Vascular adaptation to blood flow and temperature in humans

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Abstract

Recent and profound changes associated with ubiquitous exposure to television, mobile devices and the internet have rapidly accelerated an underlying trend in sedentary behaviour related to urbanisation, automation and the widespread use of motorised personal transport. These technological "advances" have fundamentally altered vocational and lifestyle behaviours in the space of one or two generations. There has never been a more sedentary population of humans than 21st century western society. It was estimated that, in 2008, physical inactivity caused 6–10% of all deaths from the major non-communicable diseases (coronary heart disease, type 2 diabetes and breast and colon cancers), or more than 5.3 of the 57 million deaths that occurred worldwide. It is clear that lifestyle changes are having profound impacts on human disease and healthcare costs and one of the most predominant health conditions is atherosclerotic cardiovascular disease.

Regular exercise has been shown to significantly reduce cardiovascular risk, an impact which is partly mediated by improvements in traditional modifiable risk factors such as hypercholesterolemia, hypertension and obesity. However, recent evidence suggests there is a ‘risk factor gap’, whereby the cardio-protective benefits of exercise cannot be fully accounted for by improvements in traditional risk factors. There is growing evidence that one element that may fill the risk factor gap relates to the direct effects of exercise on the vasculature. It is now believed that increases in blood flow and attendant shear stress associated with bouts of exercise are key mechanisms capable of inducing arterial adaptation. However, exercise is a complex stimulus associated with the release of hormones, metabolic by-products from working muscles and neural activation. The independent role of shear stress as the principal modulator of endothelial and vascular adaptation is therefore difficult to dissect in exercise studies. The broad aim of this thesis was to investigate the effects of changes in shear stress, induced by exercise-independent modalities, on both macro- and micro-vascular adaptation. The thesis is presented in two sections, containing data on conduit artery adaptation and changes in the cutaneous microvessels.

Section 1

Study 1: Effects of shear rate manipulation on conduit artery diameter

This chapter provided evidence that increases in shear stress through the brachial and radial arteries, induced by both exercise-dependent and –independent modalities, induce
similar changes in conduit diameter. When blood flow and shear stress were attenuated by partial inflation of a cuff on one arm in these experiments, no changes in diameter were observed. The findings of this study demonstrate that shear is a potent vasoactive stimulus. This reinforces the suggestion that shear is involved in vascular adaptation as a consequence of exercise. The similarity in shear and diameter responses between the exercise and exercise-independent interventions provided the impetus to investigate the chronic effects of exercise-independent increases in shear on vascular function, presented in subsequent chapters.

Study 2: Conduit vascular adaptation to repeated forearm heating

In this chapter, repeated bouts of forearm heating (to 42°C) significantly enhanced brachial artery function after 2 weeks exposure. Whilst artery function data returned to baseline values by week 8, they were superseded by structural adaptations, as evidenced by an enhanced dilator capacity. This biphasic response in vascular function is similar to that previously reported following exercise training. Throughout each heating bout, one forearm was cuffed to attenuate increases in shear. No adaptation was observed in this limb, strongly implying that shear is a primary stimulus for vascular adaptation. These findings pertaining to repeated passive forearm heating are consistent with the exercise literature and indicate that episodic increases in shear induce changes in vascular function and structure in vivo.

Study 3: Conduit vascular adaptation to repeated lower limb heating

The purpose of this Chapter was to investigate whether whole body heating induces systemic vascular adaptation. Acutely, lower limb heating-induced thermoregulatory reflexes significantly increased brachial shear rate. Eight weeks of such lower limb heating induced transient improvements in brachial artery function, consistent with previous literature. However, no change in brachial structure was observed, suggesting the existence of a potential shear stress threshold effect for vascular remodelling. In the cuffed arm, no adaptation in either function or structure was observed. These findings indicate that whole body heating results in shear-mediated systemic vascular adaptation that may potentially have direct clinical implications for populations where exercise is contraindicated.
Section 2

Study 4: Cutaneous microvascular adaptation to repeated lower limb heating

Adaptations in the functional capacity of conduit arteries to deliver blood might theoretically be accompanied by adaptations in the downstream cutaneous microvasculature. This was the investigative aim of study 4. Acutely, lower limb heating (40°C) resulted in significant increases in skin temperature. Eight weeks of repetitive lower limb heating induced an increase in skin blood flow responsiveness to a local heating stimulus in the forearm. No adaptation was observed in the contralateral cuffed arm in which increases in skin blood flow and skin temperature were attenuated during each heating bout. The results of this study indicate that repeated whole body heating induces localised cutaneous adaptations similar to those previously observed in response to exercise training and repeated forearm heating. These adaptations were dependent upon repeated increases in skin blood flow and/or skin temperature.

Study 5: Cutaneous microvascular adaptation to repeated lower limb heating: Isolating the impacts of skin blood flow and temperature

Local heating of the skin is a known stimulus for the release of vasodilator substances such as nitric oxide. The microvascular adaptations observed in study 4 as a result of reflex-mediated forearm vasodilation may have therefore been mediated by repeated increases in skin temperature, attendant to the changes in blood flow. The purpose of study 5 was to examine cutaneous microvascular adaptations following 8 weeks of lower limb heating, under conditions where skin temperatures in both arms were “clamped” at 30°C throughout each leg heating bout. This allowed for the examination of the role of skin blood flow in cutaneous adaptation, in the absence of changes in skin temperature. Following 8 weeks of repeated leg heating involving this form of forearm temperature “clamp”, skin blood flow responsiveness to local heating decreased. One interpretation of this data is that prolonged transit time of blood flow through the skin in response to local heating reflects increased capillarisation. When considered in the context of the results of study 4, these findings illustrate the importance of increased skin temperature attendant to cutaneous hyperaemia. It is possible that upregulation of heat shock protein 90, a molecule which facilitates eNOS expression, explains increased nitric oxide bioavailability and enhanced microvascular function when heat is applied concurrently with repeated hyperaemia. The findings of studies 4 and 5 are the first to suggest that increases in skin blood flow are obligatory for cutaneous microvascular...
adaptation and that skin temperature is an important stimulus that modulates the adaptive response. They infer that repeated increases in blood flow, combined with repeated increases in skin temperature, induce adaptations which enhance microvascular reserve and thermoregulatory capacity.

**Summary**

Taken together, the findings presented in this thesis strongly suggest that shear stress is a potent stimulus for vascular adaptation in both macro- and micro-vessels in humans. Repeated increases in blood flow and shear stress induced by exercise-independent interventions induced similar adaptations to those previously observed following exercise training. In this sense, the present findings inform previous exercise training studies as to the relevance of shear stress as a primary vascular adaptive stimulus *in vivo*. In addition, the novel findings of studies 4 and 5 illustrate the important and distinct impacts of both skin blood flow and skin temperature in cutaneous adaptation. These data also reinforce the complexity of cutaneous vascular physiology and its relevance to integrative cardiovascular control. Finally, these studies have important clinical implications as they potentially provide an alternative mode for improvement in cardiovascular health in populations where exercise is contraindicated.
Table of Contents

Abstract .................................................. ii
Acknowledgements ...................................... vii
List of Figures ........................................... viii
List of Tables ............................................. xvi
Declaration ................................................. xvii
Candidate Contribution ............................... xviii
Conference Proceedings ............................. xxi

Chapter 1  Introduction .................................. 22

Chapter 2  Literature Review ............................ 29
Conduit artery adaptation to chronic changes in shear stress in humans

Chapter 3  Study 1 ......................................... 55
Effect of shear rate manipulation on conduit artery dilation in humans

Chapter 4  Study 2 ......................................... 78
Repeated increases in blood flow, independent of exercise, enhance conduit artery vasodilator function in humans

Chapter 5  Study 3 ......................................... 93
Repeated core temperature elevation induces conduit artery adaptation in humans

Chapter 6  Literature Review 2 ......................... 105
Cutaneous microvascular adaptations to exercise and passive heating in humans

Chapter 7  Study 4 ......................................... 123
Cutaneous microvascular adaptation to repeated-passive core heating in humans: Combined impacts of skin blood flow and temperature on adaptation

Chapter 8  Study 5 ......................................... 137
Cutaneous microvascular adaptation to repeated-passive core heating in humans: Isolating the impacts of skin blood flow and temperature

Chapter 9  General Discussion ......................... 151

References .................................................. 161
Appendices .................................................. 176
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List of Figures

Figure 1.1 23
An illustration by Leonardo da Vinci showing the 2-chambered structure of the heart, in accordance with Galen’s theory.

Figure 1.2 25
Illustrations of William Harvey’s method of determining the circulation of blood from arteries to the veins using ligatures.

Figure 2.1 30
Diagram showing the chronological steps involved in atherogenesis and highlighting the importance of endothelial dysfunction in this development (Pepine, 1998).

Figure 2.2 33
The effects of increases in blood flow and infusion of acetylcholine, nitroglycerin and norepinephrine on femoral artery diameters when the endothelium is intact and denuded. Modified from (Pohl et al., 1986).

Figure 2.3 34
A figure showing different velocity profiles in the brachial artery and subsequent changes in diameter. Inflation of an occluding forearm cuff decreased brachial flow (circulatory arrest), resulting in brachial vasoconstriction. During reactive hyperaemia, following deflation of the cuff after 10 mins, brachial artery flow increased significantly, along with diameter. Modified from (Anderson & Mark, 1989).

Figure 2.4 35
A diagram showing the anatomy of a typical artery and the mechanism of flow-mediated dilation.

Figure 2.5 35
Diagrammatical representation of the purported molecular processes initiated in response to increases in shear stress on the inner wall of the artery (Gielen et al., 2010).
Figure 2.6 38
Mean brachial artery flow during incremental exercise showing a biphasic response from baseline (A), a response explained by examination of the antegrade and retrograde components of blood flow (B). Significantly different from baseline values at $P<0.05$ (*) or $P<0.01$ (†) (Modified from Green et al., 2002).

Figure 2.7 40
Shear rates (SR) during, and FMD responses pre and post, forearm heating (A), handgrip (B) and leg cycling (C) exercise. *Significantly different between the non cuffed and cuffed arms at $P<0.05$ (Tinken et al., 2009).

Figure 2.8 47
Brachial flow-mediated dilation (FMD%) responses in the non-cuffed (solid squares) and the cuffed (open squares) arms throughout 8 weeks of leg exercise training. Brachial FMD increases significantly after 2 weeks of training before returning to baseline values by week 8 (Birk et al., 2012).

Figure 2.9 48
A diagram showing the arterial adaptation in response to repeated exercise-induced increases in shear stress. In the untrained artery, there is basal release of NO (A), however short term training induces greater eNOS and NO bioavailability, enhancing arterial function (B). As exercise training continues, NO bioavailability returns to baseline levels as the enhanced artery function is superseded by a more permanent structural change, i.e. remodelling (C).

Figure 2.10 50
The graph on the left shows a reduction in the left common carotid artery diameter in rabbits following ligation that significantly reduced blood flow for 2 weeks. When the endothelium is removed (denuded), no change in diameter is evident. Modified from (Langille & O'Donnell, 1986).

Figure 2.11 51
Maximal diameter change from baseline (%) in mesenteric arteries of different flow conditions (A) and eNOS content (B) at day 2 and day 7. Modified from (Tuttle et al., 2001).
**Figure 2.12**
Flow-mediated dilation (solid square) and conduit artery dilator capacity (open diamond) in the brachial (top panel) and popliteal (bottom panel) arteries across the intervention. *Significantly different from baseline at $P<0.05$. Modified from (Tinken et al., 2008b).

**Figure 3.1**
Radial mean shear rate (A) and diameter (B) at rest and in response to exercise undertaken at 70% and 85%HRmax in the cuffed (open bars) and uncuffed (solid bars) forearms. #Significantly different at $P<0.05$. Differences existed in the impact of cycle exercise intensity between the cuffed and uncuffed arms in terms of both shear rate ($P<0.005$) and diameter ($P<0.01$). Data are mean ± SE.

**Figure 3.2**
Absolute brachial mean shear rate (A) and diameter (B) at rest and in response to exercise undertaken at 80%HRmax in the cuffed (open bars) and uncuffed (solid bars) forearms. The cuff was inflated throughout the exercise period. A significant impact of cuff placement was evident on mean shear rate responses across time ($P<0.01$) along with brachial artery diameter between limbs ($P<0.05$). #Significantly different at $P<0.05$. ‡Significantly different at $P<0.01$. Data are mean ± SE. Note: shear rate data for one subject were removed from the analysis due to inadequate edge detection of the velocity envelope (n=9).

**Figure 3.3**
Absolute brachial mean shear rate (A) and diameter (B) at rest and in response to exercise undertaken at 80%HRmax in the cuffed (open bars, cuff inflation at 15 mins cycling) and uncuffed (solid bars) forearm. A significant impact of cuff placement was evident on mean shear rate responses across time ($P<0.05$) along with brachial artery diameter ($P<0.01$). #Significantly different at $P<0.05$. Data are mean ± SE.

**Figure 3.4**
Absolute brachial mean shear rate (A) and diameter (B) at rest and in response to bilateral forearm heating at 42°C in the cuffed (open bars) and uncuffed (solid bars) forearm. A significant impact of cuff placement was evident on mean shear rate responses across time ($P<0.01$) along with a significant difference in brachial artery...
diameter between the limbs ($P<0.05$). *Significantly different at $P<0.05$. ‡Significantly different at $P<0.01$. C, cuffed; UC, uncuffed. Data are mean ± SE.

**Figure 3.5**

Absolute brachial mean shear rate (A) and diameter (B) at rest and in response to bilateral forearm heating at 42°C in the cuffed (open bars, cuff inflation at 15 mins heating) and uncuffed (solid bars) forearm. A significant impact of cuff placement was evident on mean shear rate responses across time ($P<0.01$) along with brachial artery diameter ($P<0.05$). ‡Significantly different at $P<0.01$. Data are mean ± SE.

**Figure 4.1**

Acute impact of cuff placement during bilateral forearm heating. Brachial artery shear rate (A), blood flow (B) and velocity (C) at baseline and at 5 min intervals in a subgroup of 5 subjects who underwent bilateral assessments throughout an acute bout of 30 mins bilateral forearm heating (42°C) in the non-cuffed (solid circles) and cuffed arm (open circles). *Significantly different from baseline at $P<0.05$. *Significantly different between limbs at same time point at $P<0.05$. Data is mean ± SE.

**Figure 4.2**

Chronic impact of 8 weeks of repetitive forearm heating on brachial artery responses. Relative change in FMD (A) from baseline and in response to ischaemic exercise (B) across the 8 week heating intervention in healthy young men (n=9). Data were presented for the uncuffed arm (solid squares) as well as the cuffed arm (open squares). *Significantly different from baseline at $P<0.05$. *Significantly different between limbs at same time point at $P<0.05$. Data is mean ± SE.

**Figure 5.1**

Brachial artery shear rate (1/s) in the cuffed (open squares) and uncuffed (solid squares) forearms at baseline and at 5, 15 and 25 mins during lower limb heating. Significantly different at $P<0.05$ from baseline (‡) or between the cuffed and uncuffed forearms (*). Data are mean ± SE.

**Figure 5.2**

Brachial artery responses to flow-mediated dilation (%) (A) and sublingual glyceryl trinitrate (%) (B) in the cuffed (open squares) and uncuffed (solid squares) forearms at
weeks 0, 2, 4, 6 and 8. *Significantly different at \( P<0.05 \) from baseline. Data are mean ± SE.

**Figure 6.1**
A typical biphasic skin blood flow response to local heating of the skin with an initial peak, followed by a brief nadir and a secondary prolonged vasodilation (A). Skin blood flow to the same heating stimulus following infusion of L-NAME reveals a similar initial peak however a significantly attenuated plateau phase, demonstrating the role of NO in this response (B). Modified from (Minson *et al.*, 2001).

**Figure 6.2**
A diagram showing initial increases in skin blood flow due to the withdrawal of cutaneous adrenergic vasoconstrictor outflow (VC). When core temperature continues to rise, substantial increases in skin blood flow are achieved via activation of cutaneous cholinergic vasodilator outflow (VD). Modified from (Kellogg, 2006).

**Figure 6.3**
Diagram showing the differences in the core temperature/skin blood flow relationship between exercise and passive heating. The plateau in skin blood flow occurs at a higher point during passive heating due to the absence of competition for blood flow to the working muscles as compared to exercise. Modified from (Simmons *et al.*, 2011b).

**Figure 6.4**
Changes in forearm and cutaneous vascular conductance (FVC and CVC, respectively) and core temperature relationships following 16 weeks of exercise training. Exercise training resulted in a significant leftwards shift in the threshold for forearm vasodilation, post-intervention (A). A similar leftwards shift in the threshold for cutaneous vasodilation was evident, along with a significantly higher plateau, post-intervention (B) (Thomas *et al.*, 1999).

**Figure 6.5**
Forearm skin vascular conductance (FVC) and core temperature (\( T_{es} \)) relationship before and after the aerobic exercise intervention in a euhydrated (A) and hypohydrated (B) state. Aerobic exercise training resulted in a significant increase in plasma volume and a greater FVC for a given \( T_c \) post-intervention (A), however once the increase in
plasma volume was reversed by hypohydration, this adaptation was less evident (B). Modified from (Ikegawa et al., 2011).

**Figure 6.6**

Resting plasma volume during control, following 4 and 8 days of either heat-exposure or exercise training and recovery. Plasma volume was significantly increased following 8 days in the heat exposure group whilst it was significantly increased by day 4 and 8 in the exercise training group. Modified from (Convertino et al., 1980).

**Figure 6.7**

Nitric oxide contribution to cutaneous vascular conductance (CVC) pre- and post-intervention. Exercise training (24 weeks) in elderly sedentary subjects resulted in a significant increase in NO-mediated skin blood flow in response to a gradual local heating protocol, indicating enhanced cutaneous microvascular endothelial function. Modified from (Black et al., 2008b).

**Figure 6.8**

Cutaneous vascular conductance (CVC) in response to local heating. Eight weeks of bilateral forearm warm water immersion resulted in an increased SkBF responsiveness to local heating in the uncuffed arm (A). The cuffed arm displayed no adaptation (B). Modified from (Green et al., 2010).

**Figure 7.1**

Forearm cutaneous vascular conductance (CVC) (A), skin temperature (B) and sweat rate (C) in the cuffed (open squares) and uncuffed (solid squares) forearms at baseline and during 30 mins of lower limb heating (5 min intervals). Significantly different at $P<0.05$ from baseline ($^*$) or between the cuffed and uncuffed forearms ($^*$). Data are mean ± SE.

**Figure 7.2**

Forearm cutaneous vascular conductance (CVC) (A), sweat rate (B) and core temperature (C) in response to 30 mins of lower limb heating at weeks 0 (open circle) and 8 (solid circle) in a subgroup of 8 subjects. Significantly different at $P<0.05$ from baseline ($^*$) or between weeks 0 and 8 ($^*$). Data are mean ± SE.
Figure 7.3 131
Change in forearm cutaneous vascular conductance (CVC) (y axis) (A) and sweat rate
(B) versus change in core temperature (x axis) during 30 mins of lower limb heating (5
min intervals) at weeks 0 (open circle) and 8 (solid circle). Data are derived from Figure
7.2.

Figure 7.4 132
Absolute forearm cutaneous vascular conductance (CVC) (A) and sweat rate (B) against
core temperature during 30 mins of lower limb heating (5 min intervals) at weeks 0
(open circle) and 8 (solid circle). Data are derived from Figure 7.2.

Figure 7.5 133
Change in mean arterial pressure (A), total peripheral resistance (B), cardiac output (C),
heart rate (D) and stroke volume (E) during 30 mins of lower limb heating at weeks 0
(open circle) and 8 (solid circle). Significantly different at $P<0.05$ from baseline (\#) or
between weeks 0 and 8 (*). Data are mean ± SE.

Figure 7.6 134
Forearm cutaneous vascular conductance (CVC) during the gradual heating protocol in
the cuffed (A) and uncuffed (B) arms and at weeks 0 (open circle) and 8 (solid circle).
*Significantly different from week 0 at $P<0.05$. Data are mean ± SE.

Figure 8.1 142
Forearm cutaneous vascular conductance (CVC) (A) and skin temperature (B) in the
cuffed (open squares) and uncuffed (solid squares) forearms at baseline and during 30
mins of lower limb heating (5 min intervals) in a sub-group of 6 subjects. Significantly
different at $P<0.05$ from baseline (\#) or between the cuffed and uncuffed forearms (*).
Data are mean ± SE.

Figure 8.2 144
Forearm cutaneous vascular conductance (CVC) (A) and core temperature (B)
responses to 30 mins of lower limb heating at weeks 0 (open circle) and 8 (solid circle)
in a subgroup of 6 subjects. *Significantly different between weeks at $P<0.05$.
†Significantly different between weeks at $P=0.05$. Data is mean ± SE.
**Figure 8.3**  
Change in forearm cutaneous vascular conductance (CVC) (y axis) versus change in core temperature (x axis) during 30 mins of lower limb heating (5 min intervals) at weeks 0 (open circle) and 8 (solid circle). Data are derived from Figure 8.2.

**Figure 8.4**  
Absolute forearm cutaneous vascular conductance (CVC) against core temperature during 30 mins of lower limb heating (5 min intervals) at weeks 0 (open circle) and 8 (solid circle). Data are derived from Figure 8.2.

**Figure 8.5**  
Forearm cutaneous vascular conductance (CVC) during the gradual heating protocol in the cuffed (A) and uncuffed (B) arms and at weeks 0 (open circle) and 8 (solid circle). *Significantly different from week 0 at $P<0.05$. ‡$P=0.06$. Data is mean ± SE.

