phase 1 vs. phase 4 (p = 0.019); phase 1 vs. phase 3(p = 0.209); phase 2 vs. phase 4 (p = 0.998).

‡ ANOVA test statistic with Tamhane's T2 test applied: phase 1 vs. phase 2 (p = 0.006); phase 2 vs. phase 3 (p = 1.00); phase 3 vs. phase 4 (p = 0.145); phase 1 vs. phase 4 (p = 0.001); phase 1 vs. phase 3(p = 0.008); phase 2 vs. phase 4 (p = 0.442).

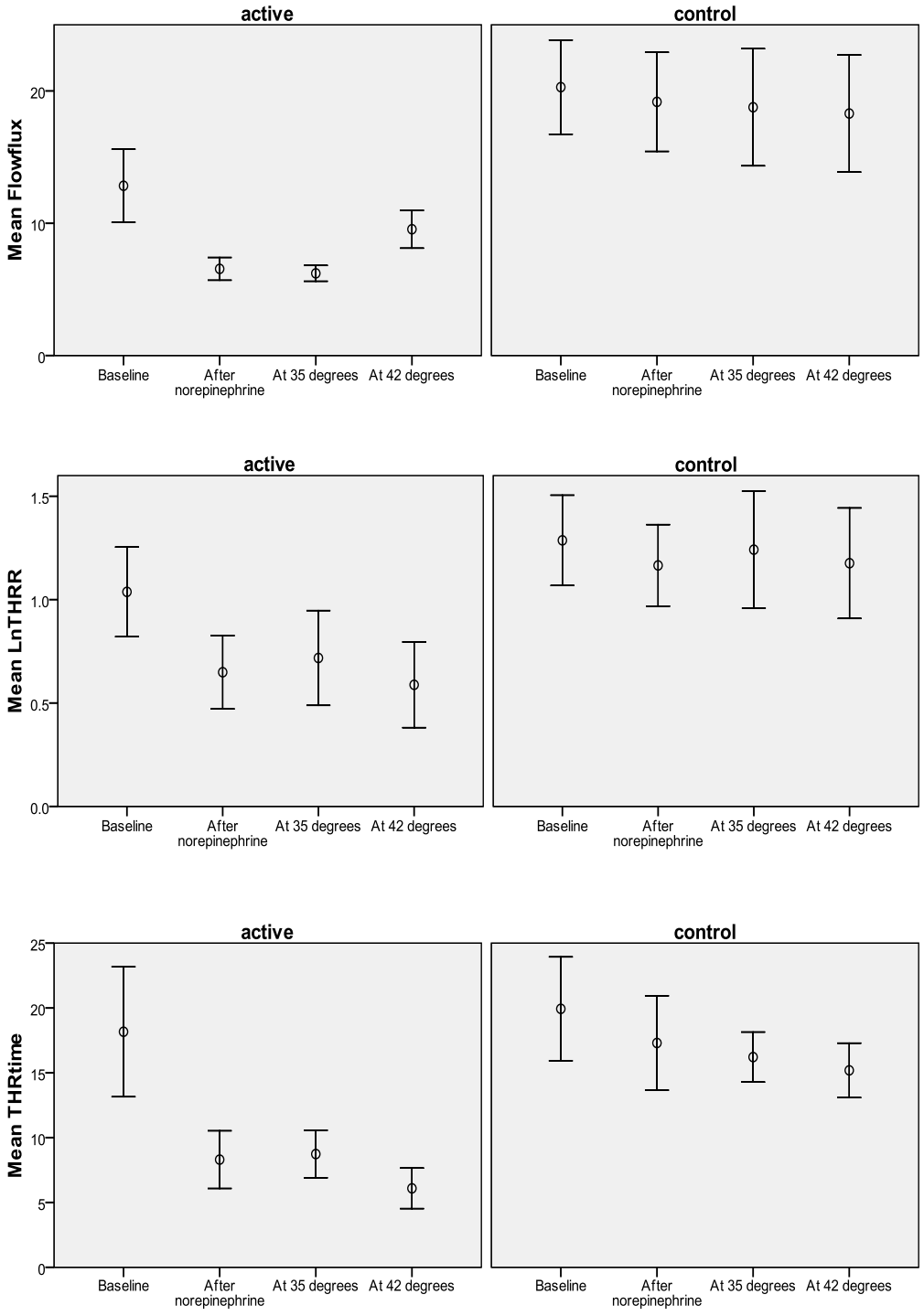

Figure 4.6 Mean flowflux, LnTHRR and THR time in volunteers after iontophoresis of norepinephrine followed by skin heating to 35°C and then 42°C; control sites had no heating or iontophoresis. Error bars represent 95% confidence intervals.

Group 2: cutaneous heating to 35°C followed by iontophoresis of norepinephrine

	Phase 1	**Phase 2**	**Phase 3**	
	Cutaneous heating		**Norepinephrine iontophoresis at 35°C**	
	Unheated	At 35°C		*P*-value
Temperature, °C (SD)	28.4 (1.1)	**35**	**35**	
Flowflux (SD)	15.52 (5.27)	19.76 (11.17)	13.69 (15.75)	0.346*
LnTHRR (SD)	0.89 (0.30)	0.96 (0.24)	0.57 (0.49)	0.012**
THR time (SD)	15.5 (7.3)	12.9 (4.9)	8.2 (4.8)	0.005†

Table 4.6 Forearm skin temperature, flowflux, LnTHRR, and THR time values in volunteers from Group 2 where cutaneous heating to 35°C was followed by iontophoresis of norepinephrine

* ANOVA test statistic with Tamhane's T2 test applied: phase 1 vs. phase 2 (p = 0.486); phase 2 vs. phase 3 (*p* = 0.552); phase 1 vs. phase 3 (*p* = 0.966).

** ANOVA test statistic with Tamhane's T2 test applied: phase 1 vs. phase 2 (*p* = 0.868); phase 2 vs. phase 3 (*p* = 0.038); phase 1 vs. phase 3 (*p* = 0.125).

† ANOVA test statistic with Tamhane's T2 test applied: phase 1 vs. phase 2 (*p* = 0.612); phase 2 vs. phase 3 (*p* = 0.039); phase 1 vs. phase 3 (*p* = 0.011).

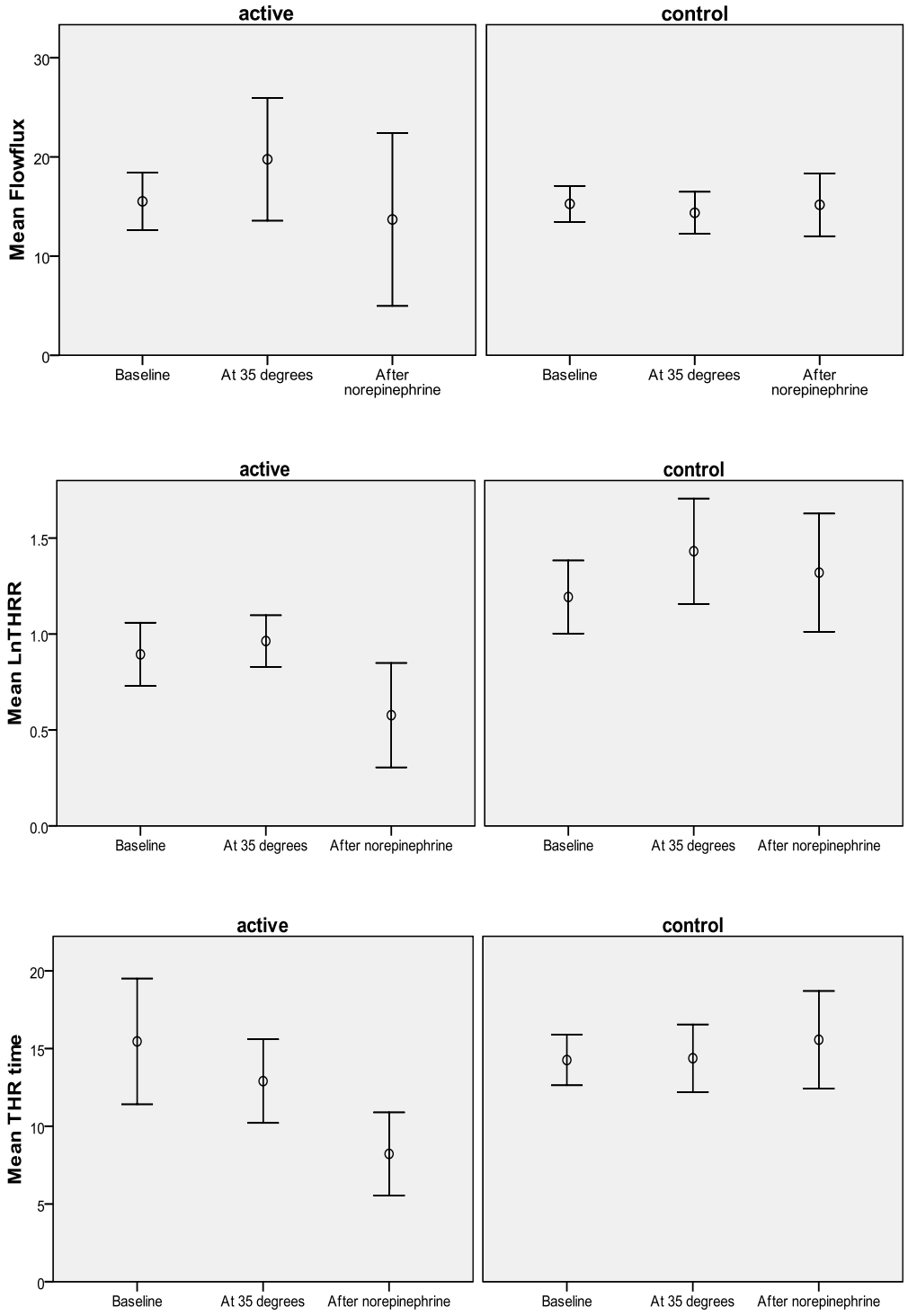

Figure 4.7 Mean flowflux, LnTHRR and THR time in volunteers after skin heating to 35°C followed by iontophoresis of norepinephrine; control sites had no heating or iontophoresis. Error bars represent 95% confidence intervals

Group 3: cutaneous heating to 42°C followed by iontophoresis of norepinephrine

	Phase 1	**Phase 2**	**Phase 3**	
	Cutaneous heating		**Norepinephrine iontophoresis at 42°C**	
	Unheated	At 42°C		*P*-value
Temperature, °C (SD)	28.2 (1.0)	**42**	**42**	
Flowflux (SD)	14.93 (7.83)	124.63 (61.40)	16.11 (8.14)	<0.001*
LnTHRR (SD)	1.08 (0.44)	- 0.53 (0.35)	0.49 (0.34)	<0.001**
THR time (SD)	15.7 (6.0)	4.7 (2.4)	8.9 (6.5)	<0.001†

Table 4.7 Forearm skin temperature, flowflux, LnTHRR, and THR time values in volunteers from Group 3 where cutaneous heating to 42°C was followed by iontophoresis of norepinephrine

* ANOVA test statistic with Tamhane's T2 test applied: phase 1 vs. phase 2 ($p < 0.001$); phase 2 vs. phase 3 ($p < 0.001$); phase 1 vs. phase 3 ($p= 0.970$).

** ANOVA test statistic with Tamhane's T2 test applied: phase 1 vs. phase 2 ($p< 0.001$); phase 2 vs. phase 3 ($p< 0.001$); phase 1 vs. phase 3 ($p= 0.001$).

† ANOVA test statistic with Tamhane's T2 test applied: phase 1 vs. phase 2 ($p< 0.001$); phase 2 vs. phase 3 ($p= 0.931$); phase 1 vs. phase 3 ($p< 0.001$).

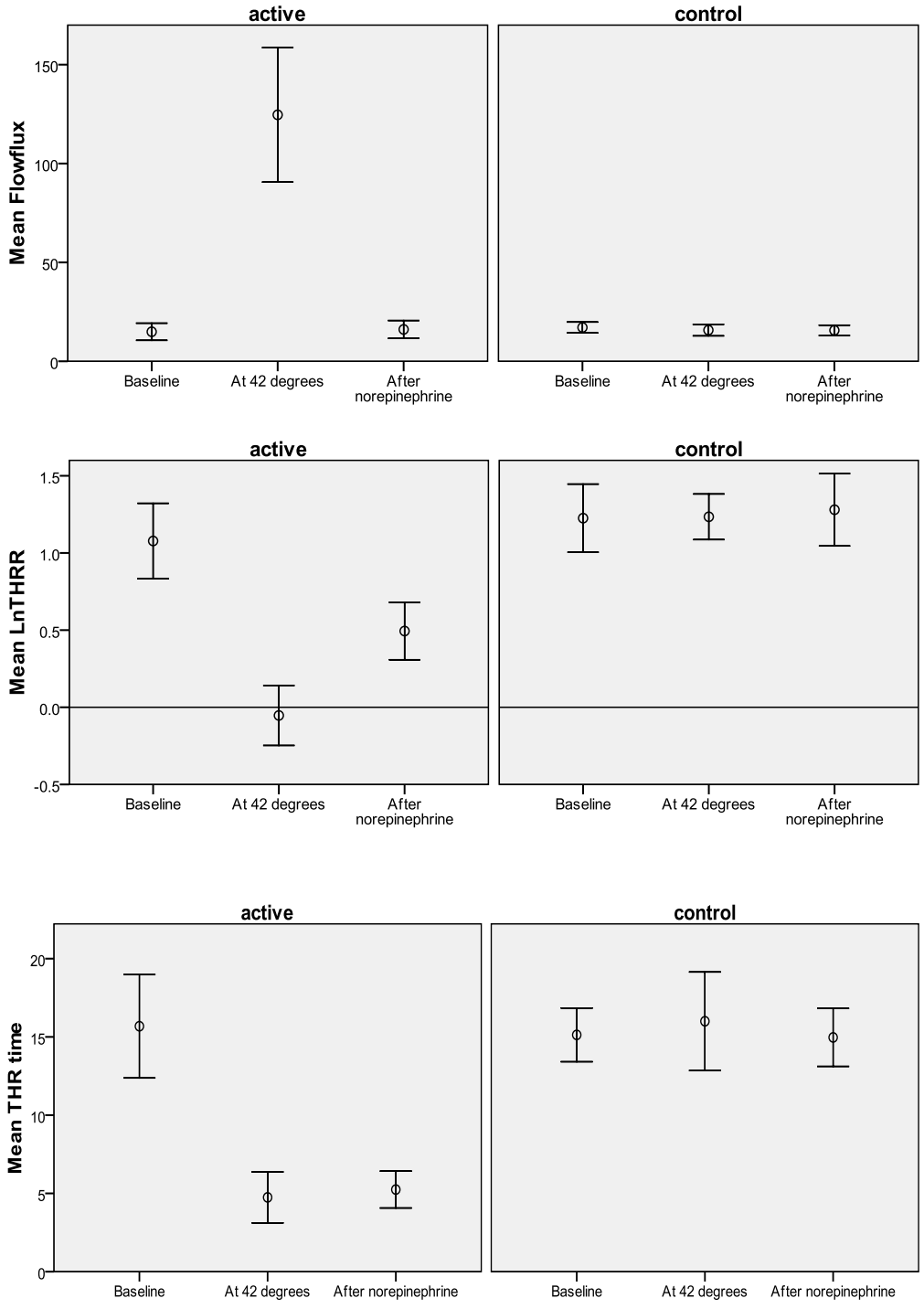

Figure 4.8 Mean flowflux, LnTHRR and THR time in volunteers after skin heating to 42°C followed by iontophoresis of norepinephrine; control sites had no heating or iontophoresis. Error bars represent 95% confidence intervals

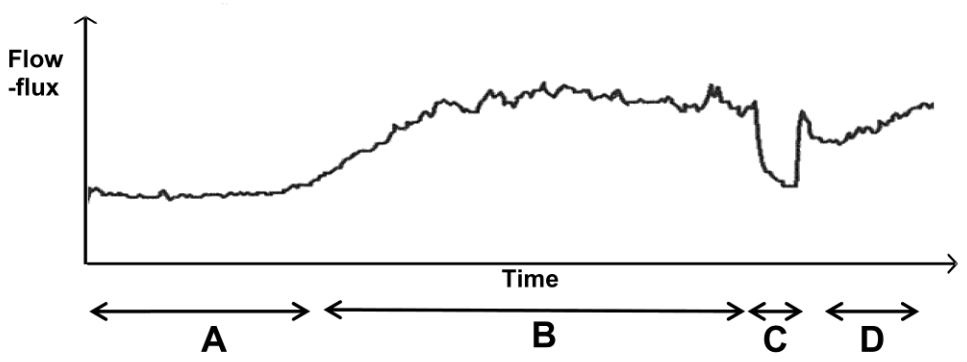

Figure 4.9 Sample trace of THR in skin heated to 42°C. A: flowflux before heating; B: flowflux during and after heating; C: biological zero during arterial compression; D: negative “hyperaemia” during THR

Discussion

The effect of PEEP on THR

The application of 10 cmH_2O PEEP in volunteers induced a fluctuating flowflux in time with respiration in most, but not all, subjects (figure 4.3). These fluctuations may have been the same as those described in chapter 3, but in this case they were obviously present at all times and did not change in magnitude following a transient hyperaemic episode. Despite these fluctuations there was no identifiable difference in mean flow flux or the THR measurements before and after the application of PEEP.

Fluctuations in flowflux associated with PEEP alter the variability of THR measurements, such that in subjects with raised mean intra-thoracic pressures they would be less reliable.

Using plethysmography limb circumference has been shown to change in phase with PEEP-induced changes in central venous pressure, and to a lesser extent arterial pressure (Christ F et al, 1995; Peters JK et al, 1997). It has been suggested that changes in vascular tone associated with PEEP are arteriolar in origin and are part of a mobilization of skin blood volume occurring at maximal right atrial transmural pressure reduction (Peters JK et al, 1997). Christ et al, 1997, also demonstrated that further vascular reactivity occurred, specifically in the form of slow-wave vasomotion (i.e. with a periodicity less than that of breathing), in hypovolaemic patients; and that this did not diminish with the removal of PEEP-related CVP changes. This would indicate that mechanisms of vascular reactivity remain intact after the application of PEEP and might explain why the transient hyperaemic response was not diminished.