**Figure 9.1**  
A schematic demonstrating the adaptive response to exercise-induced stimuli at the different segments of the coronary vascular tree. Modified from (Brown, 2003).
List of Tables

Table 3.1  
Subject characteristics for the 5 experimental protocols.

Table 3.2  
Mean, anterograde and retrograde shear rate (1/s) data during cycling exercise at different intensities.

Table 3.3  
Mean, anterograde and retrograde shear rate data (1/s) during the cycling exercise and forearm heating protocols, cuff inflated throughout.

Table 3.4  
Mean, anterograde and retrograde shear rate data (1/s) during the cycling exercise and forearm heating protocols, cuff inflated at mid-point.

Table 4.1  
Baseline subject characteristics.

Table 4.2  
Brachial artery characteristics throughout the 8 week exercise intervention, in the uncuffed and cuffed arms.

Table 5.1  
Baseline subject characteristics.

Table 5.2  
Brachial artery characteristics at weeks 0, 2, 4, 6 and 8.

Table 7.1  
Baseline subject characteristics.

Table 8.1  
Baseline subject characteristics.
Declaration

I declare this thesis is my own composition, all sources have been acknowledged and my contribution is clearly identified in the thesis. For any work in the thesis that has been co-published with other authors, I have the permission of all co-authors to include this work in my thesis.

______________________________
Howard H Carter
PhD Candidate

______________________________
Winthrop Professor Daniel J Green
Coordinating Supervisor
Publications arising from thesis

This thesis contains published work that has been co-authored. The bibliographical details of the work and where it appears in the thesis are outlined below. A statement for each publication that clarifies the contribution of the student to the work is also provided.


Candidate Contribution

Planning: Contribution to the conception of the ideas, development of the experimental designs and the establishment of laboratory techniques.

Data Collection: The candidate was the primary individual involved in data collection at UWA. This project also involved Professor Green’s laboratory at Liverpool John Moores University, which is lead by Dr Dawson. The candidate travelled to Liverpool on 3 separate occasions to ensure that experimental protocols and procedures were identical at both sites, to assist in the training of staff at Liverpool and to directly contribute to data collection at this secondary site. The applicant was primarily responsible for data collation and analysis.

Manuscript: The candidate prepared all figures and tables and drafted the first versions of all manuscripts. He was also responsible for revising manuscripts following circulation to co-authors. Finally, he led the preparation of journal rebuttals and revisions.

Candidate Contribution

Planning: Involved in the conception of the experimental ideas and the development of the study design to test the hypotheses (involving researching and purchasing equipment and installing the thermostatically maintained separate bilateral water baths).

Data Collection: This project required dual, simultaneous ultrasonography and all data collection was performed in collaboration with Dr Naylor. Similarly, analysis and interpretation of the data was performed with Dr Naylor.

Manuscript: The candidate prepared all figures and tables and drafted the first versions of all manuscripts. He was also responsible for revising manuscripts following circulation to co-authors. Finally, he led the preparation of journal rebuttals and revisions.

*Given the equivalent involvement of Dr Naylor and the candidate, it was considered joint first authorship was appropriate in this case.

Candidate Contribution
Planning: Involved in the conception of the experimental ideas aimed at progressing and extending the findings of Chapter 4. Development of the study design, including researching, purchasing and installing new laboratory equipment.

Data Collection: The candidate was the primary individual involved in the collection, analysis and interpretation of the data.

Manuscript: The candidate prepared all figures and tables and drafted the first versions of all manuscripts. He was also responsible for revising manuscripts following circulation to co-authors.


Candidate Contribution
Planning: Primarily involved in the conception of the experimental ideas, development of the experimental designs (lower limb heating bath + arm water baths, separate thermostatically controlled systems) and the establishment of laboratory techniques, including setting up a new technique for measuring sweat rate.

Data Collection: The candidate was the primary individual involved in the collection, analysis and interpretation of the data.

Manuscript: The candidate prepared all figures and tables and drafted the first version of all manuscripts. In addition, he was also responsible for revising manuscripts following circulation to co-authors. Finally, he led the preparation of journal rebuttals and revisions.
Peer-reviewed conference proceedings

Oral presentations


**Young Investigator Award**

**Carter HH**, Spence AL, Cable NT, Thijssen DHJ, Naylor LH and Green DJ (2013). “Cutaneous microvascular adaptation to repeated passive core heating in humans” presented at Australian Conference of Science and Medicine in Sport, Phuket, Thailand.

Poster presentations

Chapter 1

Introduction

1.0 Background

Whilst it could rightly be stated that all research on the cardiovascular system owes an intellectual debt to William Harvey, the studies laid down in this thesis are in some ways very related to the experiments performed nearly 400 years ago and summarised in *Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus*. The remarkable nature of Harvey’s proofs, which can be said to have introduced what we recognise today as the *Scientific Method*, are not often fully appreciated. In favouring observation or “ocular demonstration” over received Galenic wisdom, Harvey provided an exemplar of Enlightenment thinking. Even when read in the contemporary context, with all the benefit of sophisticated technological approaches, molecular biology and various –omics, these elegant proofs provide a refreshing and enduringly relevant example of what can be achieved with human insight, logic, perseverance and tools as simple as a basic ligature. This thesis therefore begins with a brief account of the history of vascular physiology, using Harvey to introduce some of the theoretical and methodological approaches adopted in the experimental chapters that follow.

1.1 The cardiovascular system before Harvey

Nowadays it seems incomprehensible that any theory other than the circulation of the blood was ever conceived of. However, the evolution in our understanding of the cardiopulmonary systems has a long history.
For almost 1,500 years prior to Harvey, educational institutions universally preached the incontestable wisdom of Galen (129-216AD), who popularised the concept that blood was formed in the liver and moved slowly throughout veins, in both directions, to provide the major organs with energy when required (Wright, 2012). Arteries however, were perceived to be a separate system that transported blood around the body to provide vitality gained from the air through the lungs. There was no operational understanding of the role or presence of a pulmonary circulation and of course, no evidence of the existence of capillaries. Galen, a physician, performed his work in Rome during a time where dissection of the human body was prohibited due to the religious beliefs pertaining to the afterlife. As a result, Galen’s understanding of anatomy was derived from dissections undertaken in animals. Nonetheless, his work was accepted universally. Even da Vinci, renowned for his precise anatomical illustrations, rendered the heart bearing two chambers with a distinct hepatic venous system, largely in accordance with Galen’s theory (Figure 1.1).

![Image](image.jpg)

**Figure 1.1.** An illustration by Leonardo da Vinci showing the 2-chambered structure of the heart, in accordance with Galen’s theory.
1.2 An example of the scientific method

Some 1,500 years later, William Harvey challenged and subsequently overturned Galenic theories regarding the function of the heart and the circulation of the blood. Key to the development of his theory were experiments he performed to quantify the volume of blood expelled from the left ventricle. He believed the amount of blood that left the heart was too great for the liver to continuously produce and, if it were capable of producing such volume, why would the body not swell and burst? Following this supposition that blood may in fact circulate around the body, he performed experiments on his servants involving tying ligatures around the upper arm ‘as tightly as was bearable’ to completely restrict arterial flow into the arm. He noticed blood ‘built up’ in the arteries above the ligature and disappearance of the radial pulse. After loosening the ligature slightly, he furthermore noted ‘coloration and distension of the hand’ along with return of the distal pulse. However, he also noted that by only loosening the ligature slightly, to the point where flow through the artery begins, the veins remained distended. From these experiments, he concluded that blow flows through arteries to the extremities of the body, and then into the veins. He followed this with the observation that blood in the veins could not be forced back down, or ‘milked’, towards the hand. It was known at this point there were ‘little doors’, as Harvey’s mentor Fabricius termed them, in the veins. Harvey realised the true role of these valves, partly as a result of anatomical dissection performed in Padua during which he noted that the valve leaflets always point to the limb root. He rightly concluded that valves function to ensure unidirectional flow of the blood back to the heart (Figure 1.2). An exert from De Motu Cordis reveals his theory of the circulation of the blood:

“…notice that the valves in the veins…were so placed that they gave free passage of the blood towards the heart, but opposed the passage the other way: I was invited to imagine, that so provident a cause as nature had not plac’d so many valves without design…I perceived that the veins should be quite emptied, and the arteries be burst with too much intrusion of blood, unless the blood did pass through arteries to the veins, and so return into the right ventricle of the heart.”
Harvey realised the importance and necessity of individuals observing his demonstrations and ‘seeing for themselves’ to make their own conclusions independent of what they’ve been taught. For example, when publically demonstrating his theory that simply too much blood leaves the heart for the liver to produce, unsuspecting patrons in the laboratory often became covered in blood, as he would simply cut the aorta of an animal in demonstration.

So using a simple ligature, used by physicians for the practice of bloodletting and removal of superficial tumours, Harvey provided evidence for the circulation of the blood. The concept of using cuffs/tourniquets to modulate blood flow through limbs remains valid and can be seen in modern investigations of cardiovascular physiology. For example, venous occlusion plethysmography has been used to measure changes in limb blood volume since the early 1900’s. This technique involves the use of a cuff around a limb that is inflated to the point where arterial flow is not impeded, while venous flow congested, thus allowing for the measurement of the change in blood
volume as the limb swells. This technique was key to providing evidence for the role of nitric oxide on vascular function in the 1980’s.

The discovery of the circulation of the blood and the function of the heart has been described as the most momentous development in anatomy (Wright, 2012), a discovery made using the insight of one man and a simple tourniquet. The studies contained in this thesis have, similarly, utilised cuff placement on the limbs to investigate the effects of changes in blood flow on both macro- and micro-vascular function in humans.

1.3 Thesis aims and hypotheses
The chapters contained in this thesis have adopted the simple and elegant solutions of Harvey to investigate the mechanisms responsible for macro- and micro-vascular adaptations, in vivo. The detailed rationale for each of the series of studies presented in sections 1 and 2 of this thesis are contained in the respective literature reviews. Briefly, exercise training has been shown to decrease the risk for developing cardiovascular disease in humans. It is also known that exercise training induces both macro- and micro-vascular adaptations in humans. Recent papers have suggested the direct action of changes in blood flow, pressure and other haemodynamics during acute bouts of exercise may, in part, explain the cardiovascular benefit of exercise. However, exercise is a highly complex stimulus, involving reflex activation, neurohumoral secretion and elicitation of circulating factors from exercising muscles. The independent and direct role of haemodynamics is therefore somewhat difficult to ascertain. The theme of the chapters presented in this thesis was to examine the impact of chronic manipulation of arterial blood flow and shear stress, independent of an exercise stimulus per se. The thesis is divided into two sections. The first lays out the rationale for investigation of the effects of changes in blood flow and shear stress, independent of exercise, on conduit artery function and structure. Section 2 summarises cutaneous microvascular adaptations induced by repeated lower limb heating, in which the independent roles of blood flow and skin temperature on the adaptive responses are examined.

Specifically;

Chapter 3
Aim: The aim of this study was to investigate the acute effects of changes in conduit blood flow and shear stress, mediated by various exercise modalities, on arterial diameter change.
Hypothesis:
Acute changes in arterial shear stress, whether exercise-mediated or –independent, will result in attendant and dose-related changes in conduit artery diameter.

Chapter 4
Aim: The aim of this study was to assess whether repeated episodic increases in brachial artery blood flow and shear stress, induced by local bilateral forearm heating and independent of exercise, would modulate vascular function and structure.
Hypotheses:
- Local forearm heating will induce an acute increase in brachial artery blood flow and shear stress.
- Repeated episodic increases in flow and shear will result in functional and structural adaptations of the brachial artery.
- Attenuation of increases in flow via the partial inflation of a pneumatic cuff on one arm will abolish the vascular adaptations associated with repeated heating and increases in shear stress.

Chapter 5
Aim: The aim of this study was to assess whether repeated episodic increases in brachial artery blood flow and shear stress, induced by systemic heating and independent of exercise, would modulate vascular function and structure.
Hypotheses:
- Lower limb heating will induce an acute increase in brachial artery blood flow and shear stress.
- Repeated episodic increases in flow and shear will result in functional and structural adaptations of the brachial artery.
- Attenuation of increases in flow via the partial inflation of a pneumatic cuff on one arm will abolish the vascular adaptations associated with repeated heating and increases in shear stress.

Chapter 7
Aim: The aim of this study was to investigate whether repeated episodic increases in skin blood flow and skin temperature, induced by systemic heating and independent of exercise, would modulate cutaneous microvascular function.
Hypotheses:
- Lower limb heating will induce an acute increase in forearm skin blood flow and temperature.
- Repeated episodic increases in skin blood flow will induce local cutaneous microvascular adaptation.
- Attenuation of increases in flow via the partial inflation of a pneumatic cuff on one arm will abolish the microvascular adaptations associated with repeated heating and flow.

Chapter 8
Aim: The aim of this study was to investigate the independent impacts of blood flow and skin temperature on cutaneous microvascular adaptation following repeated episodic increases in forearm skin blood flow induced by systemic heating.
Hypotheses:
- Lower limb heating will induce an acute increase in forearm skin blood flow.
- Repeated episodic increases in skin blood flow, independent of changes in skin temperature, will induce similar cutaneous microvascular adaptations as those observed in Chapter 7.
- Attenuation of increases in flow via the partial inflation of a pneumatic cuff on one arm will abolish the microvascular adaptations associated with repeated heating and flow.
Chapter 2

Literature Review

Conduit artery adaptation to chronic changes in shear stress in humans

2.0 Atherosclerosis and vascular function

Atherosclerosis is a disease of the blood vessels involving the build-up of lipid-laden plaque in the intima or media of medium and large sized arteries. The pathogenesis of atherosclerosis, or atherogenesis, begins in early childhood (McGill, 1990) but is typically not clinically detected for several decades when plaque rupture, haemorrhage, thrombus formation and acute myocardial infarction or ischaemic stroke can manifest. A recent report from the World Health Organisation (WHO) suggested that cardiovascular diseases (CVD) are the leading cause of death globally, associated with 17.3 million deaths in 2008 (WHO, 2011). Of concern, current projections indicate that CVD will be the cause of 23.3 million deaths by 2030 (WHO, 2011). The physiological processes underlying atherogenesis are therefore of critical concern and have been extensively studied. It is now established that endothelial dysfunction is a crucial and early atherogenic event (Davignon & Ganz, 2004) (Figure 2.1).
Figure 2.1. Diagram showing the chronological steps involved in atherogenesis and highlighting the importance of endothelial dysfunction in this development (Pepine, 1998).

2.1 Importance of the endothelium

The endothelium is a dynamic single layer of cells that lines the entire circulatory system. Its strategic position at the interface between the circulating blood and vessel wall allows it to respond to and mediate regulatory mechanisms which maintain vascular health (Green et al., 2004). The endothelium achieves these functions, in part, via the release of paracrine hormones involved in protection against lipid infiltration, sub-intimal inflammation and oxidation, chemotaxis, platelet aggregation, adhesion and thrombus formation (Rubanyi, 1993). In addition, the endothelium is involved in the regulation of vasomotor tone, blood pressure and wall shear stress (Vallance et al., 1989).

2.1.1 Role of the endothelium in vasomotor regulation

An early study by Hilton et al. (Hilton, 1959) noted that, despite lower limb denervation in cats, stimulation of the gastrocnemius muscle resulted in femoral artery dilation, suggesting that local changes in flow may be responsible. Decades later, Gerova et al. (Gerová et al., 1983) reinforced this notion by observing that controlled increases in coronary blood flow in dogs, via donor blood from the femoral artery, also resulted in an increase in coronary diameter. The mechanism/s mediating these intrinsic changes in
vascular tone were unknown at the time, although myogenic and humoral mechanisms were proposed.

2.1.2 Discovery of an ‘endothelium derived relaxing factor’

The classic study of Furchgott and Zawadzki in 1980 (Furchgott & Zawadzki, 1980) reported relaxation in strips of rabbit aorta following infusion of acetylcholine (ACh) in the presence of an intact endothelium, whereas vasoconstriction occurred in endothelium-denuded preparations. The authors concluded that relaxation of the vascular samples was dependent upon the presence of endothelial cells that, once stimulated by the action of ACh on muscarinic receptors, released a substance they termed ‘endothelium-derived relaxing factor’ (EDRF). Once released, EDRF passively diffused from the endothelium to the tunica media where it induced relaxation of vascular smooth muscle cells.

One of the first studies to suggest the EDRF could be nitric oxide (NO) was undertaken by Palmer, Ferrige and Moncada in 1987 (Palmer et al., 1987). The authors compared the relaxation induced by glyceryl trinitrate (GTN), EDRF and NO, along with their bioactivity and stability in vitro. The relaxation induced by EDRF (induced via infusion of bradykinin) and authentic NO was nearly identical, along with their transit half-lives. The authors concluded “...the biological activity of EDRF is accounted for by NO when considered in terms of the classical criteria...”. Another study from a different lab in the same year provided direct chemical evidence highlighting the similarities between EDRF and NO (Ignarro et al., 1987). Finally, after some controversy about the true identity of EDRF, the Wellcome research laboratory comprehensively established that the chemical identity of EDRF is the free radical form of NO (Moncada et al., 1991; Feelisch et al., 1994).

2.2 Endothelial function and nitric oxide in humans

Vallance, Collier and Moncada established for the first time that NO was released basally in humans. Resting forearm blood flow in young healthy individuals was assessed using strain-gauge plethysmography prior to infusion of NO\textsuperscript{G}-monomethyl-L-arginine (L-NMMA), a stereo-specific competitive inhibitor of NO synthesis. Blood flow decreased by ~30% compared to the control arm which received a saline infusion. This study demonstrated that endothelium-derived NO is involved in maintaining systemic basal blood flows at rest and in response to pharmacological stimulation (with
ACh). Indeed, systemic infusion of L-NMMA in rabbits (Rees et al., 1989) and humans (Haynes et al., 1993) results in a rise in mean arterial blood pressure, indicating that basal NO release regulates vascular resistance and blood pressure in vivo.

The molecular mechanisms involved in NO-induced vasodilation are well characterised and beyond the scope of this review. Briefly however, NO binds to and activates the enzyme guanylyl cyclase. This stimulates production of cyclic guanosine 3’,5’ monophosphate (cGMP) from guanosine-5’-triphosphate, which in turn elicits vascular smooth muscle relaxation (Loscalzo & Welch, 1995). In addition to this role in vasomotor function, endothelium-derived NO regulates numerous physiological and anti-atherogenic processes including maintaining vessel wall integrity by inhibiting platelet and leukocyte adhesion (Kubes et al., 1991), inhibition of aggregation of platelets and neutrophils (Loscalzo & Welch, 1995) and oxidation of low-density-lipoprotein (LDL). Furthermore, NO modulates the proliferation (Garg & Hassid, 1989) and infiltration of numerous cells into sub-intimal plaque, including smooth muscle cells (Loscalzo & Welch, 1995).

2.2.1 What is the physiological stimulus for the production and bioavailability of NO

Two studies published in 1986 provided key evidence regarding the physiological stimulus to NO production from the endothelium. Pohl et al. manipulated femoral artery blood flow in dogs and reported that increases in luminal flow resulted in vasodilation in the presence, but not in the absence, of the endothelium (Pohl et al., 1986) (Figure 2.2). This study concluded that increases in blood flow, and associated rises in intimal shear stress, the frictional drag force exerted by blood as it flows across the arterial wall, represent the physiological stimulus for the release of NO in animals. In the same year, Rubanyi et al. measured changes in arterial contraction and relaxation induced by changes in both steady state flow rates and pulsatile flows (Rubanyi et al., 1986). Increases in both were found to result in arterial relaxation. Finally, using an exercise model to alter blood flow and shear in dogs, Berdeaux et al. reported that incremental exercise, resulting in increases in blood flow and shear, was associated with stepwise increases in coronary artery diameter (Berdeaux et al., 1994). However, consistent with findings of Pohl et al. (Pohl et al., 1986), removal of the endothelium converted this vasodilator response to vasoconstriction.
Evidence implicating a role for changes in blood flow and shear in the regulation of vascular tone in humans was reported in 1989 by Anderson et al. (Anderson & Mark, 1989) and Sinoway et al. (Sinoway et al., 1989). In the former study, brachial blood flow and diameter were measured using Doppler ultrasound before, during and after forearm cuff inflation to suprasystolic levels (200 mmHg) for 10 mins (Figure 2.3). During cuff inflation, involving low levels of flow, brachial diameter decreased significantly from baseline, whereas during the reactive hyperaemia phase (following cuff deflation), diameter significantly increased. The latter study, involving a combination of strain-gauge plethysmography and Doppler ultrasonography, measured brachial artery blood flow and diameter following upper arm cuff inflation (240 mmHg) for 1, 3 and 10 mins. The authors observed a 37% increase in brachial diameter following 1 min of cuff inflation, and smaller graded increases beyond this following 3 and 10 mins (Sinoway et al., 1989).
A figure showing different velocity profiles in the brachial artery and subsequent changes in diameter. Inflation of an occluding forearm cuff decreased brachial flow (circulatory arrest), resulting in brachial vasoconstriction. During reactive hyperaemia, following deflation of the cuff after 10 mins, brachial artery flow increased significantly, along with diameter. Modified from (Anderson & Mark, 1989).

This technique, utilising cuff-induced post-ischaemic flow and shear manipulation, termed flow-mediated dilation (FMD), was later popularised as a non-invasive method to assess endothelial function in health and disease as a surrogate marker of relative vascular health (Celermajer et al., 1992) (Figure 2.4). For example, it was suggested that individuals with impaired endothelial function would have reduced NO bioavailability, directly impairing their vasodilator response to reactive hyperaemia compared to healthy individuals. In a recent human experiment, radial FMD was assessed before and after endothelial denudation in patients undergoing coronary angioplasty or angiographic procedures (Dawson et al., 2010). FMD following catheterisation was significantly decreased following endothelial denudation, highlighting the importance of endothelial function and NO release to the health of arteries in humans.
Figure 2.4. A diagram showing the anatomy of a typical artery and the mechanism of flow-mediated dilation.

It is now well established that shear stress is a physiological stimulus to enhance endothelium-derived NO function in humans and elements of the biochemical cascade have been fully described (Gielen et al., 2010) (Figure 2.5).

Figure 2.5. Diagrammatical representation of the purported molecular processes initiated in response to increases in shear stress on the inner wall of the artery (Gielen et al., 2010).
2.3 Exercise as a shear stress stimulus

The studies described above suggest that endothelial function is a key modulator of atherogenesis and that luminal blood flow and intimal shear stress are important physiological stimuli to modulation of endothelial function and NO bioavailability. Exercise is a stimulus which modifies arterial flow and shear, as a consequence of changes in neural vasomotor control, production of vasodilator metabolites and alterations in central haemodynamics such as cardiac output and arterial pressure. The role of exercise as a stimulus to acute and chronic shear-mediated changes in arterial function and structure is briefly reviewed below.

2.3.1 Role of NO in exercise-induced hyperaemia in humans

An early study by Gilligan et al. reported that handgrip-induced forearm hyperaemia was reduced by approximately 7% during infusion of L-NMMA (Gilligan et al., 1994). The reduction in blood flow observed in this study could be considered modest, however a separate study involving rhythmic handgrip exercise reported a much greater effect of NO inhibition on exercise induced hyperaemia, with forearm blood flow ~20-30% lower during L-NMMA infusion (Dyke et al., 1995). These studies utilised strain-gauge plethysmography to measure forearm blood flow, a technique highly sensitive to movement artefact. As a result, blood flow assessments were measured in-between repetitions of handgrip exercise, not during exercise. Hickner et al., however, examined the contribution of NO in skeletal muscle blood flow during graded leg cycle exercise, using microdialysis probes in the vastus lateralis, and reported a reduction of blood flow following infusion of L-NMMA compared to the control site (Hickner et al., 1997). Finally, Schrage et al. measured brachial blood flow and diameter during exercise using ultrasonography (Schrage et al., 2004) and, consistent with the studies above, brachial blood flow decreased by 17% during handgrip exercise following infusion of L-NAME. However, although the authors concluded NO plays a significant and consistent role in exercise hyperaemia, they also reported that NO-independent mechanisms were present which compensate for the absence of NO. For example, dual blockade of NO and prostaglandins did not significantly alter the exercise hyperaemic response, a finding the authors concluded was due to mechanisms that can compensate in the absence of the primary vasodilator pathway of NO. The authors coined the term ‘redundancy’ to describe the overlap between mechanisms. Nonetheless, in normal functioning systems, endothelium-derived NO is primarily involved in exercise-induced arterial vasodilation in conduit, resistance and skeletal muscle vessels.
2.3.2 Acute effects of exercise on blood flow and shear rate patterns

The pattern and volume of blood flow through peripheral arteries can be modulated by downstream vascular resistance as well as cardiac output and blood pressure (Green et al., 2005). It is therefore possible that distinct exercise modalities are characterised by different blood flow and shear patterns, which may in turn have different impacts on endothelial and vascular function. Green et al. reported a biphasic response in mean brachial artery blood flow in response to incremental lower limb cycling, even though peak flows increased linearly in accordance with changes in systolic function (Green et al., 2002) (Figure 2.6). The biphasic nature of the response was explained by subsequent analysis of diastolic arterial flows, which were in fact retrograde in direction. That is, under some circumstances, blood can flow backwards towards the heart during each cardiac cycle. Because this diastolic effect is more marked in the brachial artery, in a relative sense, at lower exercise intensities, mean flow apparently decreases at such workloads despite linear increases in cardiac output. Interestingly, the first evidence for such a biphasic effect of leg exercise on systemic (i.e. upper limb) blood flows dates back to the 1950’s (Bishop et al., 1957).
Figure 2.6. Mean brachial artery flow during incremental exercise showing a biphasic response from baseline (A), a response explained by examination of the antegrade and retrograde components of blood flow (B). Significantly different from baseline values at $P<0.05$ (*) or $P<0.01$ (†) (Modified from Green et al., 2002).

A recent study by Thijssen et al. further examined the impact of various forms of lower limb exercise including cycling, walking and kicking on brachial artery flow and shear stress (Thijssen et al., 2009a). Consistent with Green et al. (Green et al., 2002), cycling was associated with large increases in retrograde shear during diastole, whilst walking elicited comparable shear patterns. Bilateral kicking, which induced the largest increase in mean blood flow, was not associated with retrograde flows. In a separate study, the impact of the distinct patterns of flow and shear associated with leg cycling was compared to handgrip exercise (Green et al., 2005). This study demonstrated that both forms of exercise induced forearm hyperaemia, however the pattern of blood flow and shear were different. Interestingly, L-NMMA infusion significantly reduced forearm
blood flow during cycling, but not handgrip exercise. This study demonstrated that the pattern of shear stress directly affects the release of NO in humans.

2.3.3 Acute effect of different shear rate patterns on vascular function
To examine the impact of different shear rates on vascular function, a study by Tinken et al., (Tinken et al., 2009) had subjects complete 30 mins of recumbent cycling, forearm heating and handgrip exercise on separate days, with bilateral brachial FMDs assessed immediately before and after each intervention. Throughout each exercise bout, one forearm was cuffed to modulate shear pattern. The antegrade component of shear was similar between all interventions in the uncuffed arm, and FMD responses were enhanced. There was no change in FMD responses in the contralateral cuffed arm following forearm heating or handgrip exercise, however FMD following cycling significantly decreased. The authors related this to the impact of cuffing and cycling on retrograde shear. Whilst cuff inflation had a minor effect on retrograde flow during forearm heating and handgrip exercise, retrograde flows during cycling were significantly increased in the cuffed limb. This study suggested that retrograde shear might negatively impact upon vascular function, whereas unopposed increases in antegrade shear appeared to acutely improve function (Figure 2.7).
Figure 2.7. Shear rates (SR) during, and FMD responses pre and post, forearm heating (A), handgrip (B) and leg cycling (C) exercise. *Significantly different between the non cuffed and cuffed arms at $P<0.05$ (Tinken et al., 2009).
Thijssen et al. followed up this study by examining the impact of changes in retrograde shear on vascular function at rest (Thijssen et al., 2009b). Subjects undertook 3 separate days of 30 mins of forearm cuff inflation at different pressures (25, 50 and 75 mmHg, randomised). The increases in cuff pressure elicited a dose-dependent increase in retrograde shear rate. Furthermore, the increases in retrograde shear were associated with a similar dose-dependent decrease in FMD responses. This study indicated that acute increases in retrograde shear can have deleterious affects on endothelial function. However, such large increases in retrograde shear under normal physiological conditions are unlikely. Furthermore, as discussed previously, Tinken et al. observed large increases in antegrade shear, even in the presence of increases in retrograde, resulted in improved acute vascular function following various forms of exercise, when shear was not externally manipulated. This then raises the question: Can repeated bouts of exercise improve vascular function?

### 2.4 Impact of exercise training on vascular function

#### 2.4.1 In clinical populations

It is well established that endothelial dysfunction is a principal early event in atherogenesis. If exercise improves endothelial function, this would have important clinical implications. Higashi et al. reported that hypertensive patients have reduced endothelium-dependent vasodilator responses in conduit and resistance vessels, compared to healthy age-matched controls (Higashi et al., 1999a; Higashi et al., 1999b). However, 12 weeks of daily brisk walking (30 mins) significantly improved and normalised forearm ACh-induced and reactive hyperaemic responses. Furthermore, the adaptations were not evident during infusion of L-NMMA, suggesting that improvements were the result of enhanced NO mediated endothelium-dependent function (Higashi et al., 1999a; Higashi et al., 1999b). Impaired conduit artery endothelium-dependent function has also been observed in obese adolescents and children compared to healthy age-matched controls (Watts et al., 2004a; Watts et al., 2004b). However, 8 weeks of combined aerobic and resistance exercise in the adolescents, and whole-body exercise in the children, significantly improved endothelium-dependent vasodilation. In type 2 diabetic subjects, a comprehensive study by Maiorana et al. (Maiorana et al., 2001a) involving 8 weeks of whole body aerobic and resistance exercise training improved brachial artery FMD responses and ACh-induced forearm blood flow post-intervention, indicating enhanced endothelium-dependent vasodilation in the resistance vessels. Consistent with the above studies, 4
months of cycle training improved brachial FMD responses in type 1 diabetic patients (Fuchsjager-Mayrl et al., 2002).

A comprehensive study by Hambrecht et al. provided insight into the mechanisms associated with exercise-induced improvements in endothelial function (Hambrecht et al., 2003). Coronary artery disease patients were recruited and exercise trained for 4 weeks prior to elective bypass surgery. The exercise was hospital-based and performed on rowing and cycling ergometers. The authors observed significantly enhanced ACh- and adenosine-induced vasodilation in the left internal mammary artery following exercise training. In addition, tissue samples collected during the surgical procedure revealed the exercise group had significantly elevated eNOS expression and eNOS phosphorylation compared to the control group. This study suggested that exercise training in humans, via increases in blood flow and shear stress, upregulates eNOS expression and subsequently production of NO, thereby improving vascular function. It should be emphasised, however, that causal evidence that shear stress was directly responsible for the arterial functional adaptation could not be derived from this experiment.

The studies and others which have been comprehensively reviewed elsewhere (Green et al., 2004; Thijssen et al., 2010), suggest that individuals with clinical pathologies exhibit endothelial dysfunction which is reversible by exercise training (8-12 weeks). However, there is less evidence that exercise training enhances endothelial function in healthy subjects with normal endothelial function a priori.

2.4.2 In healthy individuals

In a study by Green et al., 4 weeks of handgrip exercise in young healthy males had no effect on resting forearm blood flow or in response to stimulated release of endothelium-derived NO. The authors did however report that peak forearm resistance decreased, suggesting adaptation in resistance vessels (Green et al., 1994). A subsequent study by Kingwell et al. in healthy young individuals reported 4 weeks of cycle exercise resulted in greater forearm vasoconstriction following intra-arterial infusion of L-NMMA, suggesting the exercise training induced an increase in basal NO bioavailability (Kingwell et al., 1997b). In support of this, 10 weeks of combined aerobic and anaerobic exercise in healthy young subjects increased brachial FMD responses modestly, but significantly, from 2.2% to 3.9%. Sensitivity to GTN
administration remained unchanged, suggesting the observed adaptation was endothelium-dependent (Clarkson *et al.*, 1999). In a separate study by Pullen *et al.*, 4 weeks of cycle exercise training in habitually sedentary healthy males also improved brachial FMD responses (Pullin *et al.*, 2004). However, the literature relating to exercise effects on vascular function in healthy individuals is not unanimous.

Contradicting the above studies, Hannukainen *et al.*, (Hannukainen *et al.*, 2007) recruited 12 pairs of young healthy monozygotic twins on the basis that there were notable differences in physical fitness within each pair, confirmed by VO$_{2\text{max}}$ tests. The authors reported no differences in brachial FMD between the fit and sedentary individuals.

Similar discrepancies have been reported following exercise training in middle aged and elderly healthy subjects. A cross sectional study by DeSouza *et al.* found maximal brachial responses to ACh infusion in healthy sedentary middle aged/older subjects was $\sim$25% lower than sedentary healthy younger subjects, suggesting that aging is associated with a decrease in endothelial function (DeSouza *et al.*, 2000). However, the authors also reported there was no difference in ACh-induced forearm vasodilation between young and middle/older endurance trained subjects, indicating that the natural decline in endothelium-dependent function may be countered by regular endurance exercise. This is in keeping with another study by Black *et al.* (Black *et al.*, 2009), that reported higher brachial FMD responses in young recreationally active individuals compared to older sedentary subjects, however no difference was evident between the young and older fit group. DeSouza *et al.* also provided evidence that exercise training in 13 sedentary middle/older subjects for 3 months increased ACh-induced forearm blood flow responses to levels similar to those in the younger and elderly trained groups (DeSouza *et al.*, 2000). In a study by Wray *et al.*, brachial FMD also improved in elderly subjects following 6 weeks of single leg kicking exercise (Wray *et al.*, 2006).

In contrast to the above studies, 8 weeks of cycle exercise training in healthy elderly subjects did not induce any change in brachial or superficial femoral FMDs (Thijssen *et al.*, 2007). Moriguchi *et al.* also reported that brachial endothelium-dependent vasodilation remained unchanged in middle-aged healthy subjects following 12 weeks of exercise training (Moriguchi *et al.*, 2005). Another study involving 8 weeks of combined aerobic and resistance exercise in healthy, sedentary middle-aged men did not
report changes in forearm endothelium-dependent (ACh-induced) or SNP-independent (SNP-induced) vascular function (Maiorana et al., 2001b). Finally, a study by Black et al. reported gender specific vascular adaptations following exercise. Following 12 weeks of aerobic exercise in older sedentary subjects, brachial FMD responses remained unchanged in men, however a significant increase was evident in women (Black et al., 2009).

2.4.3 Summary
Numerous pathological conditions are characterised by endothelial dysfunction, yet a degree of vascular plasticity is demonstrated by the consistency of studies regarding the beneficial effects of exercise training on vascular function in these clinical groups. Somewhat in contrast, studies relating to the effects of exercise in subjects with healthy endothelium are disparate. It is possible this may be due to the fact that exercise training has little additive benefit for already healthy endothelium. But, as discussed previously, acute exercise in healthy individuals enhances endothelium-dependent vasodilation (Tinken et al., 2009). Therefore, the disparity in the literature may relate to a different time-course of change in vascular function.

2.5 A time-course for functional adaptations?
2.5.1 Studies in animals
Improvements in conduit artery endothelium-mediated vasodilation were reported following as little as 7 days of exercise training in miniature swine (McAllister & Laughlin, 1997). However, this adaptation was believed to be independent of improvements in NO and prostaglandins, as blockade of these molecules did not abolish the response. It should be noted that no change was evident in the femoral artery, an observation the authors rationalised might be due to the potential relative differences in flow between the femoral and brachial arteries. Seven days of exercise training in dogs has been shown to enhance vasodilation following ACh infusion and reactive hyperaemia in the left circumflex coronary artery, compared to a sedentary group (Wang et al., 1993). Furthermore, these adaptations were thought to be due to upregulation of endothelium-derived NO, as infusion of nitro-l-arginine, a NOS inhibitor, abolished the responses. In a study reinforcing and extending the above findings, Johnson et al. (Johnson et al., 2001) noted enhanced pulmonary artery ACh-induced vasodilation in swine following 7 days of exercise, compared to the sedentary group. No difference was observed in the vasomotor sensitivity to SNP, indicating the
enhanced response was endothelium-dependent. The authors also observed increased eNOS expression in the exercise group, compared to the sedentary group, explaining the higher bioavailability of NO.

Following 2-4 weeks of exercise training in rats, skeletal muscle arterioles in the gracilis muscle displayed enhanced responsiveness to ACh and L-arginine infusion, compared to the sedentary group. No differences were evident in response to SNP infusion between the groups, suggesting enhanced endothelium-mediated vasodilation (Sun et al., 1994). Similarly, Koller et al. reported 3 weeks of exercise training in rats significantly improved skeletal muscle arteriolar vasodilation via augmented release of NO and prostaglandins, compared to the sedentary group (Koller et al., 1995). Similar improvements in endothelium-dependent vasodilation have been reported in conduit arteries following 8 weeks of exercise in rabbits (Chen & Li, 1993) and 10-12 weeks in rats (Delp et al., 1993).

A comprehensive study examining the time-course of exercise-induced vascular adaptations following 1 day, 1 week, 2 weeks, 4 weeks and 10 weeks of training was conducted by Delp and Laughlin (Delp & Laughlin, 1997a). Abdominal aorta responses to ACh infusion improved following 4 and 10 weeks of exercise. Furthermore, the authors reported greater eNOS expression in the pulmonary arteries at these time points. Increased eNOS expression was also observed by Johnson et al. (Johnson et al., 2001) following 7 days of exercise. These key findings aided in elucidating the mechanism/s mediating the functional improvements, as they suggested that exercise training upregulates eNOS expression, resulting in higher bioavailability of NO and subsequently enhanced endothelium-dependent vasodilation.

However, 16 weeks of exercise training in rats, noticeably longer than the studies above, induced no change in the sensitivity to ACh, noreadrenaline or SNP infusion between the exercise and sedentary groups (Kingwell et al., 1997a). In a separate study, no differences in the response to vasodilator (including braykinin) or vasoconstrictor substances was apparent in segments of femoral, brachial, mesenteric, renal or hepatic arteries following 16-20 weeks of exercise training in miniature swine (McAllister et al., 1996). Consistent with these longer training studies, no change in endothelium-mediated vasodilation was evident in conduit arteries following 16 weeks of exercise in swine (Johnson & Laughlin, 2000).
2.5.2 Time-course of change in response to exercise training in humans

The first study to comprehensively and directly examine a potential time-course effect on vascular function in humans was performed by Tinken et al. (Tinken et al., 2008b). The authors recruited healthy young subjects to undertake 8 weeks of cycling and treadmill based exercise with measures of both brachial and popliteal artery FMD performed at 2 week intervals. Both the brachial and popliteal arteries displayed transient improvements in FMD at weeks 2 and 4, whilst the popliteal remained elevated at week 6 of exercise training. Both arteries returned to baseline levels by week 8. This study reported, for the first time in healthy humans, that functional improvement of the endothelium can be transient in nature and may be superseded by changes in vascular structure (see below). In a follow up study, the authors examined brachial responses during 8 weeks of handgrip exercise (Tinken et al., 2010). In this experiment, a pneumatic cuff was positioned and inflated around one forearm during each training bout to unilaterally attenuate the exercise-induced increases in blood flow and shear stress during exercise training. This allowed for a within-subjects experimental design to accurately assess the effect of shear on vascular adaptation. In the uncuffed limb, brachial FMD improved significantly following 2 weeks and remained elevated at 6 weeks, before returning to baseline values. No change was observed in the cuffed arm. Reinforcing the shear-mediated transient enhancement in vascular function during exercise, even in the inactive limbs, Birk et al., utilised a similar unilateral forearm cuff inflation model during leg cycle exercise bouts. They reported that brachial artery FMDs increased significantly following 2 weeks of training in the uncuffed arm, however returned to baseline values by week 8 (Birk et al., 2012) (Figure 2.8). No change was evident in the cuffed arm.
Figure 2.8. Brachial flow-mediated dilation (FMD%) responses in the non-cuffed (solid squares) and the cuffed (open squares) arms throughout 8 weeks of leg exercise training. Brachial FMD increases significantly after 2 weeks of training before returning to baseline values by week 8 (Birk et al., 2012).

2.5.3 Summary
The studies described above, in animals and humans, indicate that exercise transiently enhances endothelial function, a response which is likely to be shear-mediated. In an attempt to explain this time-course of vascular adaptation, Laughlin suggested that exercise training induces enhancement in endothelium-mediated vasodilation to compensate for exposure to higher levels of shear (Laughlin, 1995). These functional adaptations are transient and return to ‘baseline’ levels following arterial remodeling of the vasculature, a more permanent endothelium-dependent adaptation that increases luminal diameter and structurally normalises intra-arterial shear, such that function can homeostatically return to baseline levels (Figure 2.9). Further evidence regarding the impact of exercise training on arterial remodeling is considered below.
Figure 2.9. A diagram showing the arterial adaptation in response to repeated exercise-induced increases in shear stress. In the untrained artery, there is basal release of NO (A), however short term training induces greater eNOS and NO bioavailability, enhancing arterial function (B). As exercise training continues, NO bioavailability returns to baseline levels as the enhanced artery function is superseded by a more permanent structural change, i.e. remodelling (C).

2.6 Chronic effect of exercise on vascular structure

2.6.1 Studies in animals

Tepperman and Pearlman developed a technique of assessing gross structural change in the coronary tree of animals by infusing vinyl acetate into the aorta that, once polymerised, created a cast of the ventricular and coronary artery systems (Tepperman & Pearlman, 1961). The right and left coronary trees were then separated from the cast and weighed. Using this technique, the authors reported both 5 weeks of treadmill running and 11 weeks of swimming in rats significantly increased coronary cast weight, compared to the sedentary group. In the swimming exercise group, this increase was still apparent following 8 weeks of detraining. Haslam and Stull, using the same casting
approach, also reported increased coronary weight following 8 weeks of swimming exercise in rats (Haslam & Stull, 1974).

Whereas the studies above used gross measures of structural change, a study by Leon et al. reported that 10 weeks of daily swimming in rats increased the cross-sectional lumen size of the extracoronary collateral and coronary arteries, compared to sedentary rats (Leon & Bloor, 1968). Consistent with these findings, 12 weeks of treadmill exercise in dogs significantly increased the circumflex coronary arteries from 5.73 to 6.20 mm, as measured by coronary angiograms (Wyatt & Mitchell, 1978). Finally, 42 months of exercise training in monkeys resulted in significantly larger coronary arteries compared to the sedentary group (Kramsch et al., 1981).