The effects of iontophoresed chemicals on THR

All of the chemicals iontophoresed caused an increase in microcirculatory blood flow which was exhibited as an increase in flowflux. One of the disadvantages of iontophoresis

is that although there were differences between chemicals in terms of the degree of change in flowflux it is impossible to know whether these were dose related. All of the drugs chosen were known, or suspected, to have vasodilatory effects or effects on skin perfusion.

Hypertonic saline (HTS) is typically used clinically in the treatment of hypovolaemic states and is known to result in increased capillary blood flow measured by skin Doppler flowmetry, which is thought to be associated with the increased systemic perfusion due to volume resuscitation (Boldt et al, 1991). This cannot be the mechanism of action in this instance. Likewise the reversal of venule endothelial oedema by HTS is unlikely to play a significant role in causing the increased flow seen within this experiment, as post-capillary endothelial oedema is associated with shocked states which were not present in the volunteers studied (Oliviera RP et al, 2002). HTS, and other hyperosmolar solutions, have been shown to decrease peripheral vascular resistance in uninjured animals in a manner consistent with either the dilatation of vascular beds or the opening up of new channels (Read et al, 1960). One method by which this may occur is by the release of vasodilators, in particular the release of prostacyclin (PGI_2) combined with a decrease in thromboxane B_2, which is associated with HTS infusion (Oliviera RP et al, 2002). Evidence exists that hyperosmolality of lymph may cause arteriolar vasodilatation, at least in intestinal vessels, via the release of endothelial-derived relaxing factor (EDRF), and therefore may be related to nitric oxide (NO) or prostacyclin release (Steenbergen JM et al, 1993).

Although the effects of mannitol on the renal vasculature are thought to also be mediated by prostaglandins (Johnstone PA et al, 1981), previous experiments using 3% mannitol have failed to demonstrate an increase in flowflux (Morris SJ et al, 1995), and the effects of mannitol on the skin microcirculation are largely unknown.

Midazolam is thought to exhibit a vasodilatory effect via the release of NO and the inhibition of Ca^{2+} channels (Chang KSK et al, 1994). Although propofol might exhibit direct vasodilatory effects, possibly also via the inhibition of Ca^{2+} channels, other studies suggest that sympathetic inhibition is the most likely cause at therapeutic doses (which cannot be assumed here) (Robinson BJ et al, 1997; Chang KS et al, 1993; Nakamura K et al, 1992).

As previously noted, in chapter 2, the iontophoresis of de-ionized water or saline have been associated with vasodilatation, and this has been termed the galvanic effect (i.e. related to current or potential difference, rather than a chemical effect per se) causing hyperpolarisation of cell membranes and smooth muscle relaxation. The protocol for current delivery used within this study was designed to reduce the likelihood of this occurring, particularly as it has been most associated with cathodal iontophoresis (Droog EJ et al, 2003; Abou-Elenin K et al, 2002); all the chemicals here were iontophoresed using anodal iontophoresis except midazolam. In fact the anodal iontophoresis of hypertonic saline solutions is not strongly associated with vasodilatation at all (Asberg A et al, 1999; Abou-Elenin K et al, 2002). The iontophoresis of de-ionized water, however, is associated with significant vasodilatation and it may be that the tonicities of carrier solutions (or HTS itself) used within this experiment were low enough to replicate this effect. The vasodilatation associated with both anodal and cathodal iontophoresis of de-ionized water and tap water has previously been shown to be attenuated by prostaglandin inhibition (Abou-Elenin K et al, 2002; Durand S, 2002; Berliner M, 1997). The solution of mannitol used was an aqueous solution, and although propofol is an oil-based solution it does contain water, as well as sodium hydroxide, and it is impossible to know whether or not the carrier solution, or any other chemicals it contained, might have caused the vasodilatation seen. If it is the carrier solutions which are responsible for the degree of vasodilatation seen it is surprising that this occurred despite different carrier solutions and iontophoresis polarity. This would not be in keeping with the aforementioned studies or

the study by Brown et al, 2003, which found insignificant vasodilatation after iontophoresed saline.

In each case the increase in flowflux was accompanied by a decrease in the THRR with increased levels of vasodilatation probably being associated with greater attenuation of the hyperaemic response. Iontophoresis of endothelium-dependent (sodium nitroprusside) and endothelium-independent (acetylcholine) vasodilators have been shown in previous studies to abolish the transient hyperaemic response (Webster VL et al, 2002; Brown H et al, 2003). In this study only propofol and mannitol nearly abolished the response. Regardless of the mechanism of vasodilatation this study proves that the THR can be partially depressed by the iontophoresis of exogenous chemicals.

The duration of THR was only decreased after the iontophoresis of midazolam and propofol. Given the wide range of variability associated with these measurements a degree of caution must be taken when interpreting these results. Nevertheless if these results are accurate it would suggest that the effect of chemicals on the amplitude of the THR may be decoupled from the ability to sustain the response, which has not been previously described.

The effect of localized cutaneous heating and the co-administration of iontophoresed vasopressors on THR

Local heating of skin produced a vasodilatory response and abolished THRR at 42°C consistent with previous studies (O'Connor et al, 2003). Previous studies have shown that hyperaemia associated with prolonged ischaemia may be increased by local warming (Johnson JM et al, 1986), and that this is in part mediated by the abolition of sympathetic adrenergic vasoconstriction, a process which can be mimicked by the iontophoresis of bretylium (Kellogg DL et al, 1989; Pergola PE et al, 1993).

Adrenergic inhibition is not the only mechanism by which vasodilatation occurs with cutaneous heating; denervated skin, either by sympathectomy or contained within a skin graft, still demonstrates the ability to vasodilate (Bliss M, 1998; Freund PR et al, 1981). Despite this it would seem that the predominant mode of vasodilatation is adrenergic inhibition, as although hyperaemia associated with prolonged ischaemia may be increased by local warming the reverse is not true (i.e. hyperaemia associated with local warming is not increased after an additional period of ischaemia - Johnson JM et al, 1986). This would explain why exogenous norepinephrine partially restored the transient hyperaemic response in skin heated to 42°C. It is harder to explain why the reverse was not true, in that heating skin already exposed to norepinephrine did not increase the THR. Unlike heating norepinephrine did not, however, abolish the hyperaemic response, only attenuate it. The decrease in the transient hyperaemic response seen after norepinephrine appeared to suggest a scenario in which the microcirculation became resistant, but not impervious to, hyperaemic vasodilatation. The duration of hyperaemia remained decreased after the iontophoresis of norepinephrine.

Apart from in heated skin the application of norepinephrine diminished vasodilatation and hyperaemia. It is unclear why norepinephrine affected the microcirculation in this experiment whilst epinephrine and phenylephrine failed to affect the THR in previous experiments (Brown H et al, 2003). Changing the agent or iontophoresis protocol may have resulted in better drug delivery.

The “negative” hyperaemic responses seen with skin heating, (figure 4.9), indicate either new vasoconstriction or diminished vasodilatory effect occurring following transient ischaemia which was unexpected and was not present in previous studies where vasodilatation was chemically induced (Webster VL et al, 2002; Brown H et al, 2003).

Conclusion

The preceding studies have demonstrated that the application of PEEP alters flowflux measurements in a manner consistent with the vasomotion associated with changes in intra-thoracic pressure seen in earlier studies. It does not appear to alter the transient hyperaemic response, but the aforementioned changes in flowflux will increase the degree of variability seen within the technique.

The effects of the iontophoresis of novel chemicals on THR are difficult to interpret. At the most basic level it has been demonstrated that moderate vasodilatation through chemical iontophoresis is possible and affects THR. It has been also conclusively demonstrated that hypertonic solutions can cause vasodilatation after iontophoresis. It is highly likely that many of the drugs caused vasodilatation in their own right, otherwise these results would contradict findings found in earlier studies and results demonstrated later in this thesis (where the same diluents were used but no, or minimal, vasodilatation occurred)7-7-=83339, but proving this is not possible using the current results. In future all iontophoresis of vasodilators should be performed alongside a control(s) consisting of the diluents used.

The iontophoresis of norepinephrine was shown to provoke vasoconstriction, both in heated and unheated skin, and in heated skin this resulted in a partial return of THR. The logical next step would be to identify whether or not norepinephrine can restore the THR in other vasodilated states where adrenoceptor resistance is thought to be at least partially responsible, for example in systemic inflammation.

The “negative” hyperaemic response seen after localized heating to 42°C is unexplained at this time, but further investigation may yield information regarding the vasodilator response which occurs with temperature.

CHAPTER FIVE

The THR test in patients with evidence of a systemic inflammatory response: a pilot study

Introduction

The systemic inflammatory response syndrome (SIRS) is a clinically-orientated definition of inflammation which can occur in critically ill patients after a range of insults including trauma, pancreatitis, poisoning and, most commonly within the intensive care environment, sepsis (Bone RC et al, 1992).Sepsis with evidence of organ failure is known to be common, with a surprisingly high population incidence in the United States of approximately 3 cases per 1000; and an approximate mortality of 28.6% (Angus DC et al, 2001). Although the incidence of SIRS is not known, in a study of the natural history of SIRS 26% of patients developed sepsis, 18% developed sepsis with associated organ failure and 4% developed septic shock; and the mortality associated with each definition increased in a stepwise manner with 7% of SIRS patients dying compared with 16% of septic patients and 46% of patients with septic shock (Rangel-Frausto MS et al, 1995). Whilst the criteria for SIRS have been criticized as being over-sensitive and non-specific,

no alternative clinical definitions or biomarkers have yet been identified (Vincent J-L, 1997; Levy MM et al, 2003).

Microcirculatory changes present in the tongue in patients with septic shock have previously been identified, and it has been demonstrated that perfusion increased rapidly in patients who went on to survive, but did not improve in non-survivors. Lack of improvement in non-survivors appeared to occur even after the shocked state was corrected (Sakr et al, 2004).

Reactive hyperaemia has previously been investigated in critically ill patients, although not using the transient hyperaemic response. Young JD et al, 1995, identified an increase in skin perfusion in patients with sepsis which was accompanied by a decrease in the hyperaemic response to post-occlusive ischaemia. Reactive hyperaemia has also been shown to become diminished in patients with other inflammatory conditions as well as sepsis and, but that diminution does not universally occur in either state. The reduction in hyperaemic response was associated with increased mortality, but was not predictive of it (Hartl et al, 1988).

In a study by Haisjackl et al, 1990, the reactive hyperaemic response of skin in critically ill patients was diminished, as was carbon dioxide elimination, when compared with healthy subjects. The finding of a diminished hyperaemic response was associated with the degree of physiologic derangement measured by the Acute Physiology and Chronic Health Evaluation score (APACHE II).

Kienbaum P et al, 2008, have previously demonstrated that forearm vascular resistance is reduced in patients with septic shock, and can be reduced still further by the introduction of sodium nitroprusside, similar to that seen in healthy volunteers. They also

demonstrated and that the normally vasoconstrictive response to phenylephrine was impaired in septic shock, although the response to vasopressin was not.

This study was designed as a pilot in order to examine whether the transient hyperaemic response could be successfully measured in critically ill patients within the intensive care unit (ICU). As decreased vascular reactivity has been shown to be present in patients with inflammatory responses other than sepsis, and also has been shown to occur before clinically identifiable evidence of vascular derangement in patients with sepsis, it was decided to examine the vascular reactivity in patients who presented with evidence of SIRS regardless of the cause (Hartl WH et al, 1988).

In order to evaluate the effects of exogenous vasoconstrictors and vasodilators a secondary aspect of the investigation was to examine the effects on the transient hyperaemic response of iontophoresed norepinephrine and acetylcholine.

Methods

All patients admitted to the ICU at Nottingham City Hospital over a 6 month period between February and July 2004 were screened for the presence of systemic inflammatory response syndrome criteria (SIRS). Where patients were found to have evidence of SIRS they were further screened to ensure suitability for admission into the trial. Where patients were found to be suitable they were asked for their consent to take part. Where patients were unable to give consent, for example where they were unconscious or sedated, the informed assent of their relatives was sought instead.

The presence of SIRS criteria was defined using the guidelines suggested by the American College of Chest Physicians/ Society of Critical Care Medicine Consensus Conference 1992 (Bone et al, 1992), with patients defined as having SIRS where 2 or more of the following are present:

- Core temperature > 38^{o}C or < 36^{o}C
- Heart rate > 90 beats/min
- Respiratory rate > 20 breaths/min or $PaCO_2$ < 4.3 kPa *
- White blood cell count > 12,000 cells/mm^3, < 4000 cells/mm^3, or > 10% immature (band) forms

* For the purposes of this study the presence of mandatory mechanical ventilation via an endotracheal tube or tracheostomy tube was also used as a surrogate marker of impaired ventilation, except where short-term ventilation was required in the immediate post-operative period.

These same criteria were also used to identify patients with sepsis (SIRS plus the presence of bacteraemia or an infected body cavity) or septic shock (sepsis associated with a systolic blood pressure of <90mmHg or the requirement for inotropes to maintain blood pressure) (Bone et al, 1992).

Patients were excluded where consent / assent could not be obtained. Other exclusion criteria were those defined in chapter 2. Patients in whom further ICU treatment was deemed to be futile and / or was in the process of being withdrawn were also excluded prior to enrolment.

Demographic and clinical data, both retrospective and prospective was collected on patients during their ICU and hospital admission. Patients were visited once every three days after enrolment resulting in a minimum of three visits during their stay. On the first and second visits LDF was performed with three flowflux and THR measurements being recorded. The third and any subsequent visits consisted of routine follow-up visits which were performed in order to monitor the patient's clinical condition and to ensure that there were no adverse events which occurred as a result of the study. No attempt was made to alter or interfere with the patient's clinical management during the period of observation.

Except where it was specifically refused during consent/assent iontophoresis of either norepinephrine or acetylcholine was performed on each visit after baseline flowflux and THR measurements had already been taken. Acetylcholine was chosen as one of the iontophoresed agents as its effects on the oropharyngeal mucosal microcirculation have previously been investigated in critically ill patients. Norepinephrine was chosen as a vasoconstricting agent as its effects had previously been investigated in healthy volunteers (see chapter 4).

The order in which agents were iontophorsed into patients was done according to a predefined randomization template. Iontophoresis was performed according to the protocol laid out in chapter two and was followed by a further three flowflux and THR measurements. Skin temperature measurements were made using the LDF probes during each period of LDF recording.

Acetylcholine was iontophoresed using an anodal current (i.e. the iontophoresis chamber contained the anode), whilst norepinephrine was iontophoresed using a cathodal current.

The following clinical data was collated regarding each patient:

- Pre-morbid illnesses and medication use
- Age and gender
- Presumed diagnosis on ICU admission
- ICU and hospital outcome
- Clinical and laboratory data associated with each patient visit
    - The presence of SIRS criteria (including absolute white cell count)
    - APACHE II* and SOFA scores**
    - Cardiovascular status and level of inotropic support
    - Respiratory support
    - Renal support (either haemodialysis or continuous veno-venous haemofiltration, CVVH)
    - White cell count (WCC), and serum C-reactive protein (CRP) and lactate levels (from arterial blood samples)
    - Use of steroids or intravenous insulin

*Acute physiology and chronic health evaluation score. An ICU severity of illness scoring system usually performed within the first 24 hours of admission (Knaus W et al, 1985). – see appendix ii

**Sequential organ failure assessment score. Previously known as: sepsis organ failure assessment; an ICU severity of illness scoring system (Vincent JL et al, 1996). – see appendix iii

Results

Of 42 patients who were identified as suitable for inclusion within the study, only 14 patients or their relatives consented to take part. Of those who did not choose to take part, the majority either refused consent /assent, or were unable to give consent and had no relative available to provide assent. 2 patients agreed to take part but were not prepared to undergo iontophoresis of chemicals into the skin. Two patients who agreed to take part were unable to remain still for sufficiently long periods to allow flowflux and THR measurements to take place.

Twelve patients had flowflux and THR measurements recorded, and nine patients went on to have a second set of flowflux and THR measurements. Of the remaining three patients two died prior to the second visit, and one patient withdrew from the study. Out of the twelve patients six were male, and the median age was 62 (range 23-70). The details of the gender, age, past medical history and pre-morbid drug history of all the patients can be found in table 5.1.

All of the patients demonstrated evidence of SIRS at the time of the first set of flowflux and THR measurements. During the second set of measurements only patient 2 did not exhibit two or more symptoms of inflammation that define SIRS. Seven patients had evidence of positive microbiological samples at one or both visit, suggesting that the commonest cause of inflammation was sepsis. Six patients required inotropes, of which four also had confirmed bacteraemia, suggesting septic shock. The details of the presence or absence of the criteria for SIRS, sepsis and septic shock can be found in table 5.2.

APACHE II scores ranged from 6 to 29 (first visit: median 12, mean 12.8; second visit: median 15, mean 15.7). SOFA scores ranged from 2 to 16 (first visit: median 5.5, mean 6.5; second visit: median 5, mean 5.8). Serum CRP values were raised (>10 mg/L) in all

patients indicating the presence of inflammation; and serum lactate levels were raised (>2 mmol/L) in four patients at each visit, suggesting organ hypoperfusion. The majority of patients had demonstrable evidence of pitting oedema to a greater or lesser degree suggesting an increased level of vascular permeability and/or fluid overload. Table 5.3 contains details of individual APACHE II scores, SOFA scores, serum CRP and lactate levels, the presence of oedema, and whether or not patients were receiving renal replacement therapy, steroids or intravenous insulin.

			Pre-morbid history		Intensive Care	
Patient	**Sex**	**Age**	**Medical history**	**Drug history**	**Admission diagnosis**	**Outcome**
1	F	42	Hypertension Chronic kidney disease	Amlodipine Aspirin Doxazosin	Cardiac arrest	Died in hospital
2	F	68	Thyrotoxicosis Lymphoma	Celecoxib	Pneumonia	Died in ICU
3	M	67	Smoking COPD*	Salbutamol inhaler Ipratropium bromide inhaler Atorvastatin	COPD exacerbation	Survived to discharge
4	M	41	Chronic kidney disease		Peritoneal sepsis	Died in ICU
5	M	25	Smoking		Pneumonia	Died in ICU
6	F	54			Pneumonia	Survived to discharge
7	F	68	Hypertension Smoking COPD	Salbutamol nebuliser Ipratropium bromide nebuliser	Pneumonia	Survived to discharge
8	F	68	Hypertension Smoking NIDDM	Pravastatin Metformin Enalapril Aspirin Doxazosin	Meningitis	Died in hospital
9	M	57			Pneumonia	Survived to discharge
10	M	23	Smoking		Pneumonia	Survived to discharge
11	F	70	Smoking Hypothyroidism	Thyroxine Omeprazole Frusemide	Peritoneal sepsis	Died in ICU
12	M	67			Pneumonia	Survived to discharge

Table 5.1 Age, gender, past medical history and prior medication usage of patients

*Chronic obstructive pulmonary disease (COPD)

	HR	WCC	Pyrexia	Respiration		Blood pressure		
						Visit 1		
Patient				RR	IPPV	BP	Inotropic support	Positive culture
1	110	10.0	*	32		140/85		
2	100	5.0	Yes		Yes	100/50	Dobutamine Norepinephrine	Yes
3	150	26.8	Yes		Yes	130/75		
4	168	25.9		16		90/70	Vasopressin	
5	120	24.4			Yes	120/80		Yes
6	105	9.4	*	44		140/99	Dopamine	
7	80	20.1	Yes		Yes	150/60	Norepinephrine	Yes
8	115	11.2	Yes		Yes	150/75		Yes
9	120	9.7		27		165/85		
10	100	23.1			Yes	120/55		
11	80	20.8	Yes		Yes	115/50	Norepinephrine	Yes
12	95	25.9	Yes		Yes	105/58	Norepinephrine	
						Visit 2		
1	110	9.2	Yes	20		160/90		Yes
2	115	4.2		17		100/50	Dobutamine	
3	135	17.1	Yes		Yes	140/65		Yes
4	110	35.4		15		100/60		
6	95	19.2	Yes		Yes	120/70		Yes
7	100	17.6	Yes		Yes	110/55		Yes
8	120	14.2	Yes	19		160/75		Yes
10	120	26.0	Yes		Yes	125/54		
12	91	25.4	Yes	22		125/70		

Table 5.2 Evidence of SIRS, sepsis, or septic shock in patients on whom THR was measured; including during a second visit where appropriate.

HR: heart rate, beats / minute; WCC: white cell count, cells x10^9/L; Pyrexia: temperature > 38°C (*pyrexia not present at the time of THR measurement, but occurring within the preceding 24 hours); RR: respiratory rate, breaths / minute, in spontaneously breathing patients without assisted support other than PEEP; IPPV: intermittent positive pressure ventilation, including all forms or mandatory or assisted ventilation via an endotracheal or tracheostomy tube; BP: blood pressure, systolic / diastolic in mmHg; Positive cultures: microbial samples grown from blood or peritoneal fluid within the 24 hours prior to measurement of THR.

	Organ failure scores					Patient receiving		
Patient	**APACHE II**	**SOFA**	**CRP**	**Lactate**	**Oedema**	**RRT**	**Steroids**	**Insulin**
				Visit 1				
1	26	9	26	1.57	Present	Yes		
2	11	16	52	4.90	Present	Yes	Yes	Yes
3	18	5	315	1.56	Present		Yes	
4	20	6	249	1.55	Present	Yes	Yes	
5	6	4	†	0.77				
6	13	6	221	†	Present			
7	16	10	391	4.89		Yes	Yes	Yes
8	14	4	87	0.98	Present		Yes	
9	10	2	225	0.89			Yes	
10	6	5	66	4.55		Yes	Yes	Yes
11	7	6	†	3.42	Present			Yes
12	7	5	360	2.00	Present			
				Visit 2				
1	29	8	288	1.00	Present	Yes		
2	18	13	38	3.65	Present		Yes	
3	16	5	†	1.49	Present		Yes	
4	15	5	†	†	Present	Yes		
6	14	3	153	2.9	Present			
7	20	8	234	0.60		Yes	Yes	Yes
8	12	3	36	3.12	Present		Yes	
10	10	5	35	3.24		Yes	Yes	Yes
12	7	2	†	1.17	Present			

Table 5.3 Patient organ failure assessment scores, serum C-reactive protein (CRP) and lactate levels, the presence or absence of pitting oedema, and whether or not the patient was receiving renal replacement therapy (RRT), steroids or insulin at the time THR was measured; including during a second visit where appropriate. CRP is recorded in mg/L and serum lactate in mmol/L.

† Plasma CRP or lactate unavailable

Flowflux and THRR measurements

Flowflux and THR measurements proved difficult to perform in some patients. Patients were sometimes unable to remain still for sufficiently long periods requiring multiple THR attempts to be made to achieve a recording in which there was no background interference from movement. Also the presence of a significant degree of tissue oedema meant that multiple THR manoeuvres had to be performed in order to ensure a biological zero with no demonstrable distal pulsatile blood flow (confirmed using pulse oximetry). In one patient flowflux measurements had to be abandoned after the iontophoresis of acetylcholine because of the onset of seizure activity.

Skin temperature flowflux, LnTHRR and THR time measurements for each patient can be found in table 5.4. Only one patient had a skin temperature significantly outside the range accepted for laboratory studies of THR (patient 6 during the first visit: 24.4°C). Compared with the aggregate values from healthy volunteers measured using the standard LDF probe (see chapter 3) the mean values from patients in visits 1 and 2 demonstrated raised flowflux values and decreased LnTHRR values with a trend towards a decreased time over which the hyperaemic response was present (Figures 5.1 and 5.2).

On closer examination only patients 6 and 12 during the first visit and patients 8 and 10 during the second visit had flowflux values which were not significantly different from that of healthy volunteers. Patient 10 had values which were significantly lower than that of healthy volunteers during the first visit.

Despite this, half of all LnTHRR measurements (10 of 21) were not significantly lower than those of the aggregate standard LDF probe group (discussed in chapter 3) when compared with ; with the same being true of THR time (12 of 21). Interestingly a decreased THR response was not always associated with a shortened THR time; and

shortened THR times also occurred in patients with apparently normal THR measurements.

	Temperature	**Flowflux**		**LnTHRR**		**THR time**	
	°C, (SD)	(SD)	*P*-value	(SD)	*P*-value	Secs., (SD)	*P*-value
			Volunteers combined aggregate group (from chapter 3)				
	30.7 (0.9)	20.4 (11.8)		1.15 (0.44)		16.1 (0.72)	
			Combined means from patients during each visit				
Visit 1	30.6 (2.2)	78.87 (92.55)	0.05	0.78 (0.52)	0.028	12.4 (8.5)	0.180
Visit 2	31.1 (1.2)	80.50 (78.70)	0.05	0.73 (0.48)	0.030	9.5 (6.7)	0.026
Patient			**Individual patient values during visit 1**				
1	28.9 (0.3)	38.25 (3.12)	<0.001	1.65 (0.92)	0.245	23.0 (2.5)	0.523
2	30.7 (0.2)	26.70 (2.19)	<0.001	0.95 (0.28)	0.132	15.2 (5.8)	0.762
3	31.9 (0.1)	320.22 (11.77)	<0.001	0.00 (0.08)	<0.001	4.4 (3.9)	0.001
4	30.9 (0.1)	115.62 (15.82)	<0.001	0.92 (0.23)	0.058	2.3 (0.5)	<0.001
5	30.1 (0.3)	202.80 (20.97)	<0.001	0.21 (0.10)	<0.001	2.80 (0.8)	0.014
6	24.4 (1.6)	21.55 (14.07)	0.849	0.58 (0.39)	0.016	3.2 (2.4)	<0.001
7	30.9 (0.2)	56.35 (11.00)	<0.001	0.65 (0.71)	0.148	3.7 (2.3)	<0.001
8	32.7 (0.1)	49.45 (0.49)	<0.001	0.42 (0.26)	0.001	6.5 (0.9)	0.046
9	30.5 (1.5)	38.05 (8.59)	0.004	1.36 (0.76)	0.535	14.6 (1.0)	0.757
10	32.6 (0.2)	15.60 (0.33)	<0.001	1.51 (0.52)	0.158	20.1 (3.9)	0.047
11	31.7 (0.8)	27.75 (9.54)	0.038	0.49 (0.16)	<0.001	12.8 (4.3)	0.049
12	31.8 (0.4)	34.30 (20.66)	0.062	0.57 (0.18)	<0.001	15.0 (6.0)	0.655
			Individual patient values during visit 2				
1	29.7 (0.1)	45.40 (0.15)	<0.001	1.12 (0.24)	0.835	18.7 (4.5)	0.389
2	31.3 (0.1)	53.01 (2.57)	<0.001	1.04 (0.47)	0.716	15.1 (3.6)	0.725
3	32.7 (0.1)	230.56 (17.82)	<0.001	0.05 (0.01)	<0.001	7.9 (2.1)	0.020
4	30.3 (0.1)	191.42 (13.46)	<0.001	0.22 (0.37)	0.050	2.2 (0.9)	<0.001
6	29.3 (0.0)	48.88 (1.99)	<0.001	1.21 (0.43)	0.828	2.7 (0.8)	<0.001
7	31.9 (0.0)	97.76 (5.16)	<0.001	0.56 (0.23)	0.014	10.1 (0.9)	0.305
8	30.8 (0.1)	17.50 (1.30)	0.080	0.29 (0.16)	<0.001	0.8 (0.7)	<0.001
10	31.3 (0.0)	13.18 (3.69)	0.074	1.38 (0.44)	0.462	16.2 (0.9)	0.959
12	32.3 (0.9)	28.82 (0.21)	0.004	0.69 (0.14)	<0.001	14.5 (5.2)	0.425

Table 5.4 Patient temperature, flowflux, LnTHRR, and THR times during both visits, as well as combined mean values from all patients at both visits. *P*-values refer to independent samples t-test when compared with Aggregate standard LDF probe group (discussed in chapter 3), with equal variance not assumed

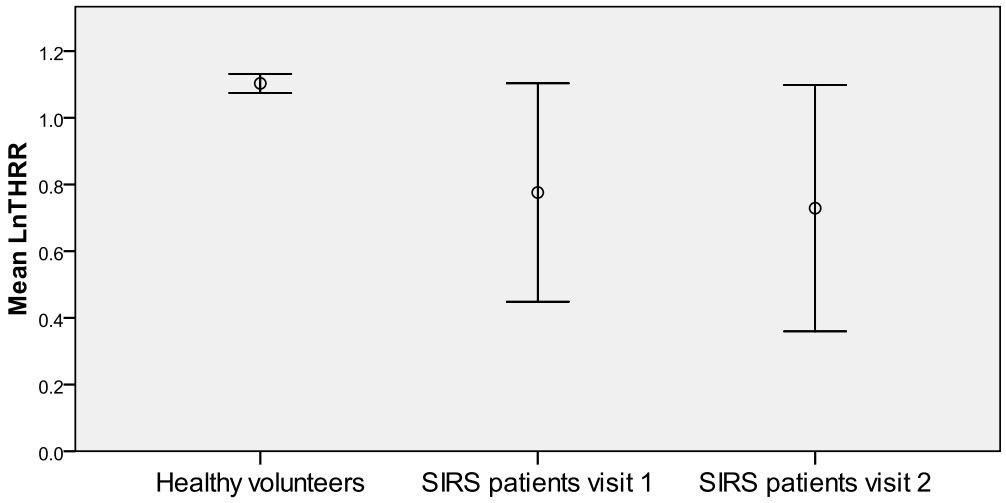

Figure 5.1 Graph of mean LnTHRR of all patients during visit 1 and visit 2 compared with mean LnTHRR value derived from the Aggregate standard LDF probe group (discussed in chapter 3). Error bars represent 95% confidence intervals.

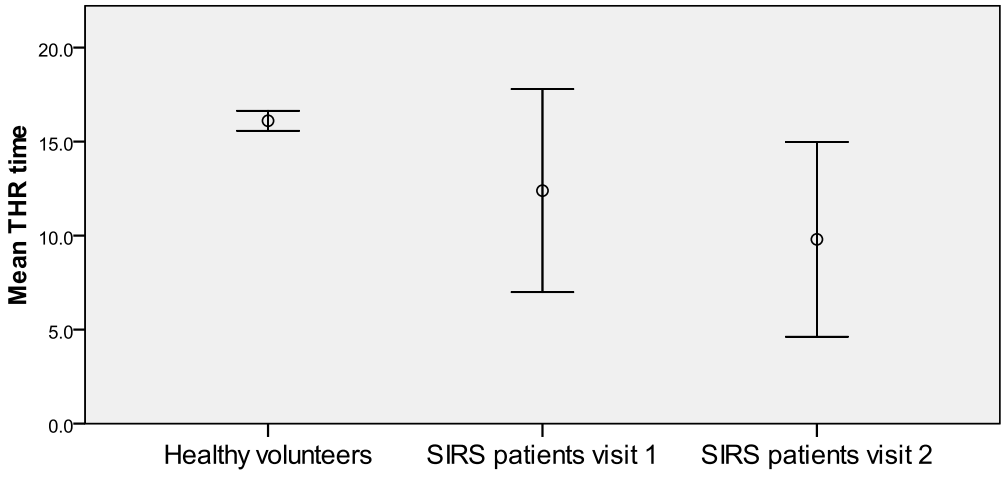

Figure 5.2 Graph of mean THR time of all patients during visit 1 and visit 2 compared with mean THR time value derived from the Aggregate standard LDF probe group (discussed in chapter 3). Error bars represent 95% confidence intervals.

Flowflux and THR measurements after iontophoresis

The values for flowflux, LnTHRR and duration of THR in patients before and after the iontophoresis of both norepinephrine and acetylcholine can be found in table 5.5. The mean overall changes associated with iontophoresis of both drugs compared with the means from the aggregate group of healthy volunteers using LDF needle-probes can be seen in figure 5.3.

In those patients in whom iontophoresis of norepinephrine was performed there was no statistically significant decrease in flowflux, LnTHRR or duration of THR (respective *P*-values 0.627, 0.282, 0.334; independent t-test with equal variances not assumed). Only patients 1, 3 and 10 showed a reduction of flowflux from baseline of more than 30%.

Whilst it was not statistically significant overall the iontophoresis of norepinephrine appeared to be associated with an increase in LnTHRR in most patients, though not all, and there was no clear association between the degree of change in flowflux and the changes in LnTHRR.

Although the iontophoresis of acetylcholine appeared to be associated with an increase in flowflux and a decrease in LnTHRR and duration of THR the mean changes overall were not statistically significant (respective *P*-values 0.159, 0.278, 0.394; independent t-test with equal variances not assumed).

In patients who already appeared to have raised flowflux the iontophoresis of acetylcholine still appeared to induce further vasodilatation. Both the LnTHRR and the THR time appeared to decrease in patients where it had been normal beforehand (patients 1, 2, 9 and 10), but remained relatively unchanged in cases where it had already been low prior to iontophoresis (patients patients 3,4,5 and 7).

In one case, patient 6, flowflux, LnTHRR and duration of THR all appeared to change in the opposite direction to what would normally be expected.

The individual changes in mean flowflux and LnTHRR before and after the iontophoresis of acetylcholine and norepinephrine can be seen in Figure 5.4.

		Flowflux (SD)		**LnTHRR** (SD)		**THR time** Secs., (SD)	
Healthy volunteers		10.35 (6.64)		1.06 (0.46)		16.1 (12.15)	
		Patients undergoing norepinephrine iontophoresis					
Patient		Before	After	Before	After	Before	After
1	Visit 2	10.00	6.70	1.12 (0.24)	0.98 (0.39)	18.7 (4.5)	3.2 (3.1)
2	Visit 2	12.29	9.8	1.04 (0.47)	1.35 (0.41)	15.1 (3.6)	17.6 (1.1)
3	Visit 1	100.63	54.7	0.05 (0.06)	0.41 (0.29)	6.0 (5.1)	6.1 (1.2)
4	Visit 1	21.91	26.5	0.84 (0.12)	0.84 (0.58)	2.1 (0.5)	2.8 (1.9)
6	Visit 2	8.48	7.3	1.21 (0.43)	2.40 (0.62)	2.7 (0.8)	2.8 (0.9)
7	Visit 1	9.80	9.0	1.24 (0.48)	1.81 (1.25)	5.7 (1.1)	3.2 (1.7)
8	Visit 1	10.10	12.8	0.41 (0.23)	1.34 (0.83)	1.6 (0.2)	3.8 (2.4)
10	Visit 1	8.22	3.3	1.64 (0.77)	0.95 (0.55)	20.7 (5.7)	6.6 (2.2)
Combined mean		22.65 (31.80)	16.26 (17.02)	0.94 (0.50)	1.26 (0.62)	9.1 (7.8)	5.8 (5.0)
		Patients undergoing acetylcholine iontophoresis					
Patient		Before	After	Before	After	Before	After
1	Visit 1	10.0	6.7	2.93 (0.56)	1.20 (0.58)	7.1 (26.1)	10.9 (8.0)
2	Visit 1	6.66	68.7	1.09 (0.02)	0.24 (0.03)	12.8 (3.8)	8.0 (7.9)
3	Visit 2	76.4	103.6	0.05 (0.01)	0.08 (0.01)	7.9 (2.1)	1.7 (3.0)
4	Visit 2	19.1	28.1	0.37 (0.22)	0.31 (0.43)	2.2 (0.9)	1.8 (1.9)
5	Visit 1	7.3	84.1	0.15 (0.09)	0.10 (0.01)	0.3 (0.1)	0
6	Visit 1	28.7	26.2	0.38 (0.36)	1.32 (0.48)	2.0 (1.7)	5.7 (3.5)
7	Visit 2	21.3	35.9	0.56 (0.23)	0.41 (0.12)	10.1 (9.4)	10.1 (7.5)
9	Visit 1	16.0	10.9	1.52 (0.81)	0.84 (0.66)	23.1 (3.9)	8.6 (9.7)
10	Visit 2	6.0	12.6	1.38 (0.44)	0.43 (0.22)	16.2 (9.7)	12.1 (11.3)
Combined mean		21.27 (22.06)	41.87 (35.07)	0.94 (0.91)	0.55 (0.46)	9.0 (7.4)	6.5 (4.4)

Table 5.5 Patient, flowflux, LnTHRR, and THR times before and after the iontophoresis of norepinephrine and acetylcholine combined mean values from all patients before and after iontophoresis. Results from the Aggregate group of healthy volunteers performed using a needle-probe LDF (see chapter 3) are included for comparison.