There is conclusive evidence that exercise training results in structural remodelling of the vasculature in animals. Furthermore, numerous studies suggest that the response is shear mediated and endothelium-dependent. In an elegant study by Langille and O’Donnell (Langille & O’Donnell, 1986), the authors reported that blood flow through the left common carotid artery in rabbits reduced by up to 70% following ligation of the external carotid artery. Following 2 weeks, this decrease in flow resulted in a significant reduction in arterial size, compared to the contralateral common carotid artery (Figure 2.10). When the same experiment was undertaken following denudation of the endothelium, no change in structure was observed, strongly suggesting that the adaptive response was endothelium-dependent.
Figure 2.10. The graph on the left shows a reduction in the left common carotid artery diameter in rabbits following ligation that significantly reduced blood flow for 2 weeks. When the endothelium is removed (denuded), no change in diameter is evident. Modified from (Langille & O'Donnell, 1986).

In a comprehensive study by Tuttle et al., the authors ligated mesenteric arteries to allow for 3 different controlled blood flow conditions where some arteries were exposed to ~50%, 200% and 400% of ‘normal’ flow, while one artery acted as a control and did not have flow altered (Tuttle et al., 2001). In the 200% and 400% flow conditions, maximally induced diameters (via adenosine and SNP) were higher at day 7 compared to day 2. Concurrently, eNOS expression was greater at day 2 than day 7 (Figure 2.11). This study demonstrated that, in response to increases in blood flow and shear, vascular function increased transiently (using eNOS expression as a surrogate marker), and this preceded a structural remodelling of the arteries. Both adaptations were endothelium-dependent.
Figure 2.11. Maximal diameter change from baseline (%) in mesenteric arteries of different flow conditions (A) and eNOS content (B) at day 2 and day 7. Modified from (Tuttle et al., 2001).

2.6.2 Studies in humans

In humans, 8 weeks of treadmill running and cycling exercise in young healthy individuals resulted in an increase in the dilator capacity of the brachial and popliteal arteries by weeks 6 and 8 (Tinken et al., 2008b). These increases were accompanied by normalisation of FMD responses (Figure 2.12). This study clearly demonstrated a functional improvement in conduit arteries in response to exercise that was transient and superseded by enhanced maximal dilator responses, a surrogate marker of structural remodelling. This response was also observed in the brachial artery following 8 weeks of handgrip exercise. Consistent with the above study and the proposal by Laughlin in
1995 (Laughlin, 1995), the increased dilator capacity of the artery was observed following the return of endothelial function to baseline levels.

![Graph showing flow-mediated dilation and conduit artery dilator capacity over weeks]

**Figure 2.12.** Flow-mediated dilation (solid square) and conduit artery dilator capacity (open diamond) in the brachial (top panel) and popliteal (bottom panel) arteries across the intervention. *Significantly different from baseline at $P<0.05$. Modified from (Tinken *et al.*, 2008b).

Numerous cross-sectional studies have also reported structural adaptations in response to long term physical activity. A famous post-mortem case study in Clarence DeMar, knick-named “Mr Marathon” due to his life-long participation in long distance running, revealed his coronary arteries were approximately 2 to 3 times the size considered ‘normal’ (Currens & White, 1961). A further study in 125 top-level athletes observed increased coronary diameters in relation to left ventricular mass, suggesting exercise...
training is associated with structural augmentation of coronary vessels (Pellicia et al., 1990). These findings relating physical activity to increases in coronary structure are reinforced by other studies (Mann et al., 1972; Hildick-Smith et al., 2000).

Zeppili et al. also reported athletes had significantly greater conduit vessels compared to sedentary healthy individuals (Zeppilli et al., 1995). Consistent with this study, conduit arteries in highly active individuals were found to be significantly larger than those in healthy inactive controls (Schmidt-Trucksass et al., 2000; Huonker et al., 2003). In a more recent cross-sectional study (Rowley et al., 2012), brachial artery diameters were larger in upper limb dominant athletes (i.e. canoeists and wheelchair subjects) and superficial femoral arteries were larger in lower limb athletes (runners and cyclists) compared to healthy sedentary controls. Another study by Rowley et al. in racquet sport players reported resting brachial artery diameters were significantly larger in the player’s dominant arms, compared to their nondominant (Rowley et al., 2011). Studies have also reported structural differences in resistance arteries between physically active and sedentary individuals (Sinoway et al., 1986; Green et al., 1996).

In a longitudinal study by Rakobowchuk et al. (Rakobowchuk et al., 2005), involving 12 weeks of resistance exercise training in young healthy individuals, brachial FMDs remained unchanged following the exercise intervention. However, resting brachial artery diameter had increased from baseline by week 6 and remained larger by 12 weeks. In a more recent study, six months of resistance-only training increased resting brachial diameter, whilst endurance-only training increased resting femoral artery diameter post-intervention (Spence et al., 2013), indicating arterial remodelling occurred in the vascular regions targeted by the distinct training interventions.

2.6.3 Summary
Studies in both animals and humans support the theory that exercise training, via repeated episodic increases in blood flow and shear stress, mediates a transient improvement in endothelial and vascular function. This enhancement is characterised by increased expression of eNOS and NO bioavailability, to buffer episodic increases in shear stress associated with the exercise bouts. These functional increases resolve and are superseded by structural remodelling of the arteries (Tinken et al., 2008b). The majority of the literature suggests that both functional and structural adaptations are
dependent upon the endothelium and are mediated by the effects of increases in flow and shear.

2.7 **Relationship to current thesis**

The studies reviewed in this section of the literature review strongly implicate shear stress as a mediator of the impact of exercise on arterial adaptation. However, exercise is a complex stimulus, involving neuro-humonal and reflex changes in the control of the cardiovascular system. If exercise-mediated haemodynamic changes, which elicit direct effects on the artery wall via shear stress and other mechanical forces, are important mediators of change in arterial function and structure, then increases in shear which occur independent of exercise should theoretically induce similar changes to those observed in response to exercise training. This possibility has not previously been studied in humans and it forms the theoretical framework for the experiments contained in this thesis.

To this end, we designed the following studies.

1) Although studies have previously reported acute changes in shear stress alters endothelial function, no study has directly examined the impact of changes in shear stress on arterial diameter. Therefore, we employed a number of modalities to alter shear stress and recorded subsequent changes in diameter.

2) Previous studies have shown exercise transiently improves FMD, a response that is typically superseded by structural remodelling of the artery. The stimulus believed to be responsible for these adaptations is shear stress. To test whether shear stress, independent of exercise, can induce arterial adaptations, we recruited subjects to undertake 8 weeks of localised bilateral forearm heating (42°C). To comprehensively examine the role of shear, one forearm was cuffed (100 mmHg) throughout each heating bout to attenuate increases in blood flow and shear.

3) Finally, local forearm heating did not increase core body temperature and therefore did not elicit central thermoregulatory reflexes. To assess whether whole body heating, independent of exercise, could systemically increase conduit artery blood flows and whether repeated bouts could enhance arterial function, subjects underwent 8 weeks of lower limb heating (40°C). The role of shear was once again examined by cuffing one forearm (80 mmHg) to attenuate increases in brachial blood flow and shear.
Chapter 3

Study 1

Effect of shear rate manipulation on conduit artery dilation in humans


3.0 Abstract

The impact of manipulating shear stress on conduit artery vasodilation has not been comprehensively described in vivo. We hypothesised that manipulation of shear rate through the brachial and radial arteries would be associated with corresponding changes in diameter. We performed a series of studies involving 1) leg cycle exercise at increasing intensities (~70 and 85%HRmax) with simultaneous bilateral measurement of shear rate in the radial arteries, 2) leg cycle exercise for 30 mins at 80%HRmax with simultaneous bilateral measurement of velocity and diameter in the brachial arteries and 3) bilateral forearm heating for 30 mins with simultaneous bilateral measurement of brachial artery diameter and blood velocity. Cycling and forearm heating interventions were performed in the presence of unilateral cuff inflation throughout the experiment, or starting during the intervention (15 mins), to manipulate shear rate responses. Cuff placement was associated with lower radial artery shear rate responses (cuffed vs uncuffed: 248±49 vs 349±105 l/s 85%HRmax, P<0.01) and diameter responses were similarly attenuated (2.45±0.30 vs 2.78±0.20mm 85%HRmax, P<0.05). Exercise performed at 80%HRmax in the presence of unilateral cuff inflation also reduced
brachial artery shear rate (cuffed vs uncuffed; 258±107 vs 454±157 1/s, P<0.01) and
diameter (3.96±0.39 vs 4.20±0.45mm). Finally, cuff inflation decreased the impact of
forearm heating on brachial shear rate (cuffed vs uncuffed; 262±97 vs 440±106 1/s,
P<0.01) and diameter (4.35±0.54 vs 4.87±0.47mm P<0.05). Similar significant
differences between the cuffed and uncuffed limbs in shear rate and diameter were
observed when cuff inflation occurred during exercise or heating. Our findings strongly
implicate shear as an important stimulus to increase conduit artery diameter in humans.

3.1 Introduction

Habitual exercise decreases primary and secondary cardiovascular events (Taylor et al.,
2004; Blair & Morris, 2009), effects which cannot be entirely accounted for by
modification of traditional risk factors (Maiorana et al., 2003; Mora et al., 2007).
Animal studies implicate shear stress as a key stimulus to the release of paracrine
hormones, including nitric oxide (NO), from the endothelium during exercise bouts
(Pohl et al., 1986; Berdeaux et al., 1994; Tuttle et al., 2001). Episodic exposure to
elevations in shear stress may explain the cardioprotective effects of exercise training
through direct effect on the vascular wall (Green et al., 2008a; Joyner & Green, 2009).

Previous studies in humans have assessed the relationship between shear rate and
conduit artery diameter by increasing shear rate using heating (Newcomer & Padilla,
2011) or exercise (Pyke et al., 2008; Padilla et al., 2011) or responses to different
periods of arterial occlusion (Pyke et al., 2004; Pyke & Tschakovsky, 2007). These
studies are complimented by recent data from Padilla and colleagues (Padilla et al.,
2011), who demonstrated that matched shear rate changes associated with leg exercise
and forearm heating increase brachial artery dilation to a similar extent. Although this
evidence suggests that shear stress may play a role in conduit artery dilation in humans,
an approach which attenuates shear as a stimulus, without affecting other potential
vasodilator stimuli, is necessary to establish that shear stress is a key stimulus which
induces conduit artery diameter change in humans.

In the present study we adopted three general strategies to comprehensively examine the
hypothesis that shear stress manipulation would alter conduit artery dilation. Leg
exercise was performed at distinct intensities in the same subjects to assess dose-
response relationships, with shear and diameter measured during the exercise bouts
(study 1). We assessed the impact of increased shear and shear-attenuation by utilising
partial cuff inflation on one forearm during simultaneous bilateral measures in the radial arteries. In a second study, we assessed the impact of cycle ergometer exercise (at 80%HRmax) on brachial artery shear and dilation utilising similar techniques (study 2). Finally, to address the impact of shear manipulation in the absence of exercise, we heated both forearms simultaneously by placement in water baths (42°C) and measured consequent changes in brachial shear and diameter, with a cuff inflated to manipulate shear through one arm (study 3). We also repeated the latter studies using cuff inflation during, as against to throughout, the cycle and heating interventions. In all experiments, we utilised within-subjects, simultaneous measures to dissect the effects of shear rate manipulation on conduit artery diameter in the absence of central or reflex haemodynamic effects.

3.2 Methods

Ethical Approval

All study procedures were approved by the Human Research Ethics Committees at the University of Western Australia and Liverpool John Moores University. Written, informed consent was obtained from all subjects and studies conformed to the declaration of Helsinki.

Subject Characteristics

Forty-four young healthy male subjects (25 ± 3 years) were recruited to participate in 1 of 5 experiments (Table 3.1). Subjects were recreationally active (≤2 hrs of physical activity per week) and all were free from cardiovascular disease, diabetes, insulin resistance and cardiovascular risk factors. Subjects who smoked or were on medication of any type were excluded.
Table 3.1. Subject characteristics for the 5 experimental protocols.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cycle Intensities</th>
<th>Cycle</th>
<th>Cycle±cuff</th>
<th>Forearm Heating</th>
<th>Forearm Heating</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>±cuff (n=8)</td>
<td>±cuff (n=10)</td>
<td>(mid-point) (n=9)</td>
<td>±cuff (n=8)</td>
<td>±cuff (mid-point) (n=9)</td>
</tr>
<tr>
<td>Age</td>
<td>22 ± 1</td>
<td>24 ± 2</td>
<td>28 ± 4</td>
<td>23 ± 3</td>
<td>24 ± 2</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>134 ± 17#</td>
<td>115 ± 27</td>
<td>106 ± 28</td>
<td>119 ± 7</td>
<td>125 ± 8</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>64 ± 6</td>
<td>71 ± 11</td>
<td>66 ± 12</td>
<td>65 ± 8</td>
<td>62 ± 11</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>74 ± 7</td>
<td>85 ± 24</td>
<td>94 ± 26</td>
<td>61 ± 6</td>
<td>61 ± 5</td>
</tr>
</tbody>
</table>

SBP: Systolic blood pressure. DBP: Diastolic blood pressure. HR: Heart rate. #Systolic BP under this condition was recorded at the ankle. NS: Not significant. Values are mean ± SD.
Study Designs

*Cycle exercise, cuffed and uncuffed forearms: Dose-response and role of shear rate in radial artery dilation*

Eight subjects performed two separate bouts of exercise on a cycle ergometer (Monark 874E, Sweden) at ~70 and 85% maximal heart rate (HRmax). Attendances were separated by >48hrs, with all bouts completed within 14 days of the initial test and each session performed at the same time of day. The order of tests (70% and 85%) was randomised between subjects. Radial artery diameter and velocity were collected simultaneously in both forearms at rest, and between 10-15 mins during exercise. Immediately prior to exercise a pneumatic cuff was placed around one forearm, distal to the probe location, and inflated to 60 mmHg while the contralateral arm remained uncuffed during cycle exercise.

*Cycle exercise, forearm cuff inflation throughout exercise: role of shear rate in the brachial artery*

Ten subjects, distinct from those studied above, were recruited to undertake 30 mins of cycle exercise (Monark 874E, Sweden) at 80% HRmax. Throughout the exercise bout a pneumatic cuff was placed around one forearm immediately below the cubital crease and inflated to 60 mmHg. The contralateral arm remained uncuffed during cycle exercise. Simultaneous brachial artery diameter and velocity values were collected immediately prior to exercise and before cuff inflation, and at the 15 and 25 min marks during exercise. Previous studies have demonstrated that placement and inflation of a forearm cuff attenuates upstream brachial artery shear rate (Thijssen et al., 2009b; Tinken et al., 2009; Tinken et al., 2010).

*Cycle exercise, cuff inflation mid-exercise: role of shear rate in the brachial artery*

Nine subjects (distinct from those studied above) undertook 30 mins of cycle exercise (Monark 874E, Sweden) at 80% HRmax. A pneumatic cuff was placed around one forearm and inflated to 60 mmHg, 15 mins after the onset of cycle exercise. Simultaneous bilateral brachial artery diameter and velocity values were collected immediately before the onset of exercise and during exercise, at 10 mins (and prior to cuff inflation), and following cuff inflation (ie between 20–25 mins). This study was performed to examine whether brachial artery dilation could be reversed by locally changing shear rate levels *during* exercise.
Bilateral forearm heating, forearm cuff inflation throughout heating: role of (non-exercise) shear rate

A further eight subjects underwent 30 mins of bilateral forearm warm water immersion (42°C). A pneumatic cuff was again positioned and inflated around one forearm whilst the contralateral arm remained uncuffed. The pneumatic cuff was inflated to 100 mmHg based on pilot studies and our previous published data indicating that this level of inflation affects mean shear rate during heating (Naylor et al., 2011). Simultaneous bilateral recordings of brachial artery diameter and velocity were again recorded immediately prior to heat application, and at the 15 and 25 min marks during the intervention.

Bilateral forearm heating, forearm cuff inflation mid-heating: role of (non-exercise) shear rate

A further 9 subjects undertook a similar protocol to that described above, involving 30 mins of bilateral forearm heating. A pneumatic cuff was placed around one forearm and inflated to 100 mmHg following 15 mins of forearm immersion (42°C). Simultaneous bilateral brachial artery diameter and velocity values were collected before the onset of heating and during heating, at 10 mins (and prior to cuff inflation), and following cuff inflation (i.e. between 20–25 mins). This study was performed to examine whether brachial artery dilation could be reversed by locally changing shear rate levels during forearm heating.

Experimental Procedures
All experiments were performed in a quiet, temperature controlled laboratory. Subjects were fasted for a period of 6 hrs and abstained from alcohol consumption and exercise for a minimum of 18 hrs prior to the commencement of each intervention.

Assessment of radial and brachial artery diameter and velocity
Following 20 mins of rest, radial or brachial artery diameter and velocity were simultaneously assessed using a 10-MHz multi-frequency linear array probe attached to a high-resolution ultrasound machine (T3000; Terason, Burlington, MA). Recordings commenced following optimisation of the longitudinal B-mode image of the lumen-arterial walls. Concurrently, Doppler velocity assessments were collected using the lowest possible insonation angle (always <60°). The arms were positioned at heart level, whilst position of the arms was identical bilaterally and was not different across the
various tests. Also, the same posture was used to assess diameters before and during exercise and heating.

Diameter and shear rate analysis
Analysis of artery diameter, blood flow and shear rate was performed using custom-designed edge detection and wall-tracking software, which is independent of investigator bias and has previously been described (Woodman et al., 2001; Black et al., 2008a). From synchronized diameter and velocity data, blood flow (lumen cross-sectional area x Doppler velocity) was calculated at 30Hz. Shear rate (an estimate of shear stress without viscosity) was calculated as 4 x mean blood velocity/vessel diameter. It is well established that shear rate, rather than blood flow per se, is the physiological stimulus that modulates endothelial function and induces changes in artery remodeling (Langille & O'Donnell, 1986; Tuttle et al., 2001; Pyke & Tschakovsky, 2005). Reproducibility of diameter measurements using this semi-automated software is significantly better than manual methods, as we have previously indicated (Woodman et al., 2001). It also reduces observer error and bias, and possesses an intra-observer coefficient of variation of 6.7% (Woodman et al., 2001).

Statistics
Statistical analysis was performed using SPSS 18.0 (SPSS, Chicago, Illinois) software. All data are reported as mean ± SD unless stated otherwise, statistical significance was assumed at \( P<0.05 \). Two-factor ANOVAs with repeated measures (with time and intensity or cuff placement as the independent factors) were performed. Post-hoc analysis \( t \)-tests were used where significant values were found.

3.3 Results
Subjects recruited to each of the experimental protocols were similar in terms of age and haemodynamics (Table 3.1).

Cycle exercise, cuffed and uncuffed forearms: Dose-response and role of shear rate in radial artery dilation
Shear rate responses. Cycle exercise significantly increased mean radial artery shear rate in both arms (2-way ANOVA main effect time \( P<0.01 \)), although the increase in shear was significantly attenuated in the cuffed arm (Figure 3.1A; 2-way ANOVA
time*cuff interaction $P=0.003$). Retrograde shear rate increased significantly from baseline to cycling exercise; post-hoc analysis revealed that exercise increased retrograde shear rate in the cuffed arm, while no changes were observed in the uncuffed arm (Table 3.2).

**Radial artery diameter responses.** A significant interaction effect revealed the presence of a different time-dependent response between the limbs ($P<0.01$, Figure 3.1B). Whilst significant increases in radial artery diameter were observed in the uncuffed arm during exercise, changes in radial artery diameter in the cuffed arm did not reach statistical significance (Figure 3.1B).

**Table 3.2.** Mean, anterograde and retrograde shear rate (1/s) data during cycling exercise at different intensities.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cycling exercise: different intensities</th>
<th>$P$ value 1-way</th>
<th>2-way Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>70%</td>
<td>85%</td>
</tr>
<tr>
<td>Mean</td>
<td>Cuffed</td>
<td>122±76</td>
<td>209±82</td>
</tr>
<tr>
<td></td>
<td>Uncuffed</td>
<td>121±66</td>
<td>295±91</td>
</tr>
<tr>
<td>Antegrade</td>
<td>Cuffed</td>
<td>130±70</td>
<td>253±62</td>
</tr>
<tr>
<td></td>
<td>Uncuffed</td>
<td>130±59</td>
<td>311±79</td>
</tr>
<tr>
<td>Retrograde</td>
<td>Cuffed</td>
<td>-8±9</td>
<td>-45±22</td>
</tr>
<tr>
<td></td>
<td>Uncuffed</td>
<td>-8±9</td>
<td>-16±19</td>
</tr>
</tbody>
</table>

NS: not significant. Data are mean ± SD.
Figure 3.1. Radial mean shear rate (A) and diameter (B) at rest and in response to exercise undertaken at 70% and 85%HRmax in the cuffed (open bars) and uncuffed (solid bars) forearms. #Significantly different at P<0.05. Differences existed in the impact of cycle exercise intensity between the cuffed and uncuffed arms in terms of both shear rate (P<0.005) and diameter (P<0.01). Data are mean ± SE.
Cycle exercise, forearm cuff inflation throughout exercise: role of shear rate in the brachial artery

Shear rate responses. Shear rate data for one subject were removed from the analysis due to inadequate edge detection of the velocity envelope. A 2-way ANOVA revealed a significant impact of cuff placement on mean shear rate responses across time ($P<0.01$) and t-tests revealed significant differences between the cuffed and uncuffed arms at 15 ($P<0.05$) and 25 mins ($P<0.01$) (Figure 3.2A). A repeated measures ANOVA revealed a significant difference in retrograde shear rate across the three time points in the cuffed arm ($P<0.05$) with t-tests revealing a significant increase relative to baseline at 15 mins ($P<0.01$) and 25 mins ($P>0.01$), while no change was evident in the uncuffed arm (Table 3.3).