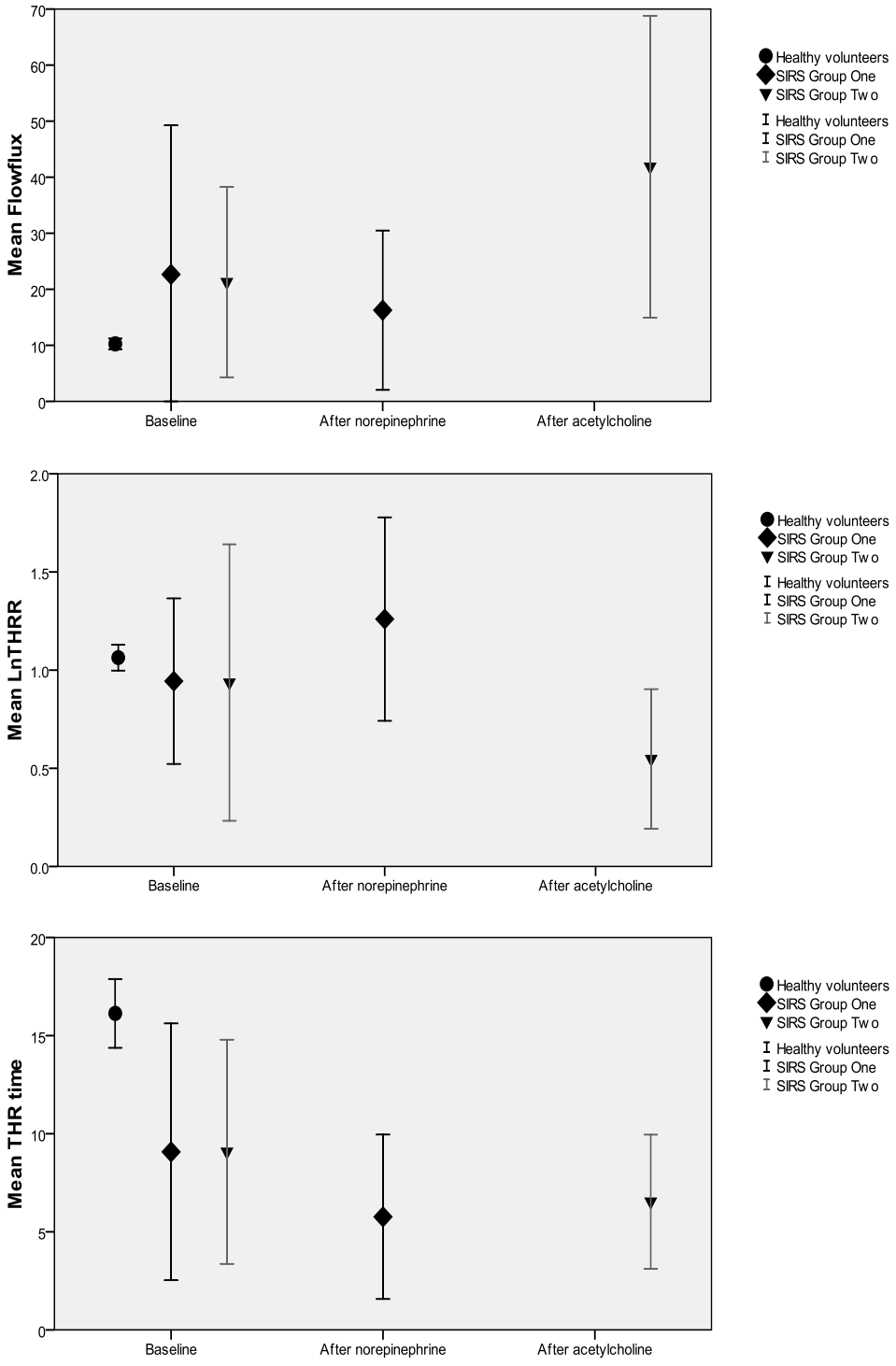

Figure 5.3 Graphs of mean flowflux, LnTHRR and THR time in patients before and after iontophoresis of norepinephrine or acetylcholine. Means from the Aggregate group of healthy volunteers using LDF needle-probes (see chapter 3) included for comparison. Error bars represent 95% confidence intervals.
SIRS Group One denotes all those patients who were exposed to noepinephrine and SIRS Group Two denotes all those patients exposed to acetylcholine.

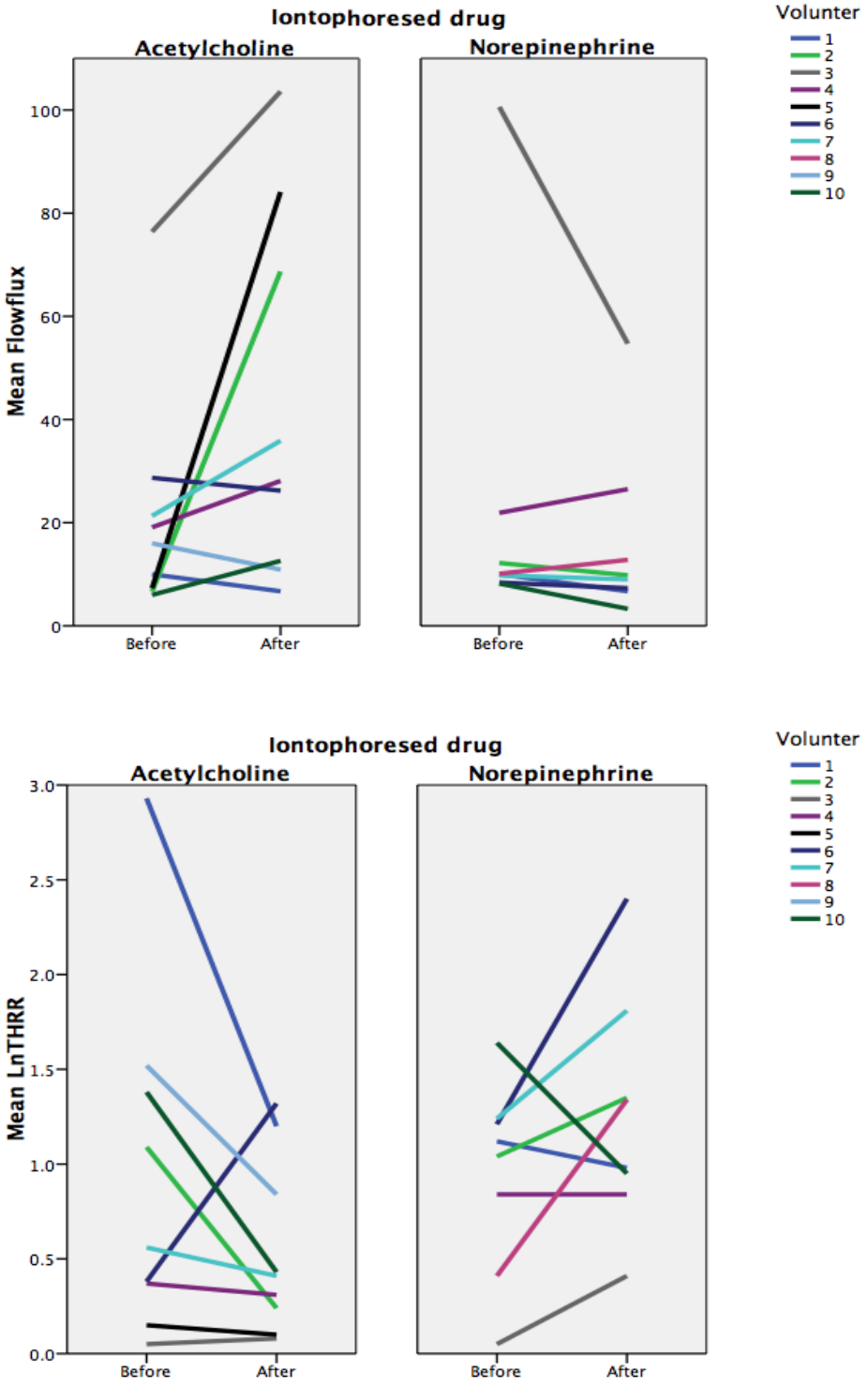

Figure 5.4 Individual changes in mean flowflux and LnTHRR before and after the iontophoresis of acetylcholine and norepinephrine

Vascular reactivity and outcome

Although eleven out of the twelve patients presented here were suspected of having sepsis on admission to the ICU (the remaining patient having been admitted following a cardiac arrest), only 7 patients were proven to have sepsis. In total 6 patients died, of whom 5 were proven to have had sepsis during their ICU stay. Those patients with sepsis all had raised flowflux measurements except one in whom flowflux was abnormally low.

There was no obvious relationship in this small study between the transient hyperaemic response and the presence of sepsis or eventual survival (table 5.6). Nor was there any clear relationship between organ failure scores, or CRP, and flowflux or THR measurements.

The responses to norepinephrine and acetylcholine were mixed and appeared unrelated to outcome (table 5.6). Overall the introduction of norepinephrine and acetylcholine did not alter flowflux, THR, or THR time.

Iontophoresis only produced a dramatic decrease in flowflux in one individual, in whom flowflux was high before-hand and in this individual the hyperaemic response was partially restored. Acetylcholine failed to cause a vasodilatory effect on flowflux in 3 patients. Where the hyperaemic response was low beforehand the introduction of acetylcholine made no difference; where it was normal beforehand acetylcholine resulted in a reduction in THR.

Patient	Proven sepsis	Visit 1		Visit 2		Outcome
		Flowflux	LnTHRR	Flowflux	LnTHRR	
1	Yes	Increased	Normal	Increased	Normal	Died
2	Yes	Increased	Normal	Increased	Normal	Died
3	Yes	Increased*	Decreased*	Increased*	Decreased*	Survived
4	No	Increased*	Normal	Increased*	Decreased*	Died
5	Yes	Increased*	Decreased*			Died
6	No	Normal	Decreased	Increased	Normal	Survived
7	Yes	Increased	Normal	Increased*	Decreased	Survived
8	Yes	Increased	Decreased	Normal	Decreased	Died
9	No	Increased	Normal			Survived
10	No	Normal	Normal	Normal	Normal	Survived
11	Yes	Decreased	Decreased			Died
12	No	Normal	Decreased	Increased	Decreased	Survived

		LnTHRR response to norepinephrine	LnTHRR response to acetylcholine	
1	Yes	Unchanged	Decreased	Died
2	Yes	Unchanged	Decreased	Died
3	Yes	Increased	Unchanged	Survived
4	No	Unchanged	Unchanged	Died
5	Yes		Unchanged	Died
6	No	Unchanged	Unchanged	Survived
7	Yes	Unchanged	Unchanged	Survived
8	Yes	Unchanged		Died
9	No		Unchanged	Survived
10	No	Unchanged	Decreased	Survived

Table 5.6 Flowflux and LnTHRR measurements when compared with healthy volunteers tabulated alongside proven evidence of sepsis and survival status at the time of hospital discharge; and statistically significant changes in LnTHRR response to iontophoresed norepinephrine and acetylcholine

*Indicates a greater increase or decrease from normal: flowflux >75 or LnTHRR <0.25

Discussion

Flowmetry measurements proved difficult to obtain during this study. Laser Doppler flowmetry is not well suited for use in critically ill patients because of the requirement for patients to avoid voluntary movements even for relatively short periods. This is clearly less of an issue for heavily sedated or paralysed patients, but even in such cases the presence of tissue oedema, where it occurs, is likely to make the THR measurements more difficult and potentially less reliable. The potential advantages of LDF, that it only requires access to skin, is portable and relatively non-invasive, are offset by these factors.

Despite these limitations this study demonstrated that overall skin flowflux measurements were raised in patients with evidence of SIRS compared with healthy volunteers. The transient hyperaemic response was also diminished in these patients as a whole.

A previous study performed in septic patients found that reactive hyperaemia provoked by prolonged ischaemia was not diminished in the early stages but only decreased when multiple organ failure ensued, even though adequate oxygenation and blood pressure were sustained through interventions (Hartl WH et al, 1988). In the study by Hartl 7 of the 9 patients with diminished vascular reactivity died. Within this study 8 patients demonstrated decreased transient hyperaemic responses, of whom half survived. Two patients in whom flowflux was raised but who had normal hyperaemic responses also died; and in these patients (and one other with a normal THR response) SOFA scores were relatively high (>8) indicating at least dual organ dysfunction.

Near-infrared spectroscopy has previously demonstrated that thenar muscle deoxygenation is significantly slower in patients with septic shock, and that the rate of decrease is closely related to organ failure as determined by the sequential organ failure assessment (SOFA) score. (Parežnik R et al, 2006). In contrast, in sepsis without shock a more rapid decline in deoxygenation was found. This has been interpreted as

mitochondrial respiration becoming dormant in the presence of septic shock (Singer M et al, 2004).

It is clear that SIRS is an imprecise definition and that sepsis is a complex entity, with different patterns of infection and inflammation being associated with different outcomes (Vincent J-L, 1997; Bossink AWJ et al, 1998; Knaus WA et al, 1992). It is probable that wide variations in vascular reactivity also occur, even amongst haemodynamically stable patients. Small studies such as this, and previous studies which have examined the microcirculation in critically ill patients, might be expected to identify a variety of responses.

Whilst the intention of this study was to recruit patients with evidence of SIRS, only seven patients had evidence of sepsis; and only three could be classified as having septic shock (albeit treated with vasopressors). With this in mind one of the most surprising results from this study was that the iontophoresis of acetylcholine did not result in an increase in flowflux caused by vasodilatation. Previous studies involving the effects of acetylcholine on the sublingual vessels of septic patients have demonstrated a preserved response (Rennie M, 2007; De Backer D, Creteur J et al, 2002). In another study by Kubli et al, 2003, the cutaneous responses to iontophoresed acetylcholine and sodium nitroprusside were preserved, but greatly diminished compared to healthy controls and a second control group of non-septic ICU patients. The effects of acetylcholine were also diminished, but to a lesser extent, in non-septic controls.

The iontophoresis protocol used by Kubli et al was different from that used in this study; in this study an iontophoresis pattern specifically designed to minimize current-induced vasodilatation was used, whilst in Kubli et al's study topical local anaesthesia was applied to achieve the same effect. It is possible that our iontophoresis protocol was simply less effective in septic patients, or, less likely, that the topical anaesthesia did not have the

desired effect on septic patients in Kubli et al's study. Some local anaesthetic creams have previously been shown to increase flowflux and decrease THRR measurements (Wiles MD et al, 2008).

The tissue oedema commonly present in treated critically ill patients might be expected to reduce the efficacy of drug iontophoresis. Although there was no obvious association between the subjective assessment that tissue oedema was present and changes in flowflux associated with acetylcholine, this remains a possibility. Oedema has also been reported as increasing the biological zero seen using laser Doppler flowmetry (Wahlberg E et al, 1992). Since THRR is calculated after the removal of biological zero this effect is unlikely to have altered the results found within this study.

Within this study there was no clear relationship between the need for inotropic infusions and vascular reactivity as assessed by the THRR. Iontophoresed norepinephrine only caused a statistically significant increase in the transient hyperaemic response in one patient. In a study by Keinbaum et al, 2008, forearm vascular resistance was increased by phenylephrine in patients with septic shock, but to a much lesser extent than in healthy volunteers. This would be consistent with the findings in our study and would suggest that patients were either already exposed to a sufficiently large dose of systemic or endogenous catecholamines, or had already developed a degree of resistance to norepinephrine, such that the dose of iontophoresed norepinephrine was insufficient to cause further vasoconstriction.

In conclusion it would appear that the transient hyperaemic response is much harder to perform and interpret when used within the ICU environment than was anticipated. An overall increase in flowflux was demonstrated, as had been predicted in other similar studies, and THRR was found to be reduced. No overall change in THRR could be demonstrated after the iontophoresis of norepinephrine or acetylcholine.

CHAPTER SIX

The effects of the prior administration of statins and cardiovascular medications in critically ill patients

Introduction

Previous studies within the thesis have concentrated on the application of the transient hyperaemic response in volunteers and patients with a view to further understanding its potential to investigate the microcirculatory changes associated with critical illness and sepsis. In the last study the difficulties in applying the THR to patients with evidence of SIRS were exposed. With this in mind it was decided to attempt to identify other agents with known cardiovascular actions, or speculated to have cardiovascular actions, which may alter vascular reactivity in sepsis; in particular agents which can be, in the first instance, administered safely, via ingestion, to volunteers in whom vascular reactivity can be measured. NSAIDs have previously been investigated in this regard (Moppett IK, Davies JA et al, 2003), but their effects in critical illness are far from certain (Bernard GR, Wheeler AP et al, 1997; Winning J et al, 2010; Mahajan RP et al, 2010).

Statins (HMG CoA reductase inhibitors) are currently being investigated to evaluate their effects in a wide range of illnesses. They are widely-used well tolerated drugs which are taken orally. Although side-effects with statins are rare, their safety record is such that changing their status from a prescription-only medication to an over-the-counter medication has even been considered (MHRA 2004;Strom BL, 2005). For these reasons this study attempts to better define the effects of prior statin usage on the outcomes of critically ill patients within intensive care, including survival and cardiovascular status immediately after admission to the ICU.

Statins are widely used in the treatment of hypercholesterolaemia and the prevention of ischaemic heart disease, but are suspected of having clinically useful therapeutic effects independent of their accepted lipid-lowering effects. These "pleiotropic" actions may include immunomodulatory, anti-inflammatory, and antithrombotic properties, as well as vascular endothelial effects (Almog Y, 2003; Mekntso-Dessap A, 2006). The effects of statins on a wide range of disease processes are currently being investigated (Bonovas S et al, 2006; Marti-Fàbregas J, 2004; Glynn RJ, 2009).

Two groups of patients of particular interest to intensive care practitioners are those with infections and those patients undergoing major surgery. Previous studies have suggested that statins may improve survival in patients with bacteraemia and may decrease the likelihood of their progression to develop sepsis or need intensive care unit (ICU) treatment (Liappis AP et al, 2001; Almog Y et al, 2004; Kruger P et al, 2006; Falagas ME et al, 2008). Studies have also suggested that statins may also reduce the morbidity and mortality associated with major cardiac and non-cardiac elective surgery (Clark LL et al, 2006; Poldermans D et al, 2003; O'Neil-Callahan K et al, 2005; Lindenauer PK et al, 2004).

Few studies have specifically examined the effects of prior statin use on critically ill patients already requiring ICU, and from those that have there have been mixed results (Fernandez R et al, 2006; Schmidt H et al, 2006). By collecting data on patients admitted to ICU with a presumed diagnosis of sepsis, or as a planned admission following major elective surgery, and retrospectively identifying those who had been taking statins prior to their admission to hospital, we aimed to examine in a pragmatic manner whether or not statins had been responsible for any measurable differences in outcome.

As statin use is strongly associated with the presence of patient co-morbidities other than hypercholesterolaemia, and with concomitant use of other medications with cardiovascular effects, we attempted to control for the presence of these factors. We also attempted to examine individual statins to ensure that any effect seen was a class-effect true of all statins.

Methods

After obtaining approval from the local ethics committee we retrospectively identified all adult patients admitted to one of two teaching hospital intensive care units (City Hospital or Queens Medical Centre, Nottingham, UK) with either a presumed diagnosis of sepsis, or as a planned admission following major elective surgery. Patients were identified between January 2000 and September 2005 using a combination of four continually maintained databases. The first two databases were local APACHE II databases supported run in conjunction with the Intensive Care National Audit and Research Centre (ICNARC) with data input by the data-clerks on both ICUs; the other two databases were internally designed Microsoft Access databases, one designed to capture ICU data input by nursing staff for the Critical Care Minimum Dataset (CCMDS), and the other designed and maintained by the local microbiology departments as a means of recording positive microbiology results. The ICNARC data contains information about all ICU admissions including the basic demographics, admission and discharge dates, and admitting diagnosis, as well as the information regarding the status of the patient during the first 24 hours of ICU admission required to calculate APACHE II scores (see appendix ii). The CCMDS data (DoH DSCN 02, 2005) was used to identify all planned and unplanned admissions to the ICU as well as the source of those admissions (i.e. theatre recovery, ward, etc), the level of care required once admitted to the ICU, and length of stay. CCMDS data was used to cross-check and ensure that no admissions were missed. Critically CCMDS data does not carry any information about admission diagnoses and so where there was any doubt as to whether the patient had either a presumed diagnosis of sepsis, or was a planned admission following major elective surgery, the patient's notes were analysed. The microbiology database only contained details about positive microbiology results and was used to ensure that no patients with a presumed diagnosis of sepsis were missed. In all cases where it was felt that patients were likely to fulfil the inclusion criteria a retrospective casenote analysis was performed.

Of the post-operative cohort only patients where ICU admission was planned prior to surgery were included. Patients whose admission was a result of surgical or anaesthetic complications, or where surgery was performed semi-electively or as an emergency (for example, surgery for abscess drainage or perforated viscus) were specifically excluded.

Where patients with sepsis were re-admitted to intensive care within thirty days of discharge, only the first admission was counted. Patients who had no evidence of sepsis on admission but who developed sepsis during their ICU stay were not included. Patients were also excluded where casenotes were unavailable or incomplete.

Casenotes were reviewed in order to confirm the cause of admission, and identify any evidence of pre-existing chronic disease and prescribed medication usage prior to hospital admission. Statin usage was recorded along with other cardiovascularly active medications, including angiotensin converting enzyme inhibitors (ACEi), angiotensin receptor antagonists (ARAs), long-acting nitrates, beta-blockers, and calcium channel antagonists. It was not possible to identify the indication, dosing, length of use, or compliance associated with any individual medication; nor was it possible to determine the efficacy of pre-admission medication usage, and where statins were prescribed recent lipid levels were only rarely available.

Patients were defined as having a significant co-morbidity if it was recorded in the notes as being part of the patients past medical history on admission to hospital or on admission to intensive care. Those co-morbidities recorded included ischaemic heart disease (any history of angina, heart failure, previous myocardial infarctions or ischaemic heart disease), other vascular disease (including cerebrovascular disease and peripheral vascular disease), diabetes (both non-insulin dependent and insulin-dependent diabetes), and chronic renal insufficiency (including all patients with a history of chronic kidney disease of stage 3 or worse).

The primary outcome measured was survival to hospital discharge. Secondary outcomes included survival to ICU discharge, length of ICU stay, presence of 3 or more systemic inflammatory response syndrome (SIRS) criteria, and evidence of cardiovascular instability within the first 24 hours of admission.

Univariate analysis was used to compare the two groups of prior statin usage and no-prior statin usage. After testing for multi-collinearity, multivariate logistic regression was also performed to calculate odds ratios adjusted for age, gender, APACHE II score at presentation, presence of pre-morbid diabetes or cardiovascular illness, and pre-morbid use of other cardiovascularly active medications (as defined above). Logistic regression was performed for: any statin usage, simvastatin usage, atorvastatin usage, and other statin usage (i.e. not simvastatin or atorvastatin). All analyses were carried out using SPSS version 14.

Logistic regression was also applied to four pre-defined subgroups: patients with presumed sepsis and proven bacteraemia at the time of admission, patients with presumed sepsis and shock at the time of admission (defined as a systolic blood pressure < 90 mmHg or already on inotropes), elective patients having undergone an oesophagectomy, and elective patients prescribed inotropes within 24 hours of admission to ICU.

Results

Over 1100 patients were identified, of whom only 15 had casenotes which were incomplete or unavailable for review. 414 patients had a presumed diagnosis of sepsis and 672 were planned admissions following elective major surgery. The overall mean age was 62 years old; and in total 138 patients (12.7%) were prescribed statins prior to their admission to hospital. The most commonly prescribed statins were simvastatin (43% of all statins prescribed), atorvastatin (38%), pravastatin (10%), and fluvastatin (7%). Only two patients were prescribed rosuvastatin and one was prescribed cerivastatin (prior to the drug being withdrawn by the manufacturers). The relative distribution of statins was the same in both post-operative patients and those presumed to have sepsis. Only one patient was identified as having been prescribed a non-statin cholesterol lowering drug (bezafibrate).

For both elective and emergency admissions statins were discontinued at least during the first part of any ICU stay, and often for the duration of stay. This probably reflects the difficulty of administering statin therapy to patients who are kept nil-by-mouth, or who are fed enterally using naso-gastric tubes.

In both cohorts those patients taking statins were on average 6-10 years older, had marginally higher APACHE II scores, and were more likely to have a history of ischaemic heart disease, vascular disease, or diabetes (tables 6.1 and 6.2). Statins users were also more likely to have been prescribed other cardiovascularly active medications. There were proportionally more male patients prescribed statins within the post-operative group, though this was not found in the presumed sepsis group.

Patients presumed to have sepsis

The two most common sources of presumed sepsis were peritonitis and pneumonia. There were no statistically significant differences in the proportion of statin prescribing in patients presenting to ICU with peritonitis or pneumonia, or where patients were shocked or found to have a proven bacteraemia at the time of admission (table 6.1).

Univariate analyses revealed no outcome differences associated with prior statin use in terms of ICU mortality, hospital mortality, or length of ICU stay (table 6.3). Statin usage was also not associated with an increased likelihood of presenting to ICU in shock, with any increased prescription of inotropes within the first 24 hours of admission, or with an increased likelihood of three or more SIRS criteria being present at admission.

Where individual types of statin were analysed simvastatin was associated with a higher ICU mortality (p = 0.018) and hospital mortality (p = 0.030). This association was not true of atorvastatin or other statins, although the absolute number of patients prescribed other statins was very small. Comparing outcome over time using composite endpoint of survival or discharge from hospital at 160 days demonstrates a trend for simvastatin to be associated with worse outcomes whilst atovastatin is associated with better outcomes (figure 6.1).

Using multivariate binomial regression, no medication use, including statins, was independently associated with increased mortality (table 6.5). This was also true when multivariate analysis was applied to ICU mortality, with the exception of simvastatin (odds ratio 3.15; confidence intervals 1.03-9.65; p =0.044). As expected, a significant association existed between hospital mortality and age or APACHE II score at presentation.

No association between hospital mortality and statin usage of any type was identified when multivariate analysis was applied to the subset of patients with proven bacteraemia at the time of admission, although the small numbers of patients resulted in very large confidence intervals (table 6.6). In the subgroup of patients presenting with shock, simvastatin usage, but not usage of other statins, was again associated with increased mortality (p =0.039).

Post-operative patients

The most common procedures requiring elective ICU admission were oesophageal resection, hepato-biliary / pancreatic, vascular, and neurosurgical operations. A statistically significant number of patients undergoing vascular surgery were prescribed statins prior to their surgery; this was not true of other procedures (table 6.2).

Univariate analysis revealed statistically significant associations between prior statin usage and the likelihood of dying whilst on ICU (though not whilst in hospital), and also receiving inotropes within the first 24 hours of ICU admission (table 6.4). Where statins were examined individually only simvastatin had a statistically significant association with ICU mortality (p =0.048); and a graph of outcome over time using composite endpoint of survival or discharge from hospital at 160 days demonstrates a trend for simvastatin to be associated with worse outcomes (figure 6.2) This association was not statistically significant when multivariate analysis was used (odds ratio 1.51; confidence intervals 0.30-7.68; p =0.618).

Multivariate analysis of hospital survival identified the significance of age and APACHE II score; neurosurgical procedures were also strongly associated with hospital mortality (table 6.5). Pre-existing co-morbidities and prior medication use, including statin usage, were not associated with altered mortality. This was also true when multivariate analyses

were applied to the subgroups of patients who had undergone oesophageal resection or who had received inotropes within the first 24 hours of ICU admission (table 6.6).

Variable	Patients with presumed sepsis, n=414		
	No prior statins (n=364)	**Prior statins (n=50)**	*P*-value*
Age, mean (SD)	60.0 (16.8)	69.6 (8.1)	<0.005
Male gender, n (%)	199 (54.7)	28 (56.0)	0.881
APACHE II score, mean (SD)	21.6 (8.1)	23.3 (8.6)	0.168
Other co-morbidities and medication usage			
Ischaemic heart disease, n (%)	44 (12.1)	24 (48.0)	<0.005
Other vascular disease, n (%)	26 (7.1)	15 (30.0)	<0.005
Diabetes, n (%)	26 (7.1)	20 (40.0)	<0.005
Chronic kidney disease, n (%)	20 (5.5)	6 (12)	0.11
ACE inhibitors / ARAs, n (%)	38 (10.4)	22 (44.0)	<0.005
Ca^{2+} channel antagonists, n (%)	23 (6.3)	16 (32)	<0.005
Beta-blockers, n (%)	32 (8.8)	13 (26)	0.001
Nitrates, n (%)	5 (1.4)	6 (12.0)	0.001
Suspected site of sepsis & presenting features			
Pneumonia, n (%)	106 (29.1)	12 (24.0)	0.508
Peritonitis, n (%)	128 (35.2)	22 (44.0)	0.272
Bacteraemia, n (%)	100 (27.5)	14 (28.0)	1.000
Shock, n (%)	326 (89.6)	43 (86.0)	0.466
Type of statin			
Simvastatin, n (%)		23 (46.0)	
Atorvastatin, n (%)		22 (44.0)	
Other statin, n (%)		5 (10.0)	

Table 6.1 Characteristics of patients admitted with a presumed diagnosis of sepsis stratified by prior statin usage. **P*-values are for independent t-test results for means, or chi-squared tests for categorical data.

	Post-operative patients, n=672		
Variable	**No prior statins (n=584)**	**Prior statins (n=88)**	*P*-value*
Age, mean (SD)	61.6 (15.6)	67.7 (13.1)	0.001
Male gender, n (%)	366 (62.7)	66 (75.0)	0.024
APACHE II score, mean (SD)	13.2 (5.1)	14.5 (6.4)	0.029
Other co-morbidities and medication usage			
Ischaemic heart disease, n (%)	56 (9.6)	53 (60.2)	<0.005
Other vascular disease, n (%)	65 (11.1)	33 (37.5)	<0.005
Diabetes, n (%)	33 (5.7)	22 (25)	<0.005
Chronic kidney disease, n (%)	11 (2.8)**	1 (1.8)**	1.000
ACE inhibitors / ARAs, n (%)	57 (9.8)	41 (46.6)	<0.005
Ca^{2+} channel antagonists, n (%)	58 (9.9)	17 (19.3)	0.017
Beta-blockers, n (%)	72 (12.3)	31.8)	<0.005
Nitrates, n (%)	10 (1.7)	10 (11.4)	<0.005
Type of surgery			
Oesophagectomy, n (%)	210 (36.0)	28 (31.8)	0.476
Hepato-biliary/pancreatic, n (%)	66 (11.3)	6 (6.8)	0.267
Vascular	44 (7.5)	24 (27.3)	<0.005
Neurosurgery	33 (5.7)	2 (2.3)	0.299
Type of statin			
Simvastatin, n (%)		37 (42.0)	
Atorvastatin, n (%)		30 (34.1)	
Other statin, n (%)		21 (23.9)	

Table 6.2 Characteristics of patients having planned ICU admission after major elective surgery stratified by prior statin usage. **P*-values are for independent t-test results for means, or chi-squared tests for categorical data.

**Incomplete dataset, denominator changed: no prior statin usage, n=392; prior statin usage, n=55

Outcome	Patients with presumed sepsis, n=414		
	No prior statins (n=364)	**Prior statins (n=50)**	*P*-value*
Died in ICU, n (%)	159 (43.7)	24 (48)	0.649†
Died in hospital, n (%)	197 (54.6)	29 (58.0)	0.762
Median ICU stay, days (range)	4 (1-31)	3 (1-31)	0.203
Presenting in shock, n (%)	326 (89.6)	43 (86.0)	0.466
Inotropes prescribed within 24hours of admission, n (%)	293 (80.9)	40 (80.0)	0.849
Presence of SIRS criteria**, n (%)	321 (90.7)	44 (89.8)	0.503

Table 6.3 Crude outcome data for patients presumed to have sepsis, stratified by prior statin usage. **P*-values are for chi-squared tests except length of ICU stay where Mann-Whitney *U*-test was used

**Modified SIRS criteria (≥ 3 variables) was used

Outcome	Post-operative patients, n=672		
	No prior statins (n=584)	**Prior statins (n=88)**	*P*-value*
Died in ICU, n (%)	20 (3.4)	8 (9.1)	0.021
Died in hospital, n (%)	53 (9.1)	13 (14.8)	0.121
Median ICU stay, days (range)	2 (0-30)	2 (1-20)	0.517
Inotropes prescribed within 24hours of admission, n (%)	221 (37.9)	47 (53.4)	0.007‡
Presence of SIRS criteria**, n (%)	276 (49.2)	40 (45.5)	0.567

Table 6.4 Crude outcome data for patients having planned ICU admission after major elective surgery, stratified by prior statin usage. **P*-values are for chi-squared tests except length of ICU stay where Mann-Whitney *U*-test was used

**Modified SIRS criteria (≥ 3 variables) was used

Hospital mortality for all patients				
	Patients with presumed sepsis, n=414		**Post-operative patients, n=672**	
	Adjusted odds ratio	***P*-value**	**Adjusted odds ratio**	***P*-value**
Age	1.03 (1.01-1.04)	<0.005	1.05 (1.02-1.08)	0.001
Male gender	1.04 (0.67-1.61)	0.877	1.29 (0.70-2.41)	0.415
APACHE II score	1.09 (1.06-1.13)	<0.005	1.16 (1.10-1.08)	<0.005
Other co-morbidities and medication usage				
Ischaemic heart disease	1.37 (0.68-2.75)	0.379	0.93 (0.39-2.24)	0.877
Other vascular disease	0.92 (0.43-1.95)	0.818	1.16 (0.52-2.57)	0.719
Diabetes	0.55 (0.25-1.21)	0.138	0.93 (0.39-2.24)	0.876
Chronic kidney disease	2.59 (0.94-7.08)	0.064	*	
ACE inhibitors / ARAs	0.73 (0.37-1.46)	0.375	1.32 (0.61-2.87)	0.482
Ca^{2+} channel antagonists	0.91 (0.41-2.02)	0.816	0.73 (0.31-1.70)	0.467
Beta-blockers	1.02 (0.49-2.11)	0.957	0.60 (0.27-1.35)	0.218
Nitrates	2.23 (0.39-12.90)	0.371	1.90 (0.48-7.52)	0.359
Type of surgery				
Oesophagectomy			1.18 (0.57-2.43)	0.658
Hepato-biliary / pancreatic			1.66 (0.64-4.27)	0.296
Vascular			0.64 (0.22-1.84)	0.403
Neurosurgery			6.95 (2.29-21.15)	0.001
Statin usage				
Any statin	0.88 (0.40-1.94)	0.755	1.28 (0.53-3.11)	0.579
Simvastatin	2.29 (0.71-7.40)	0.167	1.88 (0.63-5.67)	0.261
Atorvastatin	0.43 (0.15-1.30)	0.135	0.97 (0.24-4.01)	0.966
Other statins	0.38 (0.05-2.90)	0.352	0.82 (0.17-3.90)	0.807

Table 6.5 Adjusted odds ratio for patients presumed to have sepsis and post-operative patients

*Chronic kidney disease patients excluded due to incomplete dataset. Calculated crude odds ratio for chronic kidney disease was: OR 2.03, CI0.43-9.60, p=0.371

All variables were tested to ensure tolerance of any multicollinearity

Hospital mortality for patients prescribed inotropes within 24 hours of admission				
	Patients with presumed sepsis, n=369		**Post-operative patients, n=268**	
Simvastatin	4.49 (1.08-18.70); n=20	0.039	0.59 (0.12-2.90); n=22	0.518
Atorvastatin	0.36 (0.10-1.28); n=19	0.116	0.82 (0.16-4.23); n=14	0.808
Other statins	0.51 (0.06-4.41); n=4	0.537	0.36 (0.04-3.03); n=11	0.348

Hospital mortality for patients with proven bacteraemia		
	Patients with presumed sepsis, n=144	
Simvastatin	4.16 (0.15-113.04); n=6	0.397
Atorvastatin	5.06 (0.36-70.88); n=6	0.229
Other statins	1.70 (0.00-1371.87); n=2	0.876

Table 6.6 Adjusted odds ratio data for type of statin in patients presumed to have sepsis and in post-operative patients; within the subgroups of patients who required inotropes within 24 hours of admission, or who had proven bacteraemia. All variables were tested to ensure tolerance of any multicollinearity

*Chronic kidney disease patients excluded due to incomplete dataset. Calculated crude odds ratio for chronic kidney disease was: OR 2.03, CI0.43-9.60, p=0.371