Brachial artery diameter responses. A significant interaction effect revealed the presence of a different time-dependent diameter response between the limbs ($P<0.05$, Figure 3.2B). In the uncuffed arm, diameter increased significantly compared to baseline at 25 mins ($P<0.01$), whereas increases in the cuffed arm were not significant at any time-point. Direct comparison between the limbs revealed a significant difference between the limbs at 25 mins ($P<0.05$).
Figure 3.2. Absolute brachial mean shear rate (A) and diameter (B) at rest and in response to exercise undertaken at 80%HRmax in the cuffed (open bars) and uncuffed (solid bars) forearms. The cuff was inflated throughout the exercise period. A significant impact of cuff placement was evident on mean shear rate responses across time ($P<0.01$) along with brachial artery diameter between limbs ($P<0.05$). *Significantly different at $P<0.05$. †Significantly different at $P<0.01$. Data are mean ± SE. Note: shear rate data for one subject were removed from the analysis due to inadequate edge detection of the velocity envelope (n=9).
Cycle exercise, cuff inflation mid-exercise: role of shear rate in the brachial artery

Shear rate responses. A 2-way ANOVA revealed a significant impact of cuff placement on mean shear rate responses across time ($P<0.01$) and $t$-tests revealed significant differences between the cuffed and uncuffed arms at 25 mins ($P<0.05$) (Figure 3.3A). Post-hoc $t$-tests revealed no difference in mean shear rate between the arms at rest or at 10 mins, when both arms were uncuffed. However, after cuff inflation, shear rate in the cuffed arm decreased and was significantly lower compared to the uncuffed arm at 20-25 mins ($P<0.05$) (Figure 3.3A and Table 3.4).

Brachial artery diameter responses. The changes in mean shear rate as a result of cuff placement induced significant changes in brachial artery diameter that followed a similar pattern. A 2-way ANOVA demonstrated a significant impact of (mid-point) cuff inflation on the diameter response across time ($P<0.01$). The changes in diameter were similar between both arms during the first 15 mins prior to cuff inflation ($P<0.05$). However, unilateral cuff inflation resulted in a decrease in brachial artery diameter towards resting baseline levels, whereas diameter continued to increase in the uncuffed limb (Figure 3.3B).
Figure 3.3. Absolute brachial mean shear rate (A) and diameter (B) at rest and in response to exercise undertaken at 80%HRmax in the cuffed (open bars, cuff inflation at 15 mins cycling) and uncuffed (solid bars) forearm. A significant impact of cuff placement was evident on mean shear rate responses across time ($P<0.05$) along with brachial artery diameter ($P<0.01$). *Significantly different at $P<0.05$. Data are mean ± SE.
**Bilateral forearm heating, forearm cuff inflation throughout heating: role of (non-exercise) shear rate**

*Shear rate responses.* A 2-way ANOVA revealed a significant impact of cuff placement on shear rate responses across time ($P<0.01$) and $t$-tests revealed differences between the arms at all time-points (both $P<0.01$) (Figure 3.4A). Retrograde shear rate decreased significantly in the uncuffed arm across the 30 mins ($P<0.01$) however there was no change in the cuffed arm (Table 3.3).

*Brachial artery diameter responses.* The distinct mean shear rate response as a result of cuff placement induced significant differences in brachial artery diameter between the limbs (2-way ANOVA, $P<0.05$ main effect for cuff placement, Figure 3.4B). Direct comparison between the limbs revealed differences at 15 and 25 mins (both $P<0.05$), but not at baseline.
Figure 3.4. Absolute brachial mean shear rate (A) and diameter (B) at rest and in response to bilateral forearm heating at 42°C in the cuffed (open bars) and uncuffed (solid bars) forearm. A significant impact of cuff placement was evident on mean shear rate responses across time ($P<0.01$) along with a significant difference in brachial artery diameter between the limbs ($P<0.05$). *Significantly different at $P<0.05$. †Significantly different at $P<0.01$. C, cuffed; UC, uncuffed. Data are mean ± SE.
Bilateral forearm heating, forearm cuff inflation mid-heating: role of (non-exercise) shear rate

Shear rate responses. A 2-way ANOVA revealed a significant impact of cuff placement on mean shear rate responses across time ($P<0.01$, Figure 3.5A). Post hoc $t$-tests revealed no differences in mean shear rate between the arms at rest or at 10 mins; i.e. when both arms were uncuffed. After cuff inflation, shear rate in the cuffed arm decreased significantly compared to 10 mins ($P<0.01$) and there was a significant difference between arms at 20-25 mins ($P<0.01$) (Figure 3.5A and Table 3.4).

Brachial artery diameter responses. The changes in mean shear rate as a result of cuff placement were accompanied by significant changes in brachial artery diameter that followed a similar pattern. A 2-way ANOVA demonstrated a significant impact of (mid-point) cuff inflation on the diameter change across time ($P<0.05$). The changes in diameter were similar between both arms during the first 15 mins, with significant increases in diameter evident in both arms ($P<0.01$). However, unilateral cuff inflation resulted in a decrease in brachial artery diameter compared to 10 mins ($P<0.05$) in the cuffed arm. A significant difference was therefore evident between the arms at 20-25 mins ($P<0.01$) (Figure 3.5B).
Figure 3.5. Absolute brachial mean shear rate (A) and diameter (B) at rest and in response to bilateral forearm heating at 42°C in the cuffed (open bars, cuff inflation at 15 mins heating) and uncuffed (solid bars) forearm. A significant impact of cuff placement was evident on mean shear rate responses across time ($P<0.01$) along with brachial artery diameter ($P<0.05$). †Significantly different at $P<0.01$. Data are mean ± SE.
Table 3.3. Mean, anterograde and retrograde shear rate data (1/s) during the cycling exercise and forearm heating protocols, cuff inflated throughout.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cycling Exercise 80% HR&lt;sub&gt;max&lt;/sub&gt;</th>
<th>Forearm Heating</th>
</tr>
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<tr>
<td></td>
<td>Base 15 mins 25 mins P value 2-way Interaction</td>
<td>Base 15 mins 25 mins P value 2-way Interaction</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuffed</td>
<td>136±94 254±118 258±107 P&lt;0.05 P&lt;0.01</td>
<td>149±87 230±90 262±97 P=0.06 P&lt;0.01</td>
</tr>
<tr>
<td>Uncuffed</td>
<td>141±50 380±169 454±157 P&lt;0.01</td>
<td>151±89 392±111 440±106 P&lt;0.01</td>
</tr>
<tr>
<td>Antegrade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuffed</td>
<td>146±87 305±121 304±114 P&lt;0.01 P&lt;0.01</td>
<td>153±85 231±91 264±96 P=0.06 P&lt;0.05</td>
</tr>
<tr>
<td>Uncuffed</td>
<td>153±48 390±163 462±148 P&lt;0.01</td>
<td>156±86 392±111 440±106 P&lt;0.01</td>
</tr>
<tr>
<td>Retrograde</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuffed</td>
<td>-11±11 -52±35 -46±21 P&lt;0.05 P&lt;0.01</td>
<td>-4±3 -2±3 -2±3 NS NS</td>
</tr>
<tr>
<td>Uncuffed</td>
<td>-12±17 -10±13 -8±16 NS</td>
<td>-5±6 0±0 0±0 P&lt;0.01</td>
</tr>
</tbody>
</table>

NS: Not significant. Data are mean ± SD.
Table 3.4. Mean, anterograde and retrograde shear rate data (1/s) during the cycling exercise and forearm heating protocols, cuff inflated at mid-point.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cycling Exercise 80% HR$_{max}$ (cuff inflation 15 mins)</th>
<th>Forearm Heating (cuff inflation 15 mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Base</td>
<td>10 mins</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuffed</td>
<td>93±84</td>
<td>220±135</td>
</tr>
<tr>
<td>Uncuffed</td>
<td>110±78</td>
<td>215±88</td>
</tr>
<tr>
<td>Antegrade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuffed</td>
<td>101±80</td>
<td>264±110</td>
</tr>
<tr>
<td>Uncuffed</td>
<td>123±70</td>
<td>253±82</td>
</tr>
<tr>
<td>Retrograde</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuffed</td>
<td>-8±8</td>
<td>-44±34</td>
</tr>
<tr>
<td>Uncuffed</td>
<td>-13±15</td>
<td>-38±38</td>
</tr>
</tbody>
</table>

NS: Not significant. Data are mean ± SD
3.4 Discussion

The aim of the present study was to assess the effects of shear rate manipulation on conduit artery diameter in vivo. Our experimental approaches involved within-subjects experimental designs, which minimised important sources of error. We also collected simultaneous bilateral measurements, using identical equipment and ultrasound settings, to eliminate the impact of time-related measurement variation. Incremental cycle exercise intensity, unilateral placement of a pneumatic cuff during cycling exercise and bilateral forearm heating were successful approaches in manipulating shear rate in both the radial and brachial arteries. In addition to the application of a cuff at the onset of exercise or heating, we also manipulated shear during these interventions to assess impacts of diameter change. Our principal finding is that shear rate modulation using each of these approaches induced corresponding changes in conduit artery diameter. Changes in diameter in response to these interventions were dose-dependent and directionally consistent. Interestingly, we observed upper limb diameter changes in response to leg exercise, indicative of regional effects of acute exercise on the vasculature. Our findings relating shear and conduit artery dilation in humans are reinforced by the fact that the manipulations we adopted resulting from localised and systemic, exercise-related and -independent, interventions.

Our findings are broadly consistent with recent studies performed by Padilla and colleagues (Padilla et al., 2011; Simmons et al., 2011a). These studies indicate that increases in shear stress, associated with cycle exercise (Simmons et al., 2011a) and forearm heating (Padilla et al., 2011), were accompanied by increases in brachial artery diameter and that changes in diameter were of a similar magnitude when shear rates were matched between the interventions (Padilla et al., 2011). It was concluded that shear stress is a signal for brachial artery vasodilation in response to heating and exercise. Our data extend these findings in that we have directly manipulated shear stress using cuff inflation and measured the consequent changes in brachial artery diameter. Few studies have assessed the direct effect of shear stress on diameter changes in conduit arteries during exercise in humans and none have done so under conditions in which shear rate was actively attenuated during exercise. Although Tanaka et al. used ultrasound to study the effects of leg exercise on upper limb responses, brachial artery diameter measures were not reported (Tanaka et al., 2006). Interestingly, Pyke and colleagues demonstrated no impact of brachial artery compression, using a piston device, on upstream brachial artery diameter (Pyke et al.,
2004), whereas forearm heating was associated with increased shear and brachial diameter after piston release (Pyke et al., 2008). Our data essentially agree with this finding and indicate that shear stress is a key stimulus involved in the vasodilator response to exercise in humans, including the systemic effects of leg exercise in arteries feeding inactive vessel beds (Green et al., 2008b).

Our findings raise interesting questions pertaining to dose-response relationships between shear and artery diameter. Previous studies strongly suggest that increases in shear rate are associated with larger diameter changes. Indeed, a larger shear rate stimulus, induced by cuff release after arterial occlusion, is associated with larger increases in diameter after cuff release (Pyke & Tschakovsky, 2007), whereas different combinations of stimuli to induce vasodilation (i.e. ischaemia and superimposed exercise) also induced matched changes in shear and diameter (Naylor et al., 2005). Importantly, the latter study also found that imposing a larger stimulus for ischaemia (i.e. longer ischaemia or adding handgrip exercise) did not result in further increases in shear stress, or diameter (Naylor et al., 2005). Although these studies emphasize the strong relationship between shear and diameter changes, they also suggest the presence of a ‘ceiling’ effect for shear to induce dilation, which will require further experimentation for full characterisation.

We measured changes in anterograde and retrograde shear stress in response to each of the interventions, including cuff placement. It is possible that brachial artery diameter changes may occur in response to changes in either of these variables (Thijssen et al., 2009b; Tinken et al., 2009). Our findings indicate that cuff placement had a significant impact on brachial and radial artery retrograde shear during leg exercise but not during forearm heating. Cuff placement affected antegrade shear under all conditions. Because artery diameter was similarly affected in response to these interventions, these data might imply that manipulation of antegrade flows is a more important stimulus to brachial diameter dilation than retrograde flows, but further studies that are specifically directed at answering this question will be required.

In the present study, leg exercise induced upper limb conduit artery dilation despite the likelihood of increased activation of the sympathetic nervous system (SNS) in the inactive upper limbs. Some evidence suggests that increased sympathetic nervous activity is associated with acute impairment in brachial artery vasodilator function
(Hijmering et al., 2002; Lind et al., 2002; Dyson et al., 2006). However, SNS activity is more effective in controlling resistance vessels than conduit arteries, where it has limited impact on baseline diameter (Thijssen et al., 2006). In any case, impacts of changes in the SNS or circulating factors would arguably be similar between the limbs, and our experimental approach involving simultaneous bilateral measurements effectively eliminates such factors.

The clinical relevance of our findings relates to the impact of repeated increases in shear stress on the vasculature. It has been suggested that the direct effect of repeated episodic increases in shear stress on the vasculature may contribute to the beneficial effect of exercise training on cardiovascular risk (Green et al., 2008a; Joyner & Green, 2009). In this context, Tinken et al. recently concluded that endothelium-dependent vasodilation is increased in the brachial artery immediately following acute bouts of cycle and handgrip exercise. When increases in shear during exercise were attenuated by unilateral pneumatic cuff inflation, increases in endothelial function were abolished (Tinken et al., 2009). This study suggests that increases in shear stress during exercise can be associated with enhanced endothelial function, a finding endorsed by pharmacological blockade studies, which indicate that NO-mediated vasodilation occurs in forearm conduit (Wray et al., 2011) and resistance (Green et al., 2005) arteries in response to increases in shear stress associated with handgrip and cycle exercise. It was also recently observed that increases in shear stress are an obligatory component of training-induced endothelial cell adaptation (Tinken et al., 2010). The findings of the current study indicate that manipulation of shear stress during acute interventions modifies conduit artery dilator responses, which are likely endothelium-dependent.

Although our studies successfully manipulated shear stress and modulated endothelial function, they were performed in young healthy men and we cannot extrapolate our findings to other groups. The impact of shear stress manipulations on conduit diameter responses in subjects with endothelial dysfunction has not, to our knowledge, been previously investigated. Another potential limitation of our study is that we cannot rule out the potential impact of a veno-arteriolar reflex, the proposal that venous distension elicits a local reflex increase in arterial vasoconstriction. The presence of such a response may have contributed to our findings, although evidence for such a reflex in humans is scant. Another limitation is that we reported shear rate rather than shear stress. Although changes in viscosity may have occurred between experimental
conditions, the results of our studies involved within-subject comparison with simultaneous assessments of the arterial diameter. We contend that these experimental design features have effectively controlled for any possible impact of viscosity in our study. Finally, it would have been ideal if our cuffing interventions had completely abolished increases in shear so that we could identify whether factors other than shear contribute to any residual impacts of our interventions on diameter change. Given that we were unable to achieve such abolition, we cannot definitively exclude factors other than shear stress as contributors to conduit artery dilation in response to heating and cycle exercise. Nonetheless, the consistency of our findings, across 5 distinct experimental manipulations, gives us confidence to conclude that shear is an important in vivo physiological determinant of conduit artery vasodilation in humans.

3.5 Conclusion

Our findings strongly implicate shear stress as a key stimulus to increased conduit artery diameter in humans and suggest that exercise training induces vascular adaptation via repeated shear stress effects (Green et al., 2008a; Joyner & Green, 2009; Tinken et al., 2010). The observation that upper limb diameters change during leg exercise adds weight to systemic effects of exercise on the vasculature (Green et al., 2008b). The impact of heating on shear and diameter further reinforces the role of shear modulation versus non-shear mediated impacts of exercise (Naylor et al., 2011). Our findings may have potential clinical relevance to vascular disease and, specifically, the effects of exercise training on arterial health, function, and adaptation. Future studies should examine the impact of shear stress manipulations in groups with impaired endothelial function, such as cardiovascular disease or risk factors (e.g. hypertension).
Chapter 4

Study 2

Repeated increases in blood flow, independent of exercise, enhance conduit artery vasodilator function in humans


4.0 Abstract

This study aimed to determine the importance of repeated increases in blood flow to conduit artery adaptation, using an exercise-independent repeated episodic stimulus. Recent studies suggest that exercise training improves vasodilator function of conduit arteries via shear stress-mediated mechanisms. However, exercise is a complex stimulus that may induce shear-independent adaptations. Nine healthy males immersed their forearms in water at 42°C for three 30 min sessions/week across 8 weeks. During each session, a pneumatic pressure cuff was inflated around one forearm to unilaterally modulate heating-induced increases in shear. Forearm heating was associated with an increase in brachial artery blood flow ($P<0.001$) and shear rate ($P<0.001$) in the uncuffed forearm; this response was attenuated in the cuffed limb ($P<0.005$). Repeated episodic exposure to bilateral heating induced an increase in endothelium-dependent vasodilation in response to 5 min ischaemia ($P<0.05$) and ischaemic handgrip exercise.
(P<0.005) stimuli in the uncuffed forearm, whereas the 8 week heating intervention did not influence dilation to either stimulus in the cuffed limb. Endothelium-independent glycercyl trinitrate responses were not altered in either limb. Repeated heating increases blood flow to levels which enhance endothelium-mediated vasodilator function in humans. These findings reinforce the importance of direct impacts of shear stress on the vascular endothelium in humans.

4.1 Introduction

Endothelial dysfunction is an early manifestation of atherosclerosis which independently predicts future cardiovascular (CV) events (Rossi et al., 2008; Shechter et al., 2009; Yeboah et al., 2009) and improvement in endothelial function decreases cardiovascular risk (Modena et al., 2002; Kitta et al., 2009). Exercise training is a potent physiological stimulus which enhances endothelial function and induces endothelium-dependent arterial remodelling in humans (Green et al., 2004; Laughlin et al., 2008).

The increase in blood flow associated with repeated exercise bouts may be responsible for enhancing endothelial function and increasing artery size (i.e. outward remodelling). Hambrecht et al. demonstrated increased endothelial nitric oxide synthase (eNOS) mRNA and protein content, phosphorylation of shear-sensitive eNOS moieties, and enhanced NO vasodilator function following exercise training in humans, linking shear stress and NO-mediated vasodilator function (Hambrecht et al., 2003). More recently, we used repeated forearm handgrip exercise performed simultaneously in both arms, with a cuff inflated on one forearm to unilaterally ‘clamp’ exercise-induced hyperaemia at near baseline levels (Tinken et al., 2010). Despite similar increases in grip strength, volume and girth in both limbs, vasodilator function and indices of arterial remodelling improved only in the arm which was exposed to increased blood flow during exercise. This data, combined with animal studies (Laughlin et al., 2008), strongly suggest that exercise induces improvement in endothelial function via shear-dependent mechanisms.

Nevertheless, exercise is a complex stimulus and it remains unclear whether the mechanisms responsible for training-induced changes in arterial function and remodelling are related to the modification of flow and shear. Therefore, the aim of the present study was to determine whether repeated episodic increases in blood flow, induced by forearm heating, can induce conduit artery adaptation in humans,
independent of an exercise stimulus. Using a within-subjects, between-limb design, with simultaneous measures to minimise potential sources or error, we hypothesized that the limb exposed to larger changes in blood flow as a result of repeated heat exposure would exhibit enhanced vascular function following repeated forearm heating.

4.2 Methods

Ethical Approval

All study procedures were approved by the Human Research Ethics Committee of the University of Western Australia. Written, informed consent was obtained from all subjects and studies conformed to the Declaration of Helsinki.

Subject Characteristics

Ten young recreationally active males were recruited to undertake an 8 week experimental protocol. Complete data was unavailable from one subject across the 8 week period, so analysis was undertaken on the remaining 9 men (21.5±1.4 yrs, Table 4.1). Subjects were young and healthy and a pre-participation questionnaire confirmed the absence of known cardiovascular disease or risk factors and excluded subjects with unsuitable lifestyle traits, those on any medications or drugs. Due to possible anti-atherogenic effects of oestrogen, women were excluded from this study along with individuals taking medications or drugs of any kind.

<table>
<thead>
<tr>
<th>Table 4.1. Baseline subject characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
</tr>
<tr>
<td>Height, cm</td>
</tr>
<tr>
<td>SBP, (mmHg)</td>
</tr>
<tr>
<td>DBP, (mmHg)</td>
</tr>
<tr>
<td>MAP, (mmHg)</td>
</tr>
<tr>
<td>HR, (bpm)</td>
</tr>
</tbody>
</table>

Data are mean ± SD
Study Design
Subjects underwent an initial testing session, assessing endothelium-dependent and independent brachial artery vasodilator function. These measures were then collected at weeks 2, 6 and 8 of the intervention at the same time of day (8:00-9:00 am). During the 8 week intervention period, subjects reported to the laboratory 3 times per week for a 30 min intervention consisting of bilateral forearm heating. In one arm, a pneumatic cuff was inflated (100 mmHg) to minimise increases in brachial artery shear. This enabled comparison of heat exposure alone versus heat exposure combined with increases in shear. All study and training sessions were undertaken in a thermostatically controlled laboratory with an ambient room temperature between 22 and 24°C.

Experimental Procedures
Assessments were conducted in a quiet, temperature controlled environment. All studies were conducted at the same time of day to eliminate the possible impact of circadian variation on vascular function and subjects were fasted for 8 hours and abstained from alcohol, caffeine and exercise for 24 hours prior to testing.