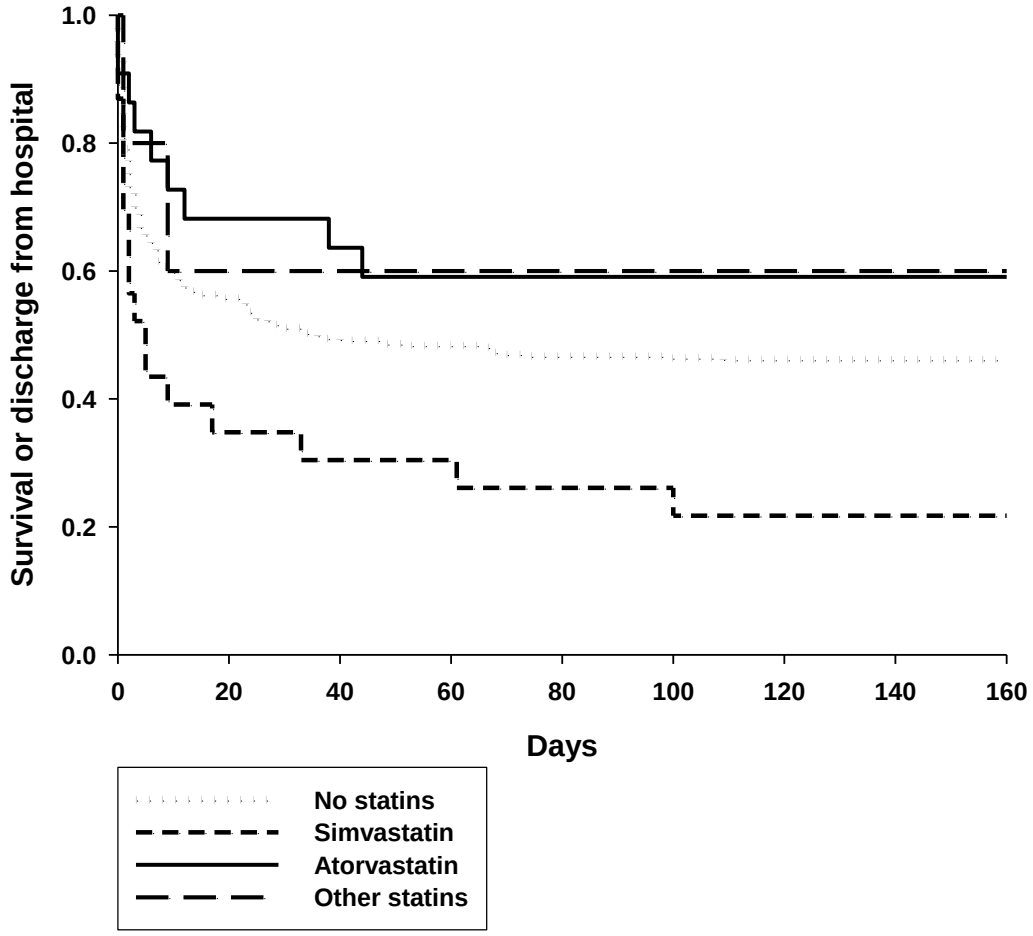

Figure 6.1 Crude 160 day survival curves for all patients with presumed sepsis, stratified by statin usage

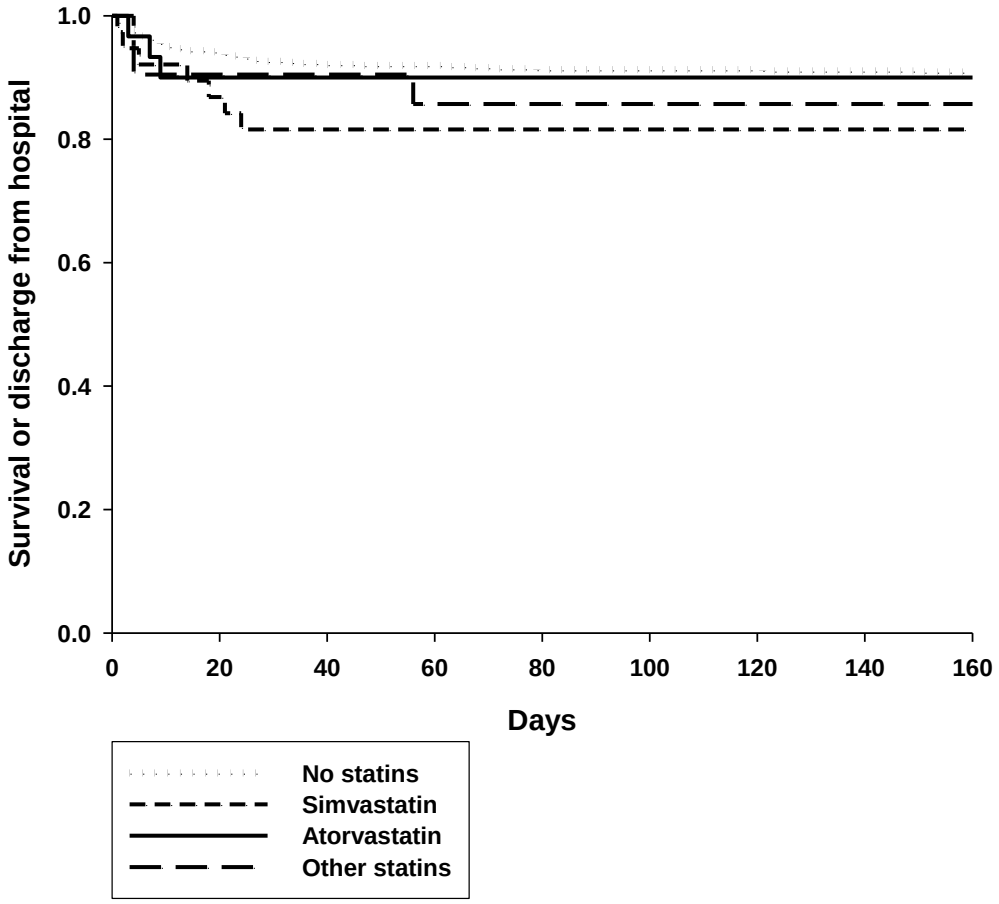

Figure 6.2 Crude 160 day survival curves for all post-operative patients, stratified by statin usage

Discussion

Previous studies have suggested potentially beneficial effects of statins in patients with bacteraemia or who have undergone major surgery. In patients with bacteraemia several studies have suggested that prior statin therapy protects against progression to severe sepsis and the need for admission to ICU; as well as decreasing overall mortality (Liappis AP et al, 2001; Almog Y et al, 2004; Kruger P et al, 2006). Other studies have suggested that statin usage confers long-term rather than short-term benefits amongst patients admitted with bacteraemia with Thomsen et al reporting unchanged mortality at 30 days, though improved mortality at 180 days (Thomsen RW, Hundborg HH et al, 2006).

When individual patient groups are selectively analysed there is also mixed evidence for any benefit from statins with regard to the development of sepsis. In the context of community-acquired pneumonia Majumdar et al found that statins were not associated with reduced mortality or reduced need for ICU admission, although Mortensen et al found did find an associated decrease in mortality at 30 days (Majumdar SR et al, 2006; Mortensen SR et al, 2005). Other studies have found a reduced incidence of sepsis in patients taking statins who had chronic kidney disease or prior cardiovascular disease (Gupta R et al, 2007; Hackam DG et al, 2006; Almog Y et al, 2007).

Several theories have been put forward to explain any potential benefit in terms of pleiotropic effects of statins, unrelated to their cholesterol-lowering abilities. Such pleiotropic effects include immunomodulation, reduction in blood thrombogenesis, and improved endothelial function and vascular reactivity (Almog Y, 2003; Mekontso-Dessap A et al, 2006).

Given this evidence it is reasonable to hypothesise that any protective effect of prior statin usage in bacteraemic patients might extend to those already admitted to the ICU. Our study found no survival benefit associated with prior statin-usage in patients admitted to

the ICU with presumed sepsis with or without a proven bacteraemia. Previous studies have suggested mixed findings amongst those admitted to ICU, with prior statin usage associated with increased mortality in patients who develop ICU-acquired infections, and decreased mortality in patients with multiple-organ dysfunction syndrome (Fernandez R et al, 2006; Schmidt H et al, 2006). Where statins have been found to be associated with worse outcome it has been suggested that this is because their use is associated with increasing age and the presence of pre-existing diseases, both of which are clearly evident in our study, and which were taken into account during the multivariate analyses.

The fact that statins users are more likely to be older with more co-morbidities may be offset by the fact that they may be a group who are more likely to receive other preventative therapies, the so-called "healthy user effect". In their study of statins-users with community-acquired pneumonia Majumdar et al found that statins decreased the crude relative risk, but that the relative risk increased once the healthy user effect was factored in (including walking unaided, quitting smoking, and up to date immunisations) (Majumdar SR et al, 2006; Thomsen RW, 2006) The strength of any healthy user effect is difficult to quantify, with one study indicating that it might even extend to statins users being at less risk of being involved in accidents (Dormuth CR et al, 2009).

There are a number of possible reasons why the design of our trial might fail to detect any beneficial effect exerted by statins in the presence of sepsis. Firstly our cohort involved patients presumed to have sepsis, rather than having proven sepsis. It is possible that any beneficial effect does not extend beyond those patients with sepsis, although we also failed to detect any benefit in patients with proven bacteraemia around the time of admission. Secondly we were unable to confirm what dose of statin patients had been taking, or for how long prior to admission. Nor could we confirm how compliant patients were with their statin medication. Lastly it was evident that statins were rapidly

discontinued following ICU admission, and it has been suggested that any benefit from statins may rapidly dissipate (Almog Y et al, 2007).

The same reasons might also explain why our study failed to find any association between statin usage and decreased mortality following major surgery. Several studies have identified an association between prior statin usage and improved morbidity and mortality after cardiac surgery, non-cardiac vascular surgery, and other non-cardiac surgery (Clark LL et al, 2006; Poldermans D et al, 2003; O'Neil-Callahan K et al, 2005; Lindenauer PK et al, 2004). The only association found by our study between statins and survival was that of an increased ICU mortality using univariate analysis, which was not present using multivariate analysis.

Where statins have been found to be beneficial it has been assumed that their pleiotropic effects, and particularly their immunomodulatory effects, have been protective; and indeed there is some in-vivo evidence that prospectively administering simvastatin reduces cytokine levels (specifically TNF-alpha and interleukin-6) amongst patients with acute bacterial infection and patients with severe sepsis (Novack V et al, 2009). It is possible that in the cohort of patients with presumed sepsis presentation to ICU meant that patients had already progressed beyond the point at which statins (either by their pleiotropic effects or by any healthy-user effect) could exert a beneficial effect. Those patients in the post-operative cohort might be considered to have too small an inflammatory response to be affected by statins. Statin usage in either cohort was associated with small, but statistically significant, increases in APACHE II scores which might indicate that any protective effect had already been lost. There was no difference in the frequency with which patients fulfilled the modified SIRS criteria, although this is admittedly a crude clinical measure of inflammation open to much criticism (Vincent J-L, 1997).

Alternatively any beneficial pleiotropic effect of statins in either group might be mediated by microvascular or antithrombotic mechanisms (Glynn RJ et al, 2009; McGown CC et al, 2007). Using the number of potentially septic patients presenting with shock, and the early requirement for inotropes in all patients, as crude measures of vascular reactivity we identified no differences between statins and non-statins users, with the exception that early inotrope use appeared more frequent amongst post-operative statins users. This association was no longer statistically significant using multivariate analysis, although a trend was still apparent (odds ratio 1.70, confidence intervals 0.95-3.05, p =0.074). Predictably beta-blockers (odds ratio 2.01, confidence intervals 1.03-3.26, p =0.005) and ACE inhibitor / ARAs (odds ratio 1.74, confidence intervals 1.04-2.91, p =0.035) were both significantly associated with inotrope requirement, as was oesophageal resection (odds ratio 2.94, confidence intervals 1.95-4.45, p <0.005) which always involved post-operative epidural analgesia combined with a fluid restrictive policy.

There is evidence that the sudden discontinuation of statins can result in a loss of endothelial benefit, and rebound cardiovascular effects that might even adversely affect outcome (Taneva E et al, 2006; Puccetti L et al, 2003; Laufs U et al, 2000; Tristano AG et al, 2007). Le Manach Y et al, 2007, found evidence of increased risk of cardiac myonecrosis when statins were discontinued after major vascular surgery. It is possible, but would seem unlikely, that any benefit from statins was offset by an equally powerful detrimental effect resulting from abrupt withdrawal. Although statins users were more likely to die on ICU following major surgery, this finding was no longer present after multivariate analysis (odds ratio 1.27, confidence intervals 0.37-4.32, p =0.704), and was not present when considering hospital mortality. It seems unlikely that this provides any support for abrupt withdrawal masking a beneficial effect, at least in the post-operative cohort. Furthermore patients undergoing oesophageal resection had prolonged withdrawal of their statins when compared with other post-operative patients and we could

identify no differences in their outcomes with regard to statins when compared with other groups.

Previous retrospective studies have mostly chosen to consider the pleiotropic effects of statins to be a class-effect and have grouped statins together. Simvastatin and pravastatin are closely related to the fungal metabolite lovastatin, whilst atorvastatin, rosuvastatin and fluvastatin are synthetic. Some statins (simvastatin, lovastatin) are pro-drug lactones, whilst others are in the active acid form (McCarey DW et al, 2005). It is unclear what influence these differences might have on poorly understood pleiotropic effects. It is also unclear how much differences in drug metabolism, particularly in critically ill patients, may affect the degree with which pleiotropic effects are present. Atorvastatin metabolism has been shown to be impaired in critically ill patients, even in the absence of known metabolic pathway inhibitors, resulting in higher than expected levels (Kruger PS et al, 2009). The degree to which other statins may be affected is unclear.

In this study we have attempted to examine statins separately, however only patients prescribed simvastatin and atorvastatin represented moderately large subgroups. Crude outcome data did identify some differences between simvastatin and atorvastatin, with simvastatin being associated with worse outcomes in the cohort with presumed sepsis. Although these findings were no longer statistically significant using multivariate analysis (except in the subgroup of patients presenting with presumed sepsis and shock) it is perhaps worth noting that the overall trend in sepsis was for simvastatin to be detrimental and atorvastatin to be protective; whilst in post-operative patients the trend was for simvastatin to be more strongly associated with worse outcome than other agents. It is unclear whether or not this represents random variation, or the variability with which different statins are prescribed for different indications, or even evidence of a subtle difference between agents in patients with altered vascular reactivity.

In conclusion it is surprising that many other retrospective studies have identified strong associations between statin usage and improved outcome, which this study has been unable to find. We are aware of several prospective trials which are underway involving patients with sepsis or critical illness. We would strongly suggest that any findings, positive or negative, are interpreted with caution and with regard to timing of intervention, underlying disease and vascular lability, and type of statin used. We would also suggest that where any findings are negative there is scope for a prospective study examining the effects of statin discontinuation in critically ill patients.

CHAPTER SEVEN

Conclusion and future proposals

In this thesis I have attempted to further explore the nature of the transient hyperaemic response, with a view to its use as an investigative tool in critically ill patients. In chapter 3 the intra- and inter-individual variability of the test was further clarified and a large dataset of results from healthy volunteers compiled. This dataset also appeared to corroborate data from earlier studies suggesting that age and gender do not cause large changes in THR. Due to the intrinsic properties of the transient hyperaemic response it is suggested that equal variances are not assumed when analyzing data, and that it may be of benefit to analyze the log-transformed THRR rather than the ratio as it stands in order to apply parametric tests.

Increased baseline flowflux fluctuations, such as was generated using PEEP in volunteers, cause an increase in variability but do not necessarily invalidate measurement of THR.

Within the context of critically ill ICU patients the transient hyperaemic response proved harder to measure and analyze than expected (chapter 5). Other techniques described in the literature do not appear to have highlighted such difficulties. The experiences of the

pilot study presented within this thesis would suggest that its use in clinical settings is restricted to highly cooperative individuals, or patients who are sedated and immobile.

Although those critical care patients known to have evidence of SIRS were found, as a group, to have altered vascular reactivity, a surprising finding within this study was the lack of effect of iontophoresed norepinephrine and acetylcholine. This would suggest that the effects described in previous studies are not clear cut, although it must be stressed that previous studies involved patients with sepsis rather than SIRS. Due to the heterogeneous nature of ICU patients it is hard to conceive a scenario in which a more homogenous group of patients could be investigated using this technique at this time. Attempts to better classify sepsis are underway, and any future attempts at investigating vascular reactivity in the clinical setting may become simpler as these develop (Levy MM et al, 2003; Lisboa T et al, 2008).

The transient hyperaemic response may still be a valuable tool for investigating models of sepsis in otherwise healthy human volunteers. Iontophoresis of chemicals into the forearm in order to induce a very localised inflammatory response is an attractive proposition, and would seem intuitively safer than triggering systemic or regional responses, such as those achieved by infusing inflammatory mediators into the brachial artery (Patel JN et al, 2002). Unfortunately the effects of iontophoresis appear to be less predictable than previously thought, and it cannot be assumed that carrier mediums are inert, despite previous evidence to the contrary. There is also evidence that prior iontophoresis of saline can enhance the effect of later vasoconstrictor iontophoresis (Drummond P, 2002). Any model involving the iontophoresis of chemicals to induce inflammation is likely to require carefully chosen control agents and/or a non-iontophoresed component.

The effects of iontophoresed norepinephrine on the THR had not been previously demonstrated prior to the investigations presented here. The finding that norepinephrine partially reversed the vasodilatation induced by localized heating may be of interest in future research involving other vasodilator mechanisms, observing the caveats already mentioned. The “negative” hyperaemic response to heat is in itself an interesting finding and a future study combining localized heating with iontophoresed adrenoceptor antagonists (with appropriate controls) is already being planned.

The effects of prior use of statins on the outcomes of critically ill patients was investigated in chapter 6 in response to a number of recent studies which have suggested a beneficial pleiotropic effect of statins in this scenario. A beneficial effect on mortality was not seen in the retrospective analysis performed within this study. There are many reasons why this could have occurred, even in the presence of a “true” beneficial effect, and randomised controlled trials into the beneficial effects of statins in sepsis and other conditions affecting critically ill patients are currently underway which may better analyse any effect.

As a hypothesis-generating exercise the retrospective analysis performed within this thesis suggests two important areas for future research. Firstly that the effects of the discontinuation of statins in critically ill patients is worthy of further investigation in its own right, regardless of whether or not statins are shown to be of benefit when given to ICU patients de novo; secondly that a class-effect of statins cannot be assumed. Although the margins of error within the research presented here are such that differences seen may be purely artefactual, there is sufficient evidence within the literature to suggest that the effects of different statins are not equal, at least as regards non-pleiotropic effects.

It was hoped that the research presented in chapter six would be complemented by further research investigating the effects of different statins on the vascular reactivity of healthy volunteers. A study measuring the effects (if any) of simvastatin usage on the

transient hyperaemic response of the cutaneous microcirculation is planned and has ethical approval Unfortunately at the time of writing this thesis that study has yet to be started. Nevertheless it is hoped that future studies looking at the effects of different statins on vascular reactivity, measured by the THR, will shed further light on the question of whether or not the pleiotropic cardiovascular effects of statins are measurable within the cutaneous microcirculation, and whether or not such effects, if they are found, represent a class-effect true of all statins.

Appendix i Abbreviations

°C	Temperature in degrees Celsius
2D	Two-dimensional (an ultrasound imaging mode)
ACF	Ante-cubital fossa
AIDS	Acquired immune deficiency syndrome
APACHE II	Acute physiology and chronic health evaluation score (version II)
ARF	Acute renal failure
AU	Arbitrary units
BP	Blood pressure
CCMDS	Critical care minimum data set
CKD	Chronic kidney disease
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
CPAP	Continuous positive-airways pressure
CRP	C-reactive protein
CVP	Central venous pressure
CVS	Cardiovascular system
CVVH	Continuous veno-venous haemofiltration
DRT4	*A laser Doppler flow controller* (Moor Instruments)
EDRF	Endothelial-derived relaxing factor(s)
EMLA	Eutectic mixture of local anaesthetics
F_0	Flowflux biological zero (during THR)
F_1	Baseline flowflux (prior to THR)
F_2	Peak flowflux during hyperaemic response
FiO_2	Fraction of inspired oxygen
GCS	Glasgow coma score
GI	Gastro-intestinal
Hct	Haematocrit
HR	Heart rate (beats per minute)
HTS	Hypertonic saline
IABP	Intra-aortic balloon pump
ICNARC	Intensive Care National Audit and Research Centre
ICP	Intra-cranial pressure
ICU	Intensive care unit
IPPV	Intermittent positive pressure ventilation

K	Potassium
LDF	Laser Doppler flowmetry
LDPI	Laser Doppler perfusion imaging
LDPM	Laser Doppler perfusion monitoring
MAP	Mean arterial pressure
MARS	Molecular adsorption recirculation system
MIC1-e	*An iontophoresis current controller* (Moor Instruments)
mmHg	Millimetres of mercury
Na	Sodium
NIDDM	Non-insulin dependent diabetes
NIRS	Near-infrared spectroscopy
NSAIDs	Non-steroidal anti-inflammatory drugs
NYHA	New York heart association
NO	Nitric oxide
PaO_2	Partial pressure of arterial oxygen
PDI	Phosphodiesterase inhibitor
PEEP	Positive end-expiratory pressure
PGI_2	Prostacyclin
RBC	Red blood cell
RR	Respiratory rate
RRT	Renal replacement therapy
RS	Respiratory system
SD	Standard deviation
SH02	*A heating control unit* (Moor Instruments)
SHP2	*A cutaneous heating probe* (Moor Instruments)
SIRS	Systemic inflammatory response syndrome
SOFA	Sequential (previously “sepsis”) organ failure assessment
SpO_2	Cutaneous pulse oximetry saturations
STROBE guidelines	Strengthening the Reporting of OBservational studies in Epidemiology guidelines
TCD	Trans-cranial Doppler
THR	Transient hyperaemic response
THRR	Transient hyperaemic response ratio
THR time	*The length of time (in seconds) a THR persists*
UOP	Urine output
WCC	White cell count

Appendix ii The APACHE II scoring system

APACHE II scoring system

Physiologic variable	Other		High abnormal range					Low abnormal range				
	+	+5	+4	+3	+2	+1	0	+1	+2	+3	+4
Core temperature (°C)			≥41	39–40.9		38.5–	36–38.4	34–	32–33.9	30–31.9	≤29.
MAP (mmHg)			≥16	130-159	110-129		70-109		50-69		≤49
HR (BPM)			≥18	140-179	110-139		70-109		50-69	40-54	≤39
RR			≥50	35-49		25-34	12-24	10-11	6-9		≤5
Oxygenation if FiO_2 > 0.5 (FiO_2 /			≥66	46.5–	26.6–		<26.6				
Oxygenation if FiO_2 <0.5 (PaO_2)							>9.3	8.1-		7.3-8	<7.3
pH			>7.7	7.6–		7.5–	7.33–		7.25–	7.15–	<7.1
Na (mmol/L)			>18	160–	155–	150–	130–		120–	111–	<110
K (mmol/L)			>7	6–6.9		5.5–5.9	3.5–5.4	3–3.4	2.5–2.9		<2.5
Creatinine (mg/L) X2 *if ARF present*			≥30	171-299		121-170	50–120		<50		
Hct (%)			≥60		50–59.9	46–49.9	30–45.9		20–29.9		<20
WCC			>40		20–39.9	15–19.9	3–14.9		1–2.9		<1
Age	≥7	65 - 74		55-64	45-54		<45				
Chronic ill health											
Liver		Emergenc		Elective							
CVS		Emergenc		Elective							
RS		Emergenc		Elective							
Renal		Emergenc		Elective							
Immunocompromised		Emergenc		Elective							
GCS	**Score equals 15 minus GCS**										

Chronic ill health is defined as follows

- Liver: Biopsy proven cirrhosis / portal hypertension / GI bleed secondary to portal hypertension / encephalopathy
- CVS: NYHA class 4
- RS: Domiciliary oxygen / pulmonary hypertension >40mmHg / fails stairs / documented hypoxia or hypercapnia / secondary polycythaemia
- Renal: Chronic dialysis
- Immunocompromise: Chemotherapy / radiation / high dose steroids / AIDS / leukaemia / lymphoma / etc

Appendix iii The SOFA scoring system

SOFA scoring system					
System	**0**	**1**	**2**	**3**	**4**
RS **PaO_2 / FiO_2 (kPa)**	>53	40.1-53	27.1-40	13.1-27	<13
Coagulation **platelets ($X10^9$/L)**	>150	100.1-150	50.1–100	20.1-50	≤20
Hepatic **bilirubin (µmol/L)**	≤20	20.1-32	32.1-101	101.1-204	≥204 or MARS
CVS	No hypotension	MAP <70 mmHg & no inotropes	Dopamine (≤5 µg/kg/min) or PDI	Dopamine (5–15 µg/kg/min) or Norepinephrine (≤0.1 µg/kg/min) or Adrenaline (≤0.1 µg/kg/min)	Dopamine (>15 µg/kg/min) or Norepinephrine (>0.1 µg/kg/min) or Adrenaline (>0.1 µg/kg/min) or IABP
CNS **Glasgow coma score**	15	13-14	10-12	6–9 or raised ICP	<6
Renal **creatinine (µmol/L)**	<110	110-170	171-299	300–440 or UOP 200-500mls/day	>400 or UOP <200mls/day

Appendix iv Volunteer information sheets and consent forms

University of Nottingham
Faculty of Medicine and Health Sciences
Division of Anaesthesia and Intensive Care
University Hospital
Queens Medical Centre
Nottingham NG7 2UH

Title of Project:

"The effect of continuous positive airways pressure on forearm skin vascular reactivity in spontaneously breathing volunteers assessed by laser Doppler flowmetry."

A study of how the blood flow in the skin responds when breathing through a mask that continually blows air to hold the airways open.

Investigators*:*

Dr Martin J Beed FRCA, **Research Fellow in Anaesthesia**
Dr Iain Moppett MRCP, FRCA **Lecturer in Anaesthesia**
Elliot Brenman **Medical Student**

Healthy Volunteer's Information Sheet

Invitation paragraph

You have been invited to take part in a research study. Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives if you wish to. Ask us if there is anything that is not clear or if you would like more information. Take the time to decide whether you wish to take part or not. If you decide to take part you may keep this leaflet. Thank you for reading this.

Background

In health blood vessels contract and relax in response to various stimuli, including the blood pressure within them. This is so that tissues receive the right amount of oxygen for their needs. This response, known as autoregulation, can be altered in various conditions, such as under anaesthesia, on the intensive care unit and with diseases such as high blood pressure and diabetes. We are developing a no-needles test to look at how skin blood vessels react in normal people, with the aim of eventually using it on patients. The test involves a technique called laser Doppler

flowmetry. This involves shining a very weak laser at your forearm skin and measuring the amount of light that comes back. From this we can get an estimate of the blood flow in your skin. In order to be sure that any changes in blood vessel autoregulation are due to a disease or illness it is important that we know whether anything else might interfere with the test. One possible procedure that commonly occurs on intensive care units is ventilation, where a patient's breathing is taken over by a ventilator, and it is possible that this might interfere with blood vessel autoregulation. Since being on a ventilator can sometimes be quite uncomfortable, with patients needing sedation, we intend to mimic ventilation with something called Continuous Positive Airways Pressure or CPAP which is much more comfortable and involves blowing air through a tight fitting mask into the mouth and nose, rather like breathing whilst facing a very strong wind. This has the effect of raising the pressure inside the chest slightly which is similar to the overall effect of being on a ventilator. CPAP is often used on fully awake patients in many of the hospital wards and is known to be very safe and well tolerated.

We hope to run this study over a period of nine months.

What does the study involve?

.
We will ask you to come to the study area and spend at least 15 minutes there before the study is to start, so that you can relax and get used to the surroundings. You will be asked to lie down on a couch with one arm comfortably outstretched on a table and pillow. A blood pressure cuff will be placed around your other arm to check your blood pressure before and during the experiment. We will then attach a special probe to the surface of your outstretched forearm with a small adhesive sticker. On the first occasion, when the blood flow measurements are steady we will perform 3 identical tests on you at 2-minute intervals. One of the investigators will feel for the artery at your upper arm and when they have found it, they will press on it firmly for 20 seconds and then release. The increase in blood flow will be recorded by the probe and the test repeated twice more.

After these initial tests we will fit a breathing mask to your face using a special harness that fits around your head and holds the mask tight. It is important that the mask is tight fitting with very little air leaking around the edges and this can mean that the mask is sometimes slightly uncomfortable as it may press slightly on the bridge of the nose. Once this is done we will then use a machine to blow air into the mask at a fixed pressure. This will make it feel as if there is a slight resistance when you are breathing out and air blowing at you when you are breathing in. Whilst it is very safe this can feel a little strange and sometimes uncomfortable. After we are sure there are no leaks we will ask you to continue breathing through the mask for 5 minutes before repeating the measurements of skin blood flow by pressing the artery in the upper arm 3 more times. During the time you are breathing through the mask we will be monitoring the gases you breathe out and the level of oxygen in your blood, this will require a monitor rather like a clothes peg being placed on a finger.

The whole process should take less than 30 minutes.

We do not expect any pain or side effects to occur as a result of this study apart from mild discomfort resulting from compression of the artery, perhaps some discomfort where the mask presses against the face and bridge of the nose, and breathing may feel uncomfortable whilst the mask is on. Occasionally people find they swallow some air with the mask on and may belch afterwards.

If you do feel uncomfortable the study will be terminated. You may withdraw from the study at any time without having to give a reason.

There is no requirement for blood tests or questionnaires either at the time of the study or in the future.

Why have you been chosen?

We need 15 healthy volunteers to complete the study.

Do you have to take part?

It is up to you to decide or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

What do I have to do?

We need you to avoid strenuous exercise before the study. Caffeine-containing substances (coffee, tea, and cola) or food should not be consumed for at least 2 hours before the study.

What is the drug or procedure that is being tested?

Laser Doppler flowmetry is a safe, painless method of measuring blood flow. It involves shining a weak laser light at the skin and measuring the light that is reflected back. It does not involve needles and does not cause any damage to the skin.

What are the side effects of any treatment or procedures received when taking part?

Pressing on the artery in your upper arm is not usually a problem, but can occasionally be a little uncomfortable.
Using the special mask which delivers continuous positive airways pressure (CPAP) can make breathing feel uncomfortable, and can occasionally lead to redness where the mask presses against the bridge of the nose, but it is not known to cause any serious side effects. Occasionally people breathing through a CPAP mask can find that they swallow more air than normal which can lead to mild stomach discomfort relieved by belching, but this normally happens after long periods of time breathing through the mask and is unlikely to occur in the short period of time which we will be using.

What are the possible disadvantages and risks of taking part?

We do not anticipate any risk to you from taking part, apart from the discomfort mentioned above.

Even so we will not include you in the study if:

a) You are allergic to any of the drugs or adhesives used to secure the probe
b) You have damaged skin on your arm
c) You have any circulatory disorders such as Raynaud's disease, systemic sclerosis, diabetes or have high blood pressure which requires medication
d) You are a smoker
e) You have recently injured your chest or have a history of collapsed lung, poorly controlled asthma or other chest disease which limits your ability to breath.

What if something goes wrong?

If taking part in this research project harms you, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain about any aspect of the

way you have been approached or treated during the course of this study you may complain to Professor A. Aitkenhead, Head of University Department of Anaesthesia in the first instance, or the Dean of the Medical School.

Will my taking part in this study be kept confidential?

All information collected about you during the course of the research will be kept strictly confidential. Any information about you that leaves the research unit will have your name and address removed so that you cannot be recognised from it.

What will happen to the results of the research study?

Once the study is completed the results will be submitted for publication to a scientific journal and will probably be published by the end of 2004. You will not be identified in any publication or report. If you wish to obtain a copy of the published results then please contact the investigators.

Who is organising and funding the research?

The investigators are all members of the University Department of Anaesthesia or medical students attached to the University Department of Anaesthesia.

Who has reviewed the study?

This study has been reviewed and approved by the University of Nottingham Medical School Ethics Committee

Contact for Further Information

You may contact any of the investigators: Dr Martin Beed or Iain Moppett.

By phone: 0115 924 9924 Ext. 42855

By email: martin.beed@nottingham.ac.uk
iain.moppett@nottingham.ac.uk

By post: University Department of Anaesthesia
C Floor, East Block
Queen's Medical Centre
Derby Road
Nottingham
NG7 2UH

Thank you for taking the time to read this information sheet and agreeing to take part in this study.

You will be given a copy of this information sheet and a signed consent form to keep.

University of Nottingham
Faculty of Medicine and Health Sciences
Division of Anaesthesia and Intensive Care
University Hospital
Queens Medical Centre
Nottingham, NG7 2UH

Title of Project:

"The effect of iontophoresed mannitol, propofol and midazolam on vascular reactivity in skin vessels using laser Doppler flowmetry of forearm skin."

A study of how the blood flow in skin responds to drugs commonly used in Intensive Care Units.

Investigators*:*

Dr Martin J Beed FRCA,	**Research Fellow in Anaesthesia**
Dr Iain Moppett MRCP, FRCA	**Lecturer in Anaesthesia**

Healthy Volunteer's Information Sheet

Invitation paragraph

You have been invited to take part in a research study. Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives if you wish to. Ask us if there is anything that is not clear or if you would like more information. Take the time to decide whether you wish to take part or not. If you decide to take part you may keep this leaflet. Thank you for reading this.

Background

In health blood vessels contract and relax in response to various stimuli, including the blood pressure within them. This is so that tissues receive the right amount of oxygen for their needs. This response, known as autoregulation, can be altered in various conditions, such as under anaesthesia, on the intensive care unit and with diseases such as high blood pressure and diabetes. We are developing a no-needles test to look at how skin blood vessels react in normal people, with the aim of eventually using it on patients in Intensive Care. The test involves a technique called laser Doppler flowmetry. This involves shining a very weak laser at your forearm skin and measuring the amount of light that comes back. From this we can get an estimate of the blood flow in your skin. We want to know whether our test will be affected by any of the drugs commonly used on Intensive Care. There are three drugs we are hoping to test two of which are commonly

used to sedate patients on intensive care; the third one is often used in the treatment of patients with head injuries. The drugs are going to be given directly to the skin by a process called iontophoresis which uses a tiny electrical current to drive a small amount of the drug into the surface of the skin. It is very safe and gives a much smaller amount of drug than any tablet or injection would, affecting only a small area of forearm skin. Given this way the drugs will have no sedative effect.
We hope to run this study over a period of nine months.

What does the study involve?

We will ask you to come to the study area and spend at least 15 minutes there before the study is to start, so that you can relax and get used to the surroundings. You will be asked to lie down on a couch with one arm comfortably outstretched on a table and pillow. A blood pressure cuff will be placed around your other arm to check your blood pressure before and during the experiment. We will then attach a special probe to the surface of your outstretched forearm with a small adhesive sticker. On the first occasion, when the blood flow measurements are steady we will perform 3 identical tests on you at 2-minute intervals. One of the investigators will feel for the artery at your upper arm and when they have found it, they will press on it firmly for 20 seconds and then release. The increase in blood flow will be recorded by the probe and the test repeated twice more.
Another probe will be attached to another part of the same forearm and the process of iontophoresis will take place with one of the drugs we are testing. Most people feel a slight tingling sensation. Once this has been completed the measurements of skin blood flow will be repeated by pressing the artery in the upper arm 3 more times. There are three drugs to test and so the process of giving a drug by iontophoresis and pressing the artery will be done on three different areas of forearm skin. The whole process should take about 45 minutes.

We do not expect any pain or side effects to occur as a result of this study apart from mild discomfort resulting from compression of the artery, a tingling sensation as previously described and a small area of redness where the drug is applied.
If you do feel uncomfortable the study will be terminated. You may withdraw from the study at any time without having to give a reason.

There is no requirement for blood tests or questionnaires either at the time of the study or in the future.

Why have you been chosen?

We need 15 healthy volunteers to complete the study with no special requirements.

Do you have to take part?

It is up to you to decide or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

What do I have to do?

We need you to avoid strenuous exercise before the study. Caffeine-containing substances (coffee, tea, and cola) or food should not be consumed for at least 2 hours before the study.

What is the drug or procedure that is being tested?

Laser Doppler flowmetry is a safe, painless method of measuring blood flow. It involves shining a weak laser light at the skin and measuring the light that is reflected back. It does not involve

needles and does not cause any damage to the skin. The drugs which we wish to investigate are ones currently used within intensive care and anaesthesia for sedation or the treatment of head injuries.