Assessment of brachial artery flow-mediated dilation
After a 20 min rest period, brachial artery diameter and velocity responses to flow-mediated dilation (FMD) were simultaneously assessed in both arms, using 10-MHz multi-frequency linear array probes, attached to high resolution ultrasound machines (T3000; Terason, Burlington, MA). A rapid inflation/deflation pneumatic cuff (AG 101 Hokanson, Bellevue USA) was placed around each arm immediately distal to the olecranon process. When an optimal B-mode image was obtained, images were collected using an insonation angle (always <60°), which did not vary during each study or within individuals across the intervention. Baseline images were recorded for 1 minute, before the forearm cuff was inflated to 220 mmHg for 5 min. Recording resumed 30 sec prior to cuff deflation, and continued for 5 min post-deflation. Heart rate and mean arterial pressure were determined from an automated sphygmomanometer (Dinamap; GE Pro 300V2, Tampa, FL). This measure provides an index of conduit artery dilation which is endothelium-dependent and which most (Joannides et al., 1995; Doshi et al., 2001; Mullen et al., 2001; Kooijman et al., 2008), but not all (Pyke et al., 2010), papers suggest is largely NO-mediated.
Assessment of brachial artery responses to ischaemic handgrip exercise

Following a subsequent 20 min rest period, we examined bilateral brachial artery dilation after 5 mins of ischaemic exercise, described in detail previously (Naylor et al., 2005). Subjects performed repeated handgrip exercise (30 contractions/min) for the middle 3 min of a 5 min ischaemic period. This protocol induces endothelium-dependent dilation, however is not NO dependent (Mullen et al., 2001) and it approximates peak dilator responses (Naylor et al., 2005), thereby providing a surrogate for arterial structural remodeling.

Assessment of brachial artery responses to glyceryl trinitrate

Subjects then rested for a further 20 mins after which brachial artery diameter and velocity were examined for 10 mins following sublingual administration of glyceryl trinitrate (GTN; 400 μg). GTN induces endothelium-independent vasodilation.

Brachial artery diameter and blood flow analysis

Analysis of brachial artery blood flow and shear rate was performed using custom-designed edge detection and wall-tracking software, which is independent of investigator bias. Specific details relating to the software and analysis procedures are provided in Chapter 3 (page 61). Peak diameter following cuff deflation was determined using an automated algorithm and FMD% was calculated as the percentage rise from the baseline diameter. In accordance with recent findings (Pyke & Tschakovsky, 2007; Black et al., 2008a), the shear rate stimulus responsible for endothelium-dependent FMD following cuff deflation was calculated. The area under the shear rate curve (AUC<sub>SR</sub>), calculated as the sum of all shear rate data up to the point of maximal post-deflation diameter (Black et al., 2008a), was calculated for each individual. A detailed and comprehensive within- and between-subject assessment of variability of measurement of %dilation using this observer-independent and automatic wall tracking system analysis system has been previously published (Woodman et al., 2001). This revealed that, assuming 80% power and an alpha of 0.05, 8 subjects would be required in an intervention study to detect an absolute 1.5% change in FMD. In addition, a power analysis was performed based on differences observed in a recent study of the impact of cuff placement on exercise training responses (Tinken et al., 2010), which used an updated version of the analysis system. This indicates that, to detect the 1.5% difference in FMD during exercise training, a sample of 6 is required. This study was therefore adequately powered.
Repeated water bath exposure protocol

Following the initial assessments, subjects attended the laboratory 3 times/week for 8 weeks for 30 min bilateral water bath exposure. A pneumatic cuff was placed on one forearm, below the elbow, and inflated to 100 mmHg using a pneumatic device (AG101 Hokanson, Bellevue, USA). Previous research suggests unilateral cuff inflation in this manner alters patterns of flow and shear (Thijssen et al., 2009b; Tinken et al., 2009; Tinken et al., 2010). The cuff pressure used in this experiment was chosen following pilot trials. The contralateral arm remained uncuffed. Left or right forearm cuff placement was randomised, but consistent for a given individual across the intervention period.

Both arms were then immersed in warm water (42°C) above the level of the elbow for 30 min. The water was maintained at a constant 42°C in a storage reservoir using a thermostatically controlled heating unit. This reservoir was connected via tubing to both forearm immersion tanks and a submersible pump ensured that water of identical temperature was continuously circulated to both tanks.

The impact of unilateral cuff placement on blood flow and shear patterns between the limbs was confirmed in a sub-study of 5 subjects who underwent direct assessments of flow and artery shear in both arms during bilateral heating. We have also previously demonstrated that a 30 min heating intervention induces improvement in FMD immediately after the heating period in the uncuffed arm, whereas placement of an inflated cuff during the heating period abolishes this acute effect (Tinken et al., 2009).

Statistical Analysis

Statistical analyses were performed using SPSS 18.0 (SPSS, Chicago, Illinois). All data are reported as mean ± SD) unless stated otherwise. Statistical significance was assumed at $P<0.05$. All variables were analysed using two factor general linear models with repeated measures. The factors were time (week of training) and group (uncuffed versus cuffed limb). Statistically significant interactions were followed up with post hoc $t$-tests.
4.3 Results

*Impact of cuff placement during bilateral forearm heating: Efficacy of independent variable manipulation*

Brachial artery measures immediately preceding water bath immersion demonstrated no significant difference in blood flow between the arms at rest (Figure 4.1).

Measures taken from each arm at 5 min intervals during the heating stimulus revealed a significant impact of cuff placement (Figure 4.1). Velocity ($P<0.001$, 1-way ANOVA), flow ($P<0.001$) and shear ($P<0.001$) were significantly higher in the uncuffed arm relative to baseline as a result of heating (Figure 4.1).

Whilst there was also an increase in the cuffed arm (velocity, flow and shear, all $P<0.001$), the magnitude of change was greater in the uncuffed limb ($P<0.005$, 2-way ANOVA, Figure 4.1). These data confirm our recent findings, which indicated forearm heating acutely increased FMD in the uncuffed, but not the cuffed forearm (Tinken *et al.*, 2009).

*Impact of 8 Weeks of Repetitive Heating on Brachial Artery Responses*

*Brachial artery responses to flow-mediated dilation*

Subjects presented with no baseline differences in resting artery diameter (Table 4.2). Repeated localised heating resulted in a significant difference between cuffed and non-cuffed dilation (%) responses (main limb effect weeks 0, 2, 8; $P=0.017$, Figure 4.2A). Post hoc analysis revealed dilation values at week 2 were significantly higher compared with baseline in the uncuffed arm ($P<0.05$). In the cuffed arm, no changes in dilation were evident at any time point relative to baseline. Comparisons between limbs revealed significant differences at week 2 ($P<0.05$), but not baseline, week 6 or 8. Shear rate following the 5 min ischemic stimulus decreased between week 0 and 2 in the uncuffed limb ($P<0.05$, Table 4.2). No differences were evident in the cuffed limb.
Figure 4.1. Acute impact of cuff placement during bilateral forearm heating. Brachial artery shear rate (A), blood flow (B) and velocity (C) at baseline and at 5 min intervals in a subgroup of 5 subjects who underwent bilateral assessments throughout an acute bout of 30 mins bilateral forearm heating (42°C) in the non-cuffed (solid circles) and cuffed arm (open circles). *Significantly different from baseline at $P<0.05$. †Significantly different between limbs at same time point at $P<0.05$. Data is mean ± SE.
**Brachial artery responses to ischaemic handgrip exercise**

In the uncuffed arm, the responses to ischaemic exercise showed an increase across the intervention period, particularly at 8 weeks (Figure 4.2B; 1-Way ANOVA, \( P<0.01 \)). In the cuffed arm no changes in response to ischaemic exercise were evident across the intervention. A 2-way ANOVA revealed a significant effect for cuffed/uncuffed limb (\( P<0.01 \)) and also an interaction effect between limb and time (\( P<0.01 \)). Post hoc \( t \)-tests revealed significant increases in the uncuffed limb between baseline and week 8 (\( P<0.005 \)), with no differences between baseline and weeks 6 or 8 in the cuffed limb. Comparisons between limbs revealed significant differences at week 2 (\( P<0.05 \)) and week 8 (\( P<0.05 \)) (Figure 4.2B). No significant effect for time, limb or interaction between these factors was evident for AUC\(_{SR}\) in response to ischaemic handgrip exercise (Table 4.2).

**Brachial artery responses to glyceryl trinitrate**

There were no significant difference in response to GTN (Table 4.2). Both cuffed and uncuffed responses remained unchanged from baseline across the intervention period.
Figure 4.2. Chronic impact of 8 weeks of repetitive forearm heating on brachial artery responses. Relative change in FMD (A) from baseline and in response to ischaemic exercise (B) across the 8 week heating intervention in healthy young men (n=9). Data were presented for the uncuffed arm (solid squares) as well as the cuffed arm (open squares). *Significantly different from baseline at $P<0.05$. †Significantly different between limbs at same time point at $P<0.05$. Data is mean ± SE.
Table 4.2. Brachial artery characteristics throughout the 8 week exercise intervention, in the uncuffed and cuffed arms.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Week 0</th>
<th>Week 2</th>
<th>Week 6</th>
<th>Week 8</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Uncuffed arm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting Diameter (mm)</td>
<td>4.0 ± 0.5</td>
<td>3.9 ± 0.6</td>
<td>4.1 ± 0.7</td>
<td>4.0 ± 0.8</td>
</tr>
<tr>
<td>5 min ischaemia AUC&lt;sub&gt;SR&lt;/sub&gt;</td>
<td>23528 ± 9327</td>
<td>16253 ± 7703*</td>
<td>20246 ± 16252</td>
<td>20368 ± 9060</td>
</tr>
<tr>
<td>Ischaemic exercise dilation AUC&lt;sub&gt;SR&lt;/sub&gt;</td>
<td>45251 ± 22492</td>
<td>35682 ± 16338</td>
<td>39002 ± 13967</td>
<td>36292 ± 16471</td>
</tr>
<tr>
<td>GTN (%)</td>
<td>19.9 ± 5.1</td>
<td>17.4 ± 9.0</td>
<td>22.5 ± 5.6</td>
<td>21.6 ± 6.4</td>
</tr>
<tr>
<td><strong>Cuffed arm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting Diameter (mm)</td>
<td>4.0 ± 0.5</td>
<td>3.8 ± 0.5</td>
<td>3.9 ± 0.6</td>
<td>3.8 ± 0.6</td>
</tr>
<tr>
<td>5 min ischaemia AUC&lt;sub&gt;SR&lt;/sub&gt;</td>
<td>24775 ± 21586</td>
<td>18297 ± 10032</td>
<td>26431 ± 11320</td>
<td>36447 ± 10872</td>
</tr>
<tr>
<td>Ischaemic exercise dilation AUC&lt;sub&gt;SR&lt;/sub&gt;</td>
<td>54731 ± 29736</td>
<td>42432 ± 14754</td>
<td>42985 ± 18279</td>
<td>37264 ± 24263</td>
</tr>
<tr>
<td>GTN (%)</td>
<td>17.7 ± 2.2</td>
<td>18.4 ± 2.4</td>
<td>19.7 ± 3.4</td>
<td>19.4 ± 3.7</td>
</tr>
</tbody>
</table>

Significant from week 0 value at *P* = 0.05. Values are means ± SD. SD, standard deviation; FMD, flow-mediated dilatation; GTN, glyceryl trinitrate; AUC<sub>SR</sub>, shear rate area under the curve from the time of cuff deflation to peak diameter detection.
4.4 Discussion

The aim of the present study was to determine whether episodic increases in blood flow, independent of an exercise stimulus, induce conduit artery adaptation in humans. Using a repeated simultaneous bilateral forearm heating stimulus, we induced increases in blood flow and shear in one forearm, whilst attenuating these responses using a cuff on the contralateral limb. This provided a within-subjects design to determine the effects of shear modulation on endothelium-dependent and -independent vasodilation. Outcome measures were simultaneously assessed in both limbs to minimise potential sources of error. Our principal findings include: 1. that a repeated exercise-independent shear-mediated stimulus can induce adaptation in endothelial function, 2. that cuff placement and shear modulation attenuates this response, 3. that different endothelial mechanisms may be involved in the above findings, 4. that adaptation is limited to the endothelium and not smooth muscle layer. These findings indicate that repeated heating increases shear to levels capable of inducing adaptation in endothelial function and that exercise-independent increases in shear can alter arterial function in vivo.

It is well established that exercise training improves endothelial function in conduit and resistance arteries (Green et al., 2004). Animal studies suggests that this is related to the repeated increases in shear which occur during exercise (Neibauer & Cooke, 1996; Laughlin et al., 2008). Furthermore, adaptation in endothelial function occurs rapidly, within one or two weeks following the onset of exercise training (Delp et al., 1993; Wang et al., 1993; Sun et al., 1994; Koller et al., 1995; Delp & Laughlin, 1997; McAllister & Laughlin, 1997). In humans, the link between exercise, repeated shear stimulation and vascular function was first suggested by Hambrecht et al. who observed improvement in NO-mediated vasodilator function alongside increases in eNOS expression and endothelial content of phospho-eNOS{Ser1177}, Akt, and phospho-Akt. They concluded that the change in endothelium-dependent vasodilatation was closely related to shear stress–induced Akt dependent phosphorylation of eNOS (Hambrecht et al., 2003). More recently, Tinken et al. reported 8 weeks of dual handgrip exercise training enhanced endothelium-dependent vasodilation in the limb exposed to increased shear during exercise, but not in the contralateral limb in which shear was clamped near baseline levels (Tinken et al., 2010). The authors concluded that exercise-induced changes in shear provide a crucial physiological stimulus to adaptation in flow-mediated endothelial function in healthy humans. As in the present study, the authors observed transient changes in NO-mediated vasodilator function which were superseded by
increases in vasodilation attributable to non-NO-dependent mechanisms or, perhaps, vascular structure, as previously discussed (Laughlin, 1995; Green et al., 2004; Tinken et al., 2008a; Tinken et al., 2010). Nonetheless, exercise may conceivably induce adaptations via mechanisms other than shear modulation (Laughlin et al., 2008).

This is the first study in humans, to our knowledge, to indicate that endothelium-dependent vasodilator function improves in response to a repeated blood flow and shear stimulus which is independent of exercise. We observed changes in endothelial function in the uncuffed limb, but no such changes in the contralateral cuffed limb which received a lower shear rate stimulus. An interesting observation is that, despite being diminished compared to the uncuffed arm, blood flow and shear rate nonetheless increased during heat exposure in the cuffed limb. The lack of change in endothelial function in the cuffed limb, despite some increase in flow and shear during heating, suggests that there may be a threshold below which endothelial adaptations are less apparent. Further studies will be required to fully address this issue.

Two distinct vasodilator stimuli were utilised in the present experiment. The vasodilator response to a 5 min period of ischaemia has been reported by most (Joannides et al., 1995; Doshi et al., 2001; Mullen et al., 2001; Kooijman et al., 2008), but not all (Pyke et al., 2010) studies to be largely NO-dependent, whereas dilation in response to ischaemic handgrip exercise is likely less NO-dependent (Mullen et al., 2001). Whatever the precise mechanisms responsible for the dilation to each stimulus, the combination of both measures provides complementary information regarding the status of endothelium-dependent and shear stress mediated conduit artery vasodilation in humans. Our findings of enhanced arterial dilation in response to these stimuli are therefore consistent with upregulation of endothelium-dependent conduit artery function. The present study reinforces recently observed changes in response to handgrip training (Tinken et al., 2010) and adds the novel finding that repeated shear stimulation, whether associated with exercise or not, may induce conduit artery endothelial adaptation. It appears that the brachial artery became somewhat "hyper-responsive" following heat training, as shear rate AUC decreased in the presence of maintained FMD and increased ischaemic handgrip responses. These findings contrast somewhat with the impact of exercise training on shear mediated dilator responses and the explanation is not entirely clear, but it may be that heat training has less impact on
resistance vessel remodeling than exercise training, even if it does modulate cutaneous microvascular function (Green et al., 2010).

There was no evidence for change in GTN responses in either limb of the subjects in the present study. This suggests that, despite evidence for change in endothelium-dependent vasodilator responses to ischaemic stimuli, repeated exercise-independent increases in shear did not alter NO-mediated smooth muscle function. Many, but not all, studies of exercise training have also reported improvement in endothelial, but not smooth muscle, vasodilator function in humans (Green et al., 2004).

We could not find many examples of the impact of repeated passive heating on conduit artery adaptation in humans. One study reported improved clinical status and brachial FMD, but not GTN, responses as a result of 2 weeks of daily sauna exposure in patients with chronic heart failure (Kihara et al., 2002). The authors suggested that the peripheral circulation improved, but they did not examine this. Our data also suggest that the purported benefits of heat therapy may be related to shear stress mediated improvement in conduit endothelial function. However, the clinical implications of our findings in response to repeated heating are not clear. While a number of studies have established the independent prognostic relevance of the dilator response to 5 mins ischaemia (Modena et al., 2002; Rossi et al., 2008; Shechter et al., 2009; Yeboah et al., 2009), possibly attributable to its dependence upon anti-atherogenic NO function, nothing is currently known regarding the prognostic relevance of the ischaemic handgrip protocol, despite is endothelium-dependence. We cannot, therefore, extend our findings to possible prognostic implications of the changes observed with heating in this study and we prefer to limit our conclusions to the impact of shear manipulation on vascular adaptation.

There are several limitations to this study. Our sample size was small. However significant differences were observed in our principal outcome variables, indicating that it was adequate. Moreover, previous studies using %dilation and interventions such as exercise have used similar sample sizes (Edwards et al., 2004; Wray et al., 2006; Wisloff et al., 2007; Tyldum et al., 2009). A post hoc power analysis revealed that the current study had 90% power to observe the difference in %dilation between baseline and week 2 for the ischaemic dilator response. This indicates that we had a strong design to detect changes in vascular function across an 8 week intervention in healthy
subjects. We chose not to normalise %dilation for its eliciting shear, due to the large variability and statistical issues associated with shear assessment and normalisation (Atkinson et al., 2009). However, it is unlikely that the conduit dilator data can be explained by changes in the shear rate stimulus, as increases in dilation were associated with no change, or decreases, in shear rate. In any event, this study clearly indicates that cuff placement and shear manipulation has an impact on endothelial function in humans and that these effects are not evident in smooth muscle. These results do not provide evidence relating to resistance vessels and larger studies, which are specifically designed to assess resistance vessel responses, would be a valuable addition to the present data set. Finally, a non-heating control group was not included to assess the effects of time on vascular responses in the present study. This is because a previous paper of this nature in a very similar group of subjects revealed no differences across 8 weeks in either FMD or ischaemic exercise responses (Tinken et al., 2008a).

4.5 Conclusion

In summary, our findings indicate that repeated heating increases shear stress to levels which enhance endothelium-mediated conduit artery vasodilator function. These findings reinforce the importance of direct impacts of shear stress on the vascular endothelium in humans and provide further insight into the mechanisms responsible for exercise training-mediated vasodilator changes.
Chapter 5

Study 3

Repeated core temperature elevation induces conduit artery adaptation in humans


5.0 Abstract

Shear stress is a known stimulus to vascular adaptation in humans. However, it is not known whether thermoregulatory reflex increases in blood flow and shear can induce conduit artery adaptation. Ten healthy young volunteers therefore underwent 8 weeks of 3 x weekly bouts of 30 mins lower limb heating (40°C) during which the upper body was not directly heated. Throughout each leg heating session, a pneumatic cuff was placed on one forearm and inflated to unilaterally restrict reflex-mediated blood flow responses. Each bout of leg heating significantly increased brachial artery shear rate in the uncuffed arm (96±97 vs 401±96 1/s, P<0.01), whereas no change was apparent in the cuffed arm (83±69 vs 131±76 1/s, P=0.67). Repeated episodic exposure to leg heating enhanced brachial artery endothelial function (measured by flow-mediated dilation) in the uncuffed arm from week 0 (5.2±1.9%) to week 4 (7.7±2.6%, P<0.05), before returning to baseline levels by week 8. No adaptation was evident in the cuffed arm. In summary, repeated increases in core temperature, induced via lower limb heating, resulted in upper limb conduit artery vascular adaptation which was dependent upon increases in shear stress, mediated by cutaneous vasodilation. To our knowledge this is the first study to establish a beneficial systemic impact of thermoregulatory reflexes on conduit artery function in humans.
5.1 Introduction

Shear stress is the frictional drag force exerted by blood as it flows across the arterial wall. Studies in animals have convincingly demonstrated that increases in shear stress result in conduit artery vasodilation, mediated by the release of vasoactive substances from the endothelium, most notably nitric oxide (NO) (Pohl et al., 1986; Laughlin et al., 2008). As demonstrated in Chapter 4, 8 weeks of bilateral forearm heating was associated with enhanced brachial artery function, measured by flow-mediated dilation (FMD), which transiently increased and was thereafter superseded by structural arterial remodelling (Naylor et al., 2011). This biphasic pattern of functional and structural adaptation is consistent with that originally suggested by Laughlin on the basis of extensive animal experimentation (Laughlin, 1995). Unilateral forearm cuff inflation, which prevented brachial shear stress from increasing during forearm heating bouts, abolished these vascular adaptations. However, most previous studies of shear-mediated effects on conduit arteries have involved exercise as a stimulus and no previous study has examined whether thermoregulatory reflex-mediated increases in blood flow and shear stress in resting subjects, akin to repeated sauna exposure, can induce arterial adaptation in humans.

Our aim in the present study was to examine the impact of repeated episodic changes in brachial artery blood flow and shear stress on conduit artery adaptation induced by a systemic heating stimulus. To this end we devised a method of isolated lower limb heating which elevates core temperature (Tc) and induces reflex thermoregulatory cutaneous vasodilation, including increased blood flow in the upper limbs. The upper body itself is not directly heated and forearm hyperaemia is therefore entirely reflex-mediated. We repeatedly exposed subjects to this isolated leg heating protocol across an 8 week intervention period. To address the role of shear stress, a cuff was partially inflated on one forearm throughout each heating bout. We hypothesised that reflex-mediated increases in brachial artery flow as a result of core body heating would result in shear stress-mediated conduit artery adaptations similar to those observed following exercise training.
5.2 Methods

Ethical Approval
This study complied with the Declaration of Helsinki and the Human Research Ethics Committee of the University of Western Australia approved the experimental protocol. All subjects provided written, informed consent before participating in the study.

Subject Characteristics
Ten recreationally active males were recruited for the study (25.8 ± 3.1 yrs, Table 5.1). A pre-participation questionnaire was administered to exclude subjects with specific lifestyle traits or medical issues such as cardiovascular disease, smoking, hypertension and hypercholesterolemia. Due to the possible vasoactive effects of oestrogen, women were excluded from this study along with individuals taking medication of any kind.

<table>
<thead>
<tr>
<th>Table 5.1. Baseline subject characteristics</th>
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<tbody>
<tr>
<td>Age (years)</td>
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<tr>
<td>Height (m)</td>
</tr>
<tr>
<td>Weight (kg)</td>
</tr>
<tr>
<td>BMI (kg.m⁻²)</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
</tr>
</tbody>
</table>

Values are mean ± SD

Study Design
Once recruited, subjects undertook 30 mins of lower limb heating (40°C), 3 times a week, for 8 weeks in a custom designed recovery bath (iCoolsport, Queensland, Australia). The water bath temperature was maintained and continuously circulated via a heating pump (IC-Heat; iCoolsport, Queensland, Australia). Subjects were submerged to the level of the waist and the custom-designed baths had a cover placed over them such that the upper limbs were not heated during the sessions and remained under ambient conditions. During each session, a pneumatic cuff was placed around one forearm and inflated (80 mmHg) to attenuate thermoregulatory reflex-mediated increases in brachial blood flow and shear. Such cuff placement has been successfully used on many occasions to unilaterally modify flow and shear (Thijssen et
al., 2009b; Tinken et al., 2009; Tinken et al., 2010; Naylor et al., 2011; Birk et al., 2012). To assess vascular adaptation to this intervention, we examined brachial artery responses to FMD, an ischemic handgrip exercise protocol (iEX) and following administration of sublingual glyceryl trinitrate (GTN; 400μg) at baseline and again at weeks 2, 4, 6 and 8. To assess the acute impact of lower body heating on upper limb haemodynamics, brachial artery shear rate was assessed during the first 30 min heating bout in a subgroup of 5 subjects.

Experimental Procedures
All studies were conducted in a quiet, temperature controlled environment. Subjects arrived at the laboratory having fasted for a minimum of 8 hours and abstained from alcohol, caffeine and vigorous exercise for at least 24 hours.

Assessment of brachial artery blood flow and shear rate during leg heating
During the initial session of lower limb heating, we assessed the acute effect of such heating on brachial artery blood flow and shear rate responses in both the cuffed and uncuffed arms. To this end brachial artery diameter and velocity were simultaneously assessed in both arms using 10-MHz multi-frequency linear array probes attached to high-resolution ultrasound machines (T3000; Terason, Burlington, MA). Recordings commenced following 20 mins of quiet seated rest and then continued throughout the 30 min bath immersion protocol, as described above.

Assessment of brachial artery responses to flow-mediated dilation, ischaemic handgrip exercise and glyceryl trinitrate
Specific details relating to these protocols can be found in Chapter 4 (Pages 81-82). Briefly, brachial artery FMD responses were assessed following 5 mins of forearm cuff inflation to 220 mmHg. After a 20 min rest period, brachial artery dilation was assessed following 5 mins of iEX. Finally, following a further 20 min rest period, brachial artery responses to sublingual administration of GTN was assessed.

Statistical Analysis
Statistical analysis was performed using SPSS 19.0 (SPSS, Chicago, IL) software. All data are reported as mean ± standard deviation unless stated otherwise and statistical significance was assumed at $P<0.05$. A 2-factor ANOVA with repeated measures (with time and cuff placement as the independent factors) was performed for the acute shear
rate data. Repeated-measures ANOVA (with time and cuff placement as independent factors) were used to assess changes in brachial artery vasodilation in response to FMD, iEX, and GTN across the 8 week intervention period. Post hoc analysis \( t \)-tests were used where significant values were found.

5.3 Results

*Acute effect of lower limb heating on brachial artery shear rate (n=5)*

There was no difference in shear rate between the arms prior to cuff inflation \((P=0.48, \text{Figure 5.1})\). Brachial artery shear rate increased in the uncuffed arm \((P<0.01)\) with significant differences from baseline at 15 and 25 mins (both \(P<0.05\)). In contrast, shear rate in the cuffed arm did not significantly change across the 30 min heating bout \((P=0.67)\). A 2-way ANOVA revealed a difference between the cuffed and uncuffed arms \((P<0.01)\) with significant differences at 5, 15 and 25 mins (all \(P<0.05\)) (Figure 5.1).

![Figure 5.1](image)

*Figure 5.1.* Brachial artery shear rate (1/s) in the cuffed (open squares) and uncuffed (solid squares) forearms at baseline and at 5, 15 and 25 mins during lower limb heating. Significantly different at \(P<0.05\) from baseline (\(^#\)) or between the cuffed and uncuffed forearms (\(*\)). Data are mean ± SE.
**Effects of repeated episodic lower limb heating on brachial artery adaptation**

*Flow-mediated dilation*

Resting brachial artery diameter did not change in the cuffed arm (week 0, 2, 4, 6 and 8; 4.3±0.6 vs 4.3±0.6 vs 4.1±0.5 vs 4.1±0.6 vs 4.1±0.5 mm, \( P=0.94 \)) or the uncuffed (4.2±0.6 vs 4.1±0.6 vs 4.1±0.5 vs 4.1±0.5 vs 4.3±0.5 mm, \( P=0.91 \)) across the intervention period. Repeated lower limb heating induced a significant increase in brachial artery FMD (2-way ANOVA, time factor, \( P<0.05 \), Figure 5.2A). Post hoc analysis revealed a significant increase in FMD between weeks 0 and 4 in the uncuffed arm (\( P<0.05 \)), before it returned to baseline values by week 8. In contrast, no change in FMD was apparent in the cuffed arm (\( P=0.73 \)). No change was evident in the shear rate stimulus (SR\(_{AUC}\)) to FMD across the 8 week intervention (Table 5.2).

*Brachial artery responses to ischaemic handgrip exercise and glyceryl trinitrate*

There was no difference in brachial artery responses to iEX between arms at baseline and the responses remained unchanged across the intervention (2-way ANOVA, \( P=0.43 \), Table 5.2). Similarly, no differences in brachial artery responses to GTN were evident between arms at baseline and responses did not change across the intervention (2-way ANOVA, \( P=0.45 \), Figure 5.2B).
Figure 5.2. Brachial artery responses to flow-mediated dilation (%) (A) and sublingual glyceryl trinitrate (%) (B) in the cuffed (open squares) and uncuffed (solid squares) forearms at weeks 0, 2, 4, 6 and 8. *Significantly different at $P<0.05$ from baseline. Data are mean ± SE.
Table 5.2. Brachial artery characteristics at weeks 0, 2, 4, 6 and 8.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Week 0</th>
<th>Week 2</th>
<th>Week 4</th>
<th>Week 6</th>
<th>Week 8</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR$_{AUC}$ (FMD%) $10^3$, s$^{-1}$</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Cuffed</td>
<td>15.6 ± 7.1</td>
<td>14.3 ± 7.7</td>
<td>14.5 ± 3.2</td>
<td>20.0 ± 12.8</td>
<td>15.7 ± 6.9</td>
<td>Time: P=0.90</td>
</tr>
<tr>
<td>Uncuffed</td>
<td>15.7 ± 10.9</td>
<td>13.2 ± 5.0</td>
<td>15.4 ± 8.0</td>
<td>18.0 ± 8.1</td>
<td>13.9 ± 7.1</td>
<td>Time*arm: P=0.68</td>
</tr>
<tr>
<td>iEX%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuffed</td>
<td>17.4 ± 4.8</td>
<td>18.9 ± 5.4</td>
<td>19.7 ± 6.8</td>
<td>17.6 ± 4.3</td>
<td>20.0 ± 4.1</td>
<td>Time: P=0.43</td>
</tr>
<tr>
<td>Uncuffed</td>
<td>17.6 ± 7.6</td>
<td>19.1 ± 3.0</td>
<td>20.2 ± 6.4</td>
<td>19.9 ± 7.5</td>
<td>16.8 ± 6.9</td>
<td>Time*arm: P=0.51</td>
</tr>
</tbody>
</table>

Values are mean ± SD. FMD, flow-mediated dilation; SR$_{AUC}$, shear rate area under the curve; iEX, ischaemic handgrip exercise.
5.4 Discussion

In the present study, we demonstrated that 30 mins of lower limb heating significantly increased brachial artery blood flow and shear rate, responses that were abolished by cuff inflation on the contralateral forearm. Eight weeks of repeated episodic lower limb heating enhanced brachial artery endothelium-dependent vasodilator (FMD) function in the uncuffed arm, exposed to large increases in blood flow and shear during each heating bout, but not the contralateral cuffed arm in which flow and shear were attenuated. Brachial artery responses to GTN did not change across the intervention in either arm, inferring that the functional adaptation in FMD was endothelium-dependent. Our findings suggest that repeated increases in Tc, induced via lower limb heating, result in vascular adaptations in upper limb conduit arteries which are dependent upon episodic increases in shear stress, mediated by cutaneous thermoregulatory vasodilation.

This study indicates that, whilst changes in conduit artery function occur as a result of repeated changes in shear, these increases in FMD ultimately resolve and return to near baseline levels by 8 weeks. This biphasic pattern of adaptation in vascular function has previously been observed in studies involving handgrip exercise training (Tinken et al., 2008a; Tinken et al., 2010), leg exercise training (Birk et al., 2012) and localized repetitive forearm heating (Naylor et al., 2011). A biphasic time-course in adaptation of artery function is also consistent with animal studies of the time-course of eNOS expression and arterial remodeling (Laughlin, 1995). Laughlin originally proposed in the mid-1990’s that changes in arterial function are transient and ultimately superseded by increases in artery size, or remodeling. The above studies are broadly consistent with this animal literature, in that the return of functional changes to baseline levels is often accompanied by evidence for increases in peak vasodilator responses in humans, a surrogate measure of outward arterial remodeling (Tinken et al., 2008a; Tinken et al., 2010; Naylor et al., 2011). However, no evidence for arterial remodeling was observed in the current study, a finding which may indicate that the levels of brachial artery shear generated were not large enough to mediate a compensatory increase in arterial size. Indeed previous studies, such as those which have noted differences in size and diameter between arms of elite racquet players, suggest that the stimulus for structural change may be localized in nature (Green et al., 1996; Rowley et al., 2011).

The mechanistic explanation for the increase in NO-mediated endothelium-dependent vasodilation we observed in this study has previously been suggested by Hambrecht et
al. (Hambrecht et al., 2003), who concluded that repeated increases in shear upregulate eNOS phosphorylation and increase the bioavailability of NO. However, the Hambrecht study correlated changes in shear with artery function, whereas our findings have directly manipulated shear levels experimentally via cuff inflation, thereby identifying shear for the first time as a causal mechanism. It is important to add that our within-subjects contralateral limb model effectively eliminates central factors from the causal pathway, as changes in circulating hormones, neural outflow or sympathetic activity would have logically been expected to express effects in both limbs. It is also important to emphasise that post cuff-deflation shear stress, the physiological stimulus to FMD (Pyke & Tschakovsky, 2007; Thijssen et al., 2011), did not change across the 8 weeks in this experiment (Table 5.2). GTN responses were also unaltered. Hence, the changes in FMD observed in this study must be due to genuine endothelial adaptation and not systematic changes in the stimulus to FMD or changes in smooth muscle function.

Whilst this is the first study, to our knowledge, which has addressed the mechanisms responsible for thermoregulatory effects on systemic conduit artery function, a previous study by Kihara et al. reported that 14 days of daily sauna treatment (60°C) in chronic heart failure patients enhanced brachial artery endothelial function (Kihara et al., 2002). This study was observational and did not isolate the heating stimulus per se, or manipulation of shear stress, to identify causal mechanisms. The current study extends the findings of Kihara et al. by isolating the heating stimulus to the lower limbs, such that the upper limbs were not directly affected by a heat stimulus. Also, by unilaterally restricting brachial flow and shear, this study experimentally manipulated the impact of shear in the vascular responses.

There are several potential limitations of this study. The sample size was small, but this is only a statistically relevant limitation under circumstances where type II errors are suspected (Batterham & Atkinson, 2005). Our findings are statistically significant and therefore, by definition, the study was adequately powered. A comprehensive published analysis of the validity and reliability of the operator-independent vascular analysis technique also indicates that our sample size was adequate (Woodman et al., 2001). In addition, this study’s methodological approaches, including the use of automated edge-detection and wall tracking software, experimental design elements such as simultaneous assessment using identical machines and settings and the use of a
contralateral within-subjects control limb, all add to the precision of our study by diminishing important sources of error. A further potential limitation of the study is the unilateral inflation of the forearm cuff during lower limb heating may have elicited a veno-arteriolar reflex (VAR) (Brothers et al., 2009). However, some previous studies suggest conduit artery diameter is not affected by the VAR (Brothers et al., 2009) and the mechanism responsible for this reflex remain largely unknown (Johnson, 2002). In any event, such a reflex, if present, would act by decreasing brachial flow and shear in the cuffed arm, thereby reinforcing our argument that these stimuli are important to vascular adaptation. Our FMD measures were also collected in both limbs using identical procedures and without venous congestion. Finally, a potential explanation for the decrease in FMD observed in the uncuffed arm from to week 4 to week 8 is a reduction in the brachial shear stimulus once subjects became acclimated to the heat exposures. However, there was no significant difference between the cuffed and uncuffed limbs in the post-occlusion $SR_{AUC}$ stimulus to FMD following cuff release across the 8 weeks of testing. This suggests the stimulus upstream remained the same throughout the intervention and discounts the possibility the return in FMD to baseline by week 8 was due to a drop in the shear stimulus.

It is well established that strong relationships exist between the behavior and function of brachial and epicardiac coronary arteries (Anderson et al., 1995; Takase et al., 1998; Takase et al., 2005), suggesting that peripheral vascular function provides a valid surrogate for systemic vascular health. Although this study did not assess subjects with risk factors or cardiovascular disease in this experiment, lower limb heating may conceivably have potential beneficial impacts in coronary disease or heart failure patients and future studies using our approach will be required to definitively answer this.

5.5 Conclusion

In summary, lower limb heating induced thermoregulatory reflex-mediated increases in upper limb cutaneous and brachial artery blood flows in this study. Repeated increases in brachial shear induced via this heating stimulus, independent of exercise, enhanced brachial NO-mediated endothelial function. These findings are the first to our knowledge to indicate that repeated thermoregulatory reflex-mediated increases in blood flow can improve systemic conduit artery function via a shear-mediated mechanism. The implications of this study relate to the potential cardiovascular
benefits of repeated episodic body heating in clinical groups or individuals in whom exercise is contraindicated. ‘Thermoregulatory training’ may provide an alternative to enhance vascular function in vivo.
Chapter 6

Publication: Literature Review 2

Cutaneous microvascular adaptations to exercise and passive heating in humans

6.0 Mechanisms of cutaneous microvascular control in humans

Blood flow to the skin typically ranges from 5-10% of cardiac output at rest and can increase to 60% during thermal stress (Rowell, 1977). This large range is indicative of the key role of skin blood flow (SkBF) in thermoregulation and the homeostatic maintenance of core temperature (Tc). Consequently, the control of SkBF is important in regulating the distribution of blood flow and the regulation of blood pressure (Rowell, 1977). As might be expected, the regulation of SkBF is highly complex and remains to be fully elucidated, however, it involves the integration of both neural and local or intrinsic mechanisms. In one of the first studies to demonstrate that the cutaneous vasculature is neurally innervated, Claude Bernard observed, in 1852, that rabbit ears become red and hot following severing of the cervical sympathetic chain (Laporte, 1996). In the same year, Brown Séquard reported that stimulation of the severed cervical sympathetic chain resulted in blanching, or lightening, of the ear, suggesting decreased blood flow (Laporte, 1996). These classic studies in animals established a basis for investigation of the existence of sympathetic neural innervation of the cutaneous vasculature in humans. It is now understood that there are 2 types of
sympathetic nerve fibres that innervate the cutaneous vasculature: adrenergic vasoconstrictor and cholinergic vasodilator nerve fibres.

6.0.1 Adrenergic vasoconstrictor nerves

Cutaneous adrenergic vasoconstrictive nerves are tonically active and can be influenced by cutaneous thermoreceptors, baroreflexes and chemoreceptors (Rowell, 1977). Numerous studies in humans have revealed that blockade of cutaneous nerves in cool environments results in marked vasodilation of the hand and forearm due to the withdrawal of vasoconstrictive outflow. For example, Lewis and Pickering noted in 1931 that blockade of the ulnar nerve using local anaesthetic resulted in an increase in skin temperature, and hence SkBF to the hand. It is now known that cutaneous vasoconstriction induced by adrenergic sympathetic nerves involves the release of noradrenaline, which binds to either $\alpha_1$- or $\alpha_2$-receptors on target blood vessels. This has been proved by studies utilising bretylium tosylate, a substance that selectively blocks the action of sympathetic adrenergic fibres (Blair et al., 1960). However, the adrenergic vasoconstrictor system is still not fully understood, with a recent study reporting that cutaneous vasoconstriction was not completely abolished following blockade of $\alpha_1$, $\alpha_2$, or $\beta$-receptors during hypothermia (Stephens et al., 2001a). A further study by this group suggested that adrenergic vasoconstriction is a result of the co-transmission of norepinephrine and neuropeptide Y (Stephens et al., 2004). However, ATP is also a known co-transmitter involved in vasoconstriction in numerous animal studies (Monge et al., 1991; Hashim & Tadepalli, 1995; Lundberg & Modin, 1995).

6.0.2 Cholinergic vasodilator nerves

In non-glabrous (hairy) skin, the cutaneous vasculature is also innervated by vasodilator nerves, a feature unique to humans. Grant and Holling, in 1938, provided evidence for the existence of cutaneous vasodilator nerves in humans after observing no change in forearm skin temperature, and therefore SkBF, during whole body heating-induced elevations in $T_c$ following blockade of cutaneous nerves in the forearm (Grant & Holling, 1938). Edholm, Fox and Macpherson subsequently measured forearm blood flow and observed a similar attenuated response during whole body heating following complete cutaneous nerve blockade (Edholm et al., 1957). It is now known that cutaneous vasodilation is mediated by the action of sympathetic cholinergic fibres. However, the precise mechanisms responsible for inducing cutaneous vasodilation
following increased cholinergic vasodilator outflow remain unclear. Current literature indicates the most likely mechanism may involve a co-transmitter, whereby sweating and cutaneous vasodilation are stimulated by the release of ACh and an as yet undetermined vasodilator substance (Kellogg, 2006; Wong, 2013). Proposed substances or pathways implicated in the vasodilator response that occurs in conjunction with ACh include vasoactive intestinal polypeptide (Bennett et al., 2003), H1 histamine receptors (Wong et al., 2004) and neurokinin-1 receptors (Wong & Minson, 2006). Previous studies have also strongly implicated NO in the reflex vasodilator responses (Kellogg et al., 1998; Shastry et al., 1998). Indeed, Wong recently reported that inhibition of sensory nerves and NO production reduced the reflex cutaneous vasodilator response to whole body heating by ~80% and that NO was obligatory for full expression of reflex-mediated cutaneous vasodilation (Wong, 2013).

6.0.3 Local control mechanisms: Local heating of the skin
Changes in the temperature of the skin also result in local regulation of SkBF. Following rapid local heating using local heater discs, SkBF responds in a biphasic manner that has been comprehensively characterised by Minson, Berry and Joyner (Minson et al., 2001). Immediately upon application of heat, SkBF peaks rapidly followed by a brief nadir, after which the cutaneous microvessels undergo a secondary prolonged vasodilation (Figure 6.1A). The authors reported blockade of local axon reflexes, via administration of topical EMLA cream, over the locally heated area significantly attenuated the initial peak in SkBF but did not affect the magnitude of the secondary vasodilation. However, infusion of an NO blocker (L-NAME) via microdialysis significantly blunted the secondary vasodilation (Figure 6.1B). The findings of this study therefore indicated that the initial rise and peak in SkBF is mediated via local axon reflexes that are not NO-dependent. It has been reported that local increases in skin temperature stimulates heat-sensitive vanilloid type 1 receptors on afferent nerves as part of the axon reflex, which causes the antidromic release of a vasodilator neurotransmitter (or neurotransmitters) that effect local SkBF increases (Stephens et al., 2001b). Another study suggested that the neurotransmitter CGRP is involved in this initial response (Schmelz et al., 1997).