What are the side effects of any treatment or procedures received when taking part?

Pressing on the artery in your upper arm is not usually a problem, but can occasionally be a little uncomfortable. The application of the two drugs to the skin may cause a little itching or redness, which may last up to 24 hours. The quantity used is too small to produce any other effects.

What are the possible disadvantages and risks of taking part?

We do not anticipate any risk to you from taking part, apart from the discomfort mentioned above.

We will not include you in the study if:

a) You are allergic to any of the drugs or adhesives used to secure the probe
b) You have damaged skin on your arm
c) You have any circulatory disorders such as Raynaud's disease, systemic sclerosis, diabetes or high blood pressure
d) You are a smoker

What if something goes wrong?

If taking part in this research project harms you, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of this study you may complain to Professor A. Aitkenhead, Head of University Department of Anaesthesia in the first instance, or the Dean of the Medical School.

Will my taking part in this study be kept confidential?

All information collected about you during the course of the research will be kept strictly confidential. Any information about you that leaves the research unit will have your name and address removed so that you cannot be recognised from it.

What will happen to the results of the research study?

Once the study is completed the results will be submitted for publication to a scientific journal and will probably be published by the end of 2005. You will not be identified in any publication or report. If you wish to obtain a copy of the published results then please contact the investigators.

Who is organising and funding the research?

The investigators are all members of the University Department of Anaesthesia or medical students attached to the University Department of Anaesthesia.

Who has reviewed the study?

This study has been reviewed and approved by the University of Nottingham Medical School Ethics Committee

Contact for Further Information

You may contact any of the investigators: Dr Martin Beed or Dr Iain Moppett,

By phone: 0115 924 9924 Ext. 42855

By email: martin.beed@nottingham.ac.uk
iain.moppett@nottingham.ac.uk

By post: University Department of Anaesthesia
C Floor, East Block
Queen's Medical Centre
Derby Road
Nottingham
NG7 2UH

Thank you for taking the time to read this information sheet and agreeing to take part in this study. You will be given a copy of this information sheet and a signed consent form to keep.

University of Nottingham
Faculty of Medicine and Health Sciences
Division of Anaesthesia and Intensive Care
University Hospital
Queens Medical Centre
Nottingham, NG7 2UH

Title of Project:

"The effect of iontophoresed norepinephrine, vasopressin and endothelin on vascular reactivity in skin vessels dilated by heat assessed using laser Doppler flowmetry of forearm skin."

A study of how the blood flow in skin that is warm responds to drugs that constrict blood vessels.

Investigators*:*

Dr Martin J Beed FRCA, **Research Fellow in Anaesthesia**
Dr Iain Moppett MRCP, FRCA **Lecturer in Anaesthesia**
Elliot Brenman **Medical Student**

Healthy Volunteer's Information Sheet

Invitation paragraph

You have been invited to take part in a research study. Before you decide whether to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends and relatives if you wish to. Ask us if there is anything that is not clear or if you would like more information. Take the time to decide whether you wish to take part or not. If you decide to take part you may keep this leaflet. Thank you for reading this.

Background

In health blood vessels contract and relax in response to various stimuli, including the blood pressure within them. This is so that tissues receive the right amount of oxygen for their needs. This response, known as autoregulation, can be altered in various conditions, such as under anaesthesia, on the intensive care unit and with diseases such as high blood pressure and diabetes. We are developing a no-needles test to look at how skin blood vessels react in normal people, with the aim of eventually using it on patients. The test involves a technique called laser Doppler flowmetry. This involves shining a very weak laser at your forearm skin and measuring the amount of light that comes back. From this we can get an estimate of the blood flow in your skin. We want to know whether our test can be used to look at the effect of various drugs that we use to raise people's blood pressure when they are unwell. So far testing these drugs has been difficult to do, so we are going to try testing them on skin that has already been warmed up to make the blood vessels expand. The drugs are going to be given directly to the skin by a process called iontophoresis which

uses a tiny electrical current to drive a small amount of the drug into the surface of the skin. It is very safe and gives a much smaller amount of drug than any tablet or injection would, affecting only a small area of forearm skin.
We hope to run this study over a period of six months.

What does the study involve?

We will ask you to come to the study area and spend at least 15 minutes there before the study is to start, so that you can relax and get used to the surroundings. You will be asked to lie down on a couch with one arm comfortably outstretched on a table and pillow. A blood pressure cuff will be placed around your other arm to check your blood pressure before and during the experiment. We will then attach a special probe to the surface of your outstretched forearm with a small adhesive sticker. On the first occasion, when the blood flow measurements are steady we will perform 3 identical tests on you at 2-minute intervals. One of the investigators will feel for the artery at your upper arm and when they have found it, they will press on it firmly for 20 seconds and then release. The increase in blood flow will be recorded by the probe and the test repeated twice more.
Another probe will be attached to another part of the same forearm and this one will gently heat a small area of skin underneath to a temperature of 38 degrees Celsius. Once the skin is warmed the process of iontophoresis will take place with one of the drugs we are testing. Most people feel a slight tingling sensation. Once this has been completed the measurements of skin blood flow will be repeated by pressing the artery in the upper arm 3 more times. There are three drugs to test and so the process of heating an area of skin, giving a drug by iontophoresis and pressing the artery will be done on three different areas of forearm skin.
The whole process should take about 45 minutes.

We do not expect any pain or side effects to occur as a result of this study apart from mild discomfort resulting from compression of the artery, a tingling sensation as previously described and a small area of redness where the drug is applied.
If you do feel uncomfortable the study will be terminated. You may withdraw from the study at any time without having to give a reason.

There is no requirement for blood tests or questionnaires either at the time of the study or in the future.

Why have you been chosen?

We need 15 healthy volunteers to complete the study with no special requirements.

Do you have to take part?

It is up to you to decide or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason.

What do I have to do?

We need you to avoid strenuous exercise before the study. Caffeine-containing substances (coffee, tea, and cola) or food should not be consumed for at least 2 hours before the study.

What is the drug or procedure that is being tested?

Laser Doppler flowmetry is a safe, painless method of measuring blood flow. It involves shining a weak laser light at the skin and measuring the light that is reflected back. It does not involve needles and does not cause any damage to the skin. The drugs which we wish to investigate are

ones currently used within intensive care and anaesthesia in order to raise the blood pressure of critically ill patients.

What are the side effects of any treatment or procedures received when taking part?

Pressing on the artery in your upper arm is not usually a problem, but can occasionally be a little uncomfortable. The application of the two drugs to the skin may cause a little itching or redness, which may last up to 24 hours. The quantity used is too small to produce any other effects.

What are the possible disadvantages and risks of taking part?

We do not anticipate any risk to you from taking part, apart from the discomfort mentioned above.

We will not include you in the study if:

a) You are allergic to any of the drugs or adhesives used to secure the probe
b) You have damaged skin on your arm
c) You have any circulatory disorders such as Raynaud's disease, systemic sclerosis, diabetes or high blood pressure
d) You are a smoker

What if something goes wrong?

If taking part in this research project harms you, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of this study you may complain to Professor A. Aitkenhead, Head of University Department of Anaesthesia in the first instance, or the Dean of the Medical School.

Will my taking part in this study be kept confidential?

All information collected about you during the course of the research will be kept strictly confidential. Any information about you that leaves the research unit will have your name and address removed so that you cannot be recognised from it.

What will happen to the results of the research study?

Once the study is completed the results will be submitted for publication to a scientific journal and will probably be published by the end of 2004. You will not be identified in any publication or report. If you wish to obtain a copy of the published results then please contact the investigators.

Who is organising and funding the research?

The investigators are all members of the University Department of Anaesthesia or medical students attached to the University Department of Anaesthesia.

Who has reviewed the study?

This study has been reviewed and approved by the University of Nottingham Medical School Ethics Committee

Contact for Further Information

You may contact any of the investigators: Dr Martin Beed or Dr Iain Moppett,

By phone: 0115 924 9924 Ext. 42855

By email: martin.beed@nottingham.ac.uk
iain.moppett@nottingham.ac.uk

By post: University Department of Anaesthesia
C Floor, East Block
Queen's Medical Centre
Derby Road
Nottingham
NG7 2UH

Thank you for taking the time to read this information sheet and agreeing to take part in this study. You will be given a copy of this information sheet and a signed consent form to keep.

The same consent form was used for all three studies, with altered titles as below:

Title of Project:

"The effect of continuous positive airways pressure on forearm skin vascular reactivity in spontaneously breathing volunteers assessed by laser Doppler flowmetry."

Title of Project:

"The effect of iontophoresed mannitol, propofol and midazolam on vascular reactivity in skin vessels using laser Doppler flowmetry of forearm skin."

University of Nottingham
Faculty of Medicine and Health Sciences
Division of Anaesthesia and Intensive Care
University Hospital
Queens Medical Centre
Nottingham, NG7 2UH

Title of Project:

"The effect of iontophoresed norepinephrine, vasopressin and endothelin on vascular reactivity in skin vessels dilated by heat assessed using laser Doppler flowmetry of forearm skin."

Name of Investigators:

Dr Martin J Beed FRCA, Research Fellow in Anaesthesia
Dr Iain Moppett MRCP FRCA Lecturer in Anaesthesia
Elliot Brenman Medical Student

Healthy Volunteer's Consent Form

Please read this form and sign it once the above named or their designated representative, has explained fully the aims and procedures of the study to you

- I voluntarily agree to take part in this study.
- I confirm that I have been given a full explanation by the above named and that I have read and understand the information sheet given to me which is attached.
- I have been given the opportunity to ask questions and discuss the study with one of the above investigators or their deputies on all aspects of the study and have understood the advice and information given as a result.

- I agree to the above investigators contacting my general practitioner [and teaching or university authority if appropriate] to make known my participation in the study where relevant.

- I agree to comply with the reasonable instructions of the supervising investigator and will notify him immediately of any unexpected unusual symptoms or deterioration of health.

- I authorise the investigators to disclose the results of my participation in the study but not my name.

- I understand that information about me recorded during the study will be kept in a secure database. If data is transferred to others it will be made anonymous. Data will be kept for 7 years after the results of this study have been published.

- I understand that I can ask for further instructions or explanations at any time.
- **I understand that I am free to withdraw from the study at any time, without having to give a reason for withdrawing.**

- I confirm that I have disclosed relevant medical information before the study.

- I have not been a subject in any other research study in the last three months which involved: taking a drug; being paid a disturbance allowance**;** having an invasive procedure (e.g. venepuncture >50ml, endoscopy) or exposure to ionising radiation.

Name: ……

Address: ……..

Telephone number: …….

Signature: …… **Date:** …….

I confirm that I have fully explained the purpose of the study and what is involved to:

…….

I have given the above named a copy of this form together with the information sheet.

Investigators Signature: …….. **Name**: ……

Study Volunteer Number:

……..

Appendix v Patient and relatives information sheets and consent / assent forms

Similar but adapted forms was used for relatives who were approached to provide assent on behalf of sedated, unconscious or otherwise incapacitated patients

Queen's Medical Centre Nottingham NHS
University Hospital NHS Trust

Nottingham City Hospital NHS
NHS Trust

Title of Project:

"To determine association between altered skin vascular reactivity and haemodynamic instability, level of inotropic support, morbidity and mortality in patients with systemic inflammatory response syndrome (SIRS)."

A study of how skin blood flow responds in people with sepsis or inflammation.

Investigators: **Dr Martin Beed** **Research Fellow**
Dr Iain Moppett **Clinical Lecturer**
Dr Sally Hancock **Clinical Lecturer**
Dr Ravi Mahajan **Reader and Consultant**

Patient Information Sheet

Invitation

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Thank you for reading this.

What is the purpose of the study?

In health blood vessels contract and relax in response to many things so that tissues receive the right amount of oxygen for their needs. This response is known as autoregulation and can be altered by many illnesses including infections or inflammation. We are developing a no-needles test to look at how skin blood vessels react in patients who are unwell, particularly with infections or inflammation. Our test uses a technique called laser Doppler flowmetry which involves shining a very weak laser at forearm skin and measuring the amount of light that comes back. From this

we can get an estimate of the skin blood flow. We want to know whether our test will show how well any treatment is working.

The study will last for about two years and involve 160 people.

Why have I been chosen?

You have been chosen because you have an infection or another condition, such as a burn or an injury to your bowel, causing inflammation, particularly to the blood vessels.

Do I have to take part?

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

What will happen to me if I take part?

We will visit you on the Intensive Care Unit or High Dependency Unit and ask you to lie with one arm comfortably outstretched on a table and pillow. A blood pressure machine will be placed on your other arm to check your blood pressure before and during the experiment. We will then attach a special probe to the surface of your outstretched forearm with a small adhesive sticker. At least 15 minutes will be spent relaxing and getting used to this before any measurements are taken. After 15 minutes we will perform 3 identical tests at 2-minute intervals. An investigator will feel for the artery at your elbow. When they have found it, they will press on it firmly for 20 seconds and then release. The increase in blood flow will be recorded by the probe and the test repeated twice more.

Once this has been completed a very small amount of a drug that alters the ability of blood vessels to dilate and expand will then be applied to the skin via the chamber holding the laser flow monitor. A tiny electric current will then be passed through the chamber for two minutes. This enables the drug to penetrate the outermost layer of skin to reach the superficial vessels underneath. The size of the current is too small to be felt other than as an itching sensation. The quantity of drug used is only able to produce effects locally in the skin. It produces no other effects in the body other than a slightly red area where it has been applied, lasting a few hours. The technique has been used for many years and been proven to be safe. Once this has been performed we will press firmly on the artery two more times.

On a second day a baseline set of blood flow readings will again be taken. For the last part of the study a drug with slightly different effects on blood vessel reactivity will be applied in the same way as previously to the forearm.

On both occasions a blood sample will be taken to test for markers of inflammation. In the vast majority of cases this can be done via intravenous drips that are already in place, and so will not require any needles.

We do not expect any pain or side effects to occur as a result of this study apart from mild discomfort resulting from compression of the artery, a tingling sensation as previously described and a small area of redness where the drug is applied. If you do feel uncomfortable the study will be terminated.

YOU MAY WITHDRAW FROM THE STUDY AT ANY TIME WITHOUT HAVING TO GIVE A REASON.

We anticipate that the study will take about 30 minutes to complete on each occasion. We will not alter your medical or nursing care in any other way, however we would like to follow your care and monitor your progress whilst in the hospital.

You will receive the same standard of care and medication for your illness whether you are part of the study or not.

What do I have to do?

If you are able to eat or drink please avoid caffeine-containing substances (coffee, tea, and cola) or food for at least 2 hours before the study. There are no other restrictions.

What is the drug or procedure that is being tested?

Laser Doppler flowmetry is a safe, painless method of measuring blood flow. It involves shining a weak laser light at the skin and measuring the light that is reflected back. It does not involve needles and does not cause any damage to the skin.
A blood sample will be taken to test for markers of inflammation both times that the test takes place, but in most cases this too can be done without any needles.

What are the alternatives for diagnosis or treatment?

There are currently no tests that will predict how well patients respond to treatment for inflammation or sepsis, which is why we wish to develop one. The results of our research will not affect your treatment or management in any way.

What are the side effects of taking part?

Pressing on the artery at the elbow is not usually a problem, but can occasionally be a little uncomfortable. The application of the two drugs to the skin may cause a little itching or redness, which may last up to 24 hours. The quantity used is too small to produce any other effects.
In the event of a problem a doctor will be present throughout the duration of the test.

What are the possible disadvantages and risks of taking part?

We do not anticipate any risk to you from taking part, apart from the discomfort mentioned above. We will not include you in the study if:

a) You are allergic to any of the adhesives used to secure the probe
b) You have damaged skin on your arm
c) You have any circulatory disorders such as Raynaud's disease, systemic sclerosis or diabetes affecting the circulation.
d) You are pregnant

What are the possible benefits of taking part?

There is no benefit to you if you take part. This study will not alter your care or management in any way.

What is something goes wrong?

If you are harmed by taking part in this research project, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a

legal action but you may have to pay for it. Regardless of this, if you wish to complain about any aspect of the way you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms should be available to you.

Will my taking part in this study be kept confidential?

All information collected about you during the course of the research will be kept strictly confidential. Any information about you that leaves the research unit will have your name and address removed so that you cannot be recognised from it. Your GP will be informed that you have participated in this research study as a matter of course.

What will happen to the results of the research study?

Once the study is completed the results will be submitted for publication to a scientific journal and will probably be published by the end of 2005. You will not be identified in any publication or report. If you wish to obtain a copy of the published results then please contact the investigators.

Who is organising and funding the research?

The investigators are all members of Nottingham University Department of Anaesthesia. The funding is from the Association of Anaesthetists of Great Britain and Ireland.

Your doctor will NOT be paid for including you in this study.

Who has reviewed the study?

This study has been reviewed and approved by the Nottingham Research Ethics Committee.

Contact for further information

You may contact any of the investigators: Dr Martin Beed and Dr Iain Moppett

By phone: 0115 9691169 Ext. 46837

By email: martin.beed@nottingham.ac.uk
iain.moppett@nottingham.ac.uk

By post: University Department of Anaesthesia
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Nottingham
NG7 2UH

Thank you for taking the time to read this information sheet.

If you agree to take part in this study you will be given a copy of this information sheet and a signed consent form to keep.

Nottingham City Hospital NHS Trust | Queen's Medical Centre Nottingham NHS University Hospital NHS Trust

CONSENT FORM

Study title:

To determine association between altered skin vascular reactivity and haemodynamic instability, level of inotropic support, morbidity and mortality in patients with systemic inflammatory response syndrome (SIRS).

A study of how skin blood flow responds in people with sepsis or inflammation.

Please ask the patient to complete the following: cross out as necessary	Please
Have you read and understood the patient information sheet?	YES/NO
Have you had an opportunity to ask questions and discuss this study?	YES/NO
Have you received satisfactory answers to all your questions?	YES/NO
Have you received enough information about the study?	YES/NO
Do you understand that you are free to withdraw from the study at any time?	YES/NO
- without giving a reason for withdrawing?	YES/NO
- and without affecting your future medical care?	YES/NO

Who explained the details of this study to you? ……

I agree to take part in this study. YES/NO

Name of patient …….……..…….

Signed ……..… Date …….

Name of researcher ……..

Signed ….…….. Date ……..

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