The secondary prolonged vasodilation phase is, in contrast, predominantly NO-mediated (Minson et al., 2001). These mechanisms explain the bimodal response to local heating until the threshold of pain (41-42°C), where infusion of higher doses of L-
NAME had no impact on SKBF (Kellogg et al., 1999; Minson et al., 2001) indicating that different mechanisms contribute to this vasodilator response. Local heating to these higher temperatures may elicit nociceptor activation, resulting in the release of vasoactive substances such as prostaglandins (Kellogg et al., 1999) or neuropeptides (Minson et al., 2001). Another study concluded that cutaneous vasodilation to local heating at 40°C and higher, but in the absence of the conscious sensation of pain, is also affected by these mechanisms (Magerl & Treede, 1996).

Figure 6.1. A typical biphasic skin blood flow response to local heating of the skin with an initial peak, followed by a brief nadir and a secondary prolonged vasodilation (A). Skin blood flow to the same heating stimulus following infusion of L-NAME reveals a similar initial peak however a significantly attenuated plateau phase, demonstrating the role of NO in this response (B). Modified from (Minson et al., 2001).
6.0.4 Summary: Cutaneous microvascular control in humans

The mechanisms described above represent efferent or effector pathways that are of intrinsic (localised) and reflex (central) origin. The contribution of each of these pathways to the microvascular response depends upon the nature of the eliciting stimulus. Common physiological conditions that elicit an integrated response involving each of these pathways include physical exercise and whole body heating, or their combination.

6.1 Skin blood flow responses during acute exercise and passive heating

Small reductions in SkBF immediately upon the commencement of exercise are common (Zelis et al., 1969; Kellogg et al., 1991) and are thought to be due to increased systemic sympathetic vasoconstrictive outflow which redirects blood flow to active vascular beds, such as the working muscles. As exercise duration and/or intensity increases and Tc begins to rise, withdrawal of vasoconstrictive tone is responsible for an initial increase in SkBF/cutaneous vasodilation. Thereafter, a Tc threshold is reached, beyond which activation of cholinergic vasodilator nerves induce significant cutaneous vasodilation (Figure 6.2). Skin blood flow increases linearly with Tc until the point at which SkBF plateaus, as a consequence of competition for blood flow to working muscles to maintain exercise intensity as well as arterial blood pressure. Previous studies have attributed this plateau to the action of cardiopulmonary reflexes, as evidenced by the effect of changes in central blood volume and cardiac filling pressures on manifestation of the plateau (Nadel et al., 1980; Nielsen et al., 1984; Simmons et al., 2011b). For example, a study by Nielsen, Rowell and Bonde-Petersen revealed that water immersion to the xiphoid process increased cardiac output and stroke volume (via an upwards shift in central blood volume) and allowed for higher forearm blood flows for a given Tc during exercise in water (35°C) compared to exercise in air (45°C) (Nielsen et al., 1984).
Figure 6.2. A diagram showing initial increases in skin blood flow due to the withdrawal of cutaneous adrenergic vasoconstrictor outflow (VC). When core temperature continues to rise, substantial increases in skin blood flow are achieved via activation of cutaneous cholinergic vasodilator outflow (VD). Modified from (Kellogg, 2006).

Skin blood flow responses to passive heating are similar in most respects to those described above, with the exception there is no initial decrease in SkBF and, as there is no competition for blood flow with working muscles, SkBF increases to a higher level before plateauing (Figure 6.3) (Simmons et al., 2011b).

Exercise and passive heating thus present substantial thermal and cardiovascular challenges to homeostasis (Rowell, 1993). Skin blood flow plays a crucial role in the integrative systems response to exercise and body heating. In the subsequent sections, the impact of repeated exposure to exercise and body heating on cutaneous microvascular control will be addressed. This review will discuss firstly central thermoregulatory and cardiovascular adaptations to exercise and passive heating, followed by peripheral and intrinsic cutaneous adaptations. An important technical note is that early studies attempting to examine changes in forearm SkBF during lower limb exercise and whole body heating utilised volumetric or venous occlusion.
plethysmography. Although these techniques are measures of gross changes in whole limb blood volume, early studies assumed that blood flow to inactive muscle during passive heating within the instrumented limb did not change (Edholm et al., 1956; Roddie et al., 1956; Fox & Edholm, 1963). Recent studies, however, indicate that blood flow to inactive skeletal muscles during passive body heating does in fact increase, albeit not to significantly high levels (Keller et al., 2010; Pearson et al., 2011). Finally, in the context of this review, heat acclimation as a result of exercise training in the heat will be referred to as ‘exercise training-heat acclimation’.

![Figure 6.3](image)

**Figure 6.3.** Diagram showing the differences in the core temperature/skin blood flow relationship between exercise and passive heating. The plateau in skin blood flow occurs at a higher point during passive heating due to the absence of competition for blood flow to the working muscles as compared to exercise. Modified from (Simmons et al., 2011b).

### 6.2 Exercise training-heat acclimation induced central thermoregulatory and cardiovascular adaptations

Early studies examining the impact of exercise training and heat acclimation reported lower SkBFs for a given workload, post-acclimation. For example, 2-3 weeks of exercise in a hot environment (~45°C dry bulb) resulted in a decrease in forearm and
hand blood flows during workloads performed in the heat (Wyndham, 1951). A further study exposed 4 subjects to exercise in hot conditions (45°C dry bulb) for 3-8 days and noted forearm volume was lower during exercise in the heat, post-intervention (Whitney, 1954). The results of such studies suggested thermoregulatory related vascular adaptations following exercise in the heat, however these experiments were difficult to interpret due to limitations in technology and relatively poor methodological control.

More recently, a cross-sectional study by Tankersley et al. reported that forearm blood flows induced by 20 mins of cycling exercise in a 30°C room were significantly lower in elderly sedentary subjects compared to young individuals, and that no difference was evident between the young and elderly fit groups (Tankersley et al., 1991). The findings of this study suggested that aging is associated with a decline in the ability to lose heat and that exercise training can normalise this response. Indeed, training enhances heat loss responses during exercise in healthy young subjects, with Ho et al. reporting significant differences in SkBF between younger sedentary and fit subjects during exercise at 35% and 60% of VO$_{2\max}$ (Ho et al., 1997). These findings were reinforced by Fritzsche and Coyle who reported forearm SkBFs at 50%, 70% and 90% VO$_{2peak}$ were higher in trained, compared to untrained, individuals (Fritzsche & Coyle, 2000). Finally, a longitudinal study by Wang involving 8 weeks of exercise training in previously sedentary young subjects, reported forearm cutaneous vascular conductance during exercise significantly increased, post-intervention (Wang, 2005). These studies suggest that exercise training is associated with improved thermoregulatory capacity, as evidenced by higher forearm SkBFs during exercise in the adapted state. However, none of these experiments measured Tc, a key afferent input to the neural control of SkBF, nor how the observed changes in forearm limb blood flow and SkBF affected the ability to counter rises in Tc. Therefore, these studies did not directly assess thermoregulatory function.

A study by Roberts et al. exercise trained 8 young healthy subjects for 20 days (10 days in 25°C followed by 10 days in a 35°C environment) (Roberts et al., 1977) and reported that the Tc threshold for forearm vasodilation shifted leftwards following the exercise and heat acclimation period and that SkBF was higher for a given Tc, e.g., an increased SkBF/Tc slope or sensitivity, during exercise. Reinforcing these findings, a study by Thomas, Pierzga and Kenney reported, in older and young individuals whose VO$_{2\max}$ improved by at least 5% following 16 weeks of aerobic exercise training, a significant
leftwards shift in the Tc thresholds for forearm vascular conductance and SkBF (Thomas et al., 1999). The authors also noted that, whilst the threshold for vasodilation shifted leftwards, the slope of the increase in SkBF and forearm vascular conductance remained unchanged whilst the plateau of SkBF was significantly higher (Figure 6.4). This study also suggested that the leftwards shift in vasodilation was the result of adaptation in the control of sympathetic vasodilator nerves, rather than the vasoconstrictor system, as the threshold for vasodilation for SkBF was unchanged following infusion of bretylium.

![Figure 6.4](image)

**Figure 6.4.** Changes in forearm and cutaneous vascular conductance (FVC and CVC, respectively) and core temperature relationships following 16 weeks of exercise training. Exercise training resulted in a significant leftwards shift in the threshold for forearm vasodilation, post-intervention (A). A similar leftwards shift in the threshold for cutaneous vasodilation was evident, along with a significantly higher plateau, post-intervention (B) (Thomas et al., 1999).

To our knowledge, no studies in humans have directly assessed the impact of heat acclimation on adaptation in the hypothalamic neural control of thermoregulation. However, a study by Shido et al. in rats reported that anterior hypothalamic temperature was lower at the onset of vasodilation in the tail following 21 days of heat acclimation, indicating that heat exposure resulted in a downward shift in the set-point for thermoregulatory activation (Shido et al., 1995). A further study, using bromodeoxyuridine to identify newly born neural cells, indicated that continuous heat exposure in rats resulted in greater proliferation of progenitor cells in the hypothalamus, compared to the control group, following 5 days. The authors reported proliferation of hypothalamic progenitor cells continued for 20 days before differentiating to neurons, i.e. neurogenesis (Matsuzaki et al., 2009). This study strongly suggests that heat
exposure improves thermoregulatory capacity, at least in part via augmentation of neural pathways in the hypothalamus. Although interesting, these findings cannot be directly translated to humans due to differences in the neural circuitry and thermoregulatory effector mechanisms between both species. Therefore, the role of neural adaptations in the enhancement of thermoregulatory capacity in humans remains unclear. However, in a recent review by Simmons et al., the authors suggested that the shift in the skin and forearm blood flow/Tc relationship observed following exercise training and/or heat acclimation may potentially be explained by concomitant increases in central blood volume (BV) (Simmons et al., 2011b).

Increases in BV allow for a greater stroke volume (thus cardiac output), greater O₂ delivery to working muscles and a delay in the competition between muscle and SkBF during exercise, which ultimately improves aerobic performance. Numerous studies have reported increases in BV following 2-4 months of exercise training (Holmgren et al., 1960; Akgün et al., 1974; Ray et al., 1990; Convertino et al., 1991). Interestingly, changes in BV have been observed following very brief periods of exercise with a study by Green et al. reporting that 3 days of supramaximal exercise training increased total BV by 4.5% (Green et al., 1984), with further studies reporting increases following 8 (Convertino et al., 1980) and 10 days (Takeno et al., 2001). Blood volume has even been reported to increase following a single prolonged bout (8-10 hrs) of exercise (Pugh, 1969). However, the time-course of BV change is biphasic and dependent upon alterations in its constituents. In a comprehensive review of the literature by Convertino (Convertino, 1991), increases in plasma volume were reported to plateau following approximately 7 days of exercise. In studies where the exercise training interventions were longer in duration (>4 weeks), increases in BV were due to more evenly apportioned changes in both plasma volume and red cell mass.

As discussed above, BV increases can occur rapidly and may explain the cardiovascular and thermoregulatory adaptations observed following exercise training and/or heat acclimation. A recent study by Ikegawa et al. sought to determine whether increases in plasma volume were responsible for vascular and thermoregulatory adaptations, by assessing thermoregulatory capacity whilst in a euhydrated and hypohydrated state prior to, and following, 5 days of aerobic training in a 30°C/50% relative humidity room (Ikegawa et al., 2011). Consistent with previous literature, exercise training increased plasma volume. In addition, greater forearm vascular conductance for a given Tc was
observed following the intervention when subjects were euhydrated (Figure 6.5A). However, this adaptation was not as apparent if the exercise training-induced increases in plasma volume were reversed by hypohydration (Figure 6.5B). This study supports the suggestion that the mechanism behind enhanced SkBF during exercise in the acclimated state is central in origin and due to increased BV. However, a study by Takeno, Kamijo and Nose reported that despite similar increases in plasma and blood volume in 4 groups (simulated low-altitude cool and warm and high-altitude cool and warm environments) following 10 days of exercise training were observed, there was a significant difference between the slope of the forearm skin vascular conductance/Tc relationship in the high-altitude warm condition compared to the others. This study therefore suggests that the mechanisms for improved thermoregulatory/cardiovascular responses are not entirely central in origin (Takeno et al., 2001).

**Figure 6.5.** Forearm skin vascular conductance (FVC) and core temperature (T<sub>es</sub>) relationship before and after the aerobic exercise intervention in a euhydrated (A) and hypohydrated (B) state. Aerobic exercise training resulted in a significant increase in plasma volume and a greater FVC for a given T<sub>es</sub> post-intervention (A), however once the increase in plasma volume was reversed by hypohydration, this adaptation was less evident (B). Modified from (Ikegawa et al., 2011).
6.3 Passive heating induced central thermoregulatory and cardiovascular adaptations

Fewer studies have been performed on cardiovascular and thermoregulatory adaptations following repeated bouts of whole body passive heating. However, a study by Fox et al. assessed changes in Tc and forearm and hand blood flow before and after 12-24 days of passive heating (Fox et al., 1963). The authors reported that Tc was lower during the early stages of heat exposure, post-intervention. Furthermore, forearm and hand blood flows were higher during a standardised heat stress test following the intervention. This study therefore suggests that passive heating alone can provide a stimulus for adaptation of the SkBF/Tc relationship. However, it did not provide any insight into the mechanisms responsible. Interestingly, a study by Convertino, Greenleaf and Bernauer revealed that repeated passive heating, independent of exercise, can induce an increase in BV (Convertino et al., 1980). In an attempt to determine the independent impact of thermal stress on BV changes, the authors recruited two groups: an exercise and a passive heating group. Subjects in the exercise group performed 8 days (2 hrs/daily) of exercise in a controlled environment (25°C at 60% relative humidity), whereas subjects in the heating group sat in a 42°C (dry bulb) room at 98% relative humidity. The increases in Tc induced by each modality were matched. Plasma volume was found to increase by 427ml in the exercise group whilst the passive heating group increased by 177ml, post-intervention (Figure 6.6). The authors concluded that the effects of thermal stress induced by exercise accounts for approximately 40% of the resultant hypervolemia.
6.3.1 Summary

The literature described above suggests that exercise training in the heat, and repeated passive heating, are associated with a shift in the relationship between SkBF and Tc. Furthermore, studies have suggested that this response is due to central adaptations, and in particular, increased blood volume. However, the studies above assessed SkBF responses to systemic stimuli (exercise and heat exposure) and it is difficult to ascertain, from such studies, whether the differences in SkBF observed following exercise-heat acclimation are due, at least in part, to intrinsic peripheral cutaneous adaptations. The subsequent section of this review will address the question of whether repeated exercise and/or passive whole body heating induce functional or structural changes in the cutaneous microvasculature.

6.4 Exercise training-heat acclimation induced cutaneous microvascular adaptations

A cross sectional study by Kvernmo et al. reported that endurance trained athletes exhibit larger localised ACh-induced forearm SkBF responses, compared to healthy controls (Kvernmo et al., 1998). However, no difference in SkBF was evident between the groups following infusion of SNP, suggesting that exercise training is associated
with enhanced cutaneous microvascular endothelium-dependent vasodilator function. In a further study, larger increases in SkBF on the dorsum of the hand were observed following iontophoresis of ACh in endurance trained cyclists compared to sedentary subjects (Lenasi & Strucl, 2004). Lastly, enhanced endothelium-dependent cutaneous vasodilation was observed in the legs of trained Tai Chi Chuan individuals, compared to age matched sedentary controls (Wang et al., 2002). Whilst these cross-sectional studies reported enhanced cutaneous endothelium-dependent function, Boegli et al. reported no difference in forearm SkBF responses to iontophoresis of ACh between trained and sedentary young and older individuals. These authors also reported SNP responses were higher in trained, compared to sedentary, subjects (Boegli et al., 2003). Using a different measure of cutaneous vasodilator function, Roche et al. reported that trained adolescents exhibited greater forearm SkBFs in response to rapid local heating (44°C), compared to sedentary controls (Roche et al., 2010). As mentioned earlier in this review, the secondary rise in SkBF in response to rapid local heating is primarily mediated by NO (Minson et al., 2001), therefore this study is consistent with the theme that exercise training improves intrinsic cutaneous endothelium-mediated vasodilator function. Skin blood flows following a reactive hyperaemic test were also found to be higher in trained adolescents, however once presented as percent (%) change from baseline the increase was non-significant. In support of the notion that exercise training can enhance cutaneous vascular function, a cross-sectional study by Tew et al. observed no difference between the older trained subjects and the young untrained subjects in the initial SkBF peak response to rapid heating (Tew et al., 2011), indicating that exercise training is associated with enhanced sensory nerve mediated function. The disparity between these studies may highlight the important limitation inherent in cross-sectional comparisons between individuals as a means of assessing the impacts of exercise training.

A longitudinal study involving 8 weeks of cycle exercise training in healthy, previously sedentary, subjects reported that forearm cutaneous vasodilation in response to ACh was significantly enhanced post-intervention, whilst no difference was evident in response to SNP (Wang, 2005). A mechanistic study performed by Black et al. (Black et al., 2008b) involving a cross-sectional comparison between young and elderly sedentary individuals revealed that aging is associated with a decline in NO-mediated microvascular vasodilation in response to both local heating and ACh infusion. However, no difference in NO-mediated microvascular function was evident between
recreationally active young and elderly fit individuals, suggesting that exercise training can prevent this natural decline. The authors also reported that in the elderly sedentary individuals who initially displayed impaired microvascular endothelium function, 24 weeks of exercise training increased the NO-mediated contribution to SkBF in response to local heating (Figure 6.7). Reinforcing the finding that exercise training improved cutaneous microvascular endothelial function, the NO-contribution to ACh induced SkBF significantly increased post-intervention.

![Nitric oxide contribution to skin blood flow](image)

**Figure 6.7.** Nitric oxide contribution to cutaneous vascular conductance (CVC) pre- and post-intervention. Exercise training (24 weeks) in elderly sedentary subjects resulted in a significant increase in NO-mediated skin blood flow in response to a gradual local heating protocol, indicating enhanced cutaneous microvascular endothelial function. Modified from (Black *et al.*, 2008b).

In a recent study, Lorenzo and Minson recruited highly trained cyclists for a 10 day heat acclimation intervention (40°C at 30% relative humidity). Cutaneous microvascular function was enhanced, as evidenced by increased forearm SkBF in response to incremental ACh infusions, post-intervention. The authors also reported that maximal SkBF did not change following the heat acclimation period, consistent with Wang (Wang, 2005), suggesting there were no structural adaptations in the cutaneous microvasculature. However, structural adaptations following just 10 days would be unlikely and the subjects recruited were highly trained athletes at entry. These studies confirm the majority of the cross-sectional literature above, suggesting that exercise training improves endothelium-dependent vasodilation of the local cutaneous
vasculature. To date, no study has thoroughly examined cutaneous structural changes in recreationally active young subjects following a longer exercise protocol.

6.5 Passive heating induced cutaneous microvascular adaptations

To date, no study has directly assessed the effects of repeated systemic passive heating on peripheral cutaneous microvascular adaptation in humans. However, a recent study by Green et al. revealed that 8 weeks of bilateral forearm heating (using separate water baths thermostatically controlled at 42°C) resulted in a significant increase in SkBF responsiveness to a local heating protocol, post-intervention (Figure 6.8) (Green et al., 2010). The local heating protocol used in this study induced a largely NO-mediated vasodilator response (Black et al., 2008b), thereby indicating that the 8 week repeated passive heating protocol induced changes in intrinsic microvascular NO-mediated vasodilator function, a finding consistent with responses induced by exercise training (Black et al., 2008b). Furthermore, a pneumatic cuff was placed around one forearm and inflated to 100 mmHg during each heated bath exposure of that forearm to unilaterally restrict SkBF. No cutaneous microvascular adaptation was evident in the cuffed arm, suggesting that repeated increases in SkBF are obligatory for microvascular adaptations. However, this study involved local heating of the forearms and did not evoke central thermoregulatory reflexes or increases in Tc.
Figure 6.8. Cutaneous vascular conductance (CVC) in response to local heating. Eight weeks of bilateral forearm warm water immersion resulted in an increased SkBF responsiveness to local heating in the uncuffed arm (A). The cuffed arm displayed no adaptation (B). Modified from (Green et al., 2010).
6.6 Summary
Regulating blood flow to the skin is the body’s primary effector mechanism in the homeostatic control of core body temperature, a physiological function that is imperative and if not tightly controlled has deleterious effects on systemic cellular activity and function. The control of SkBF is also vital in the maintenance of blood pressure. The regulation of SkBF is achieved via complex interplay between both neural and local mechanisms and a scenario in which these mechanisms would be active is exercise training and passive heating. There are numerous cardiovascular and thermoregulatory adaptations in response to exercise training-heat acclimation and passive heating, both central and peripheral in origin. The literature summarised in this review suggests that the primary central adaptation is an increase in BV, allowing for greater thermoregulatory capacity and enhanced performance by prolonging the point at which SkBF is compromised by competition with working muscles. The role, or even presence, of hypothalamic neural adaptation in enhancing thermoregulation in humans is currently unknown. Heat acclimation and passive heating have also been shown to induce cutaneous microvascular adaptation. Whilst the mechanisms responsible for mediating these intrinsic improvements are largely unknown, stimuli such as SkBF (shear stress) and skin temperature are potential candidates. Notably, no previous study has examined the integrative response of central and peripheral adaptations to repeated Tc elevations. In experiments contained in this thesis, we aimed to investigate the independent effects of SkBF and skin temperature on cutaneous microvascular adaptation to repeated increases in Tc induced by lower limb heating. We also assessed SkBF responses during a bout of lower limb heating, before and after repeated Tc elevation to determine whether there are central changes in the control of SkBF.
Chapter 7

Study 4

Cutaneous microvascular adaptation to repeated-passive core heating in humans: Combined impacts of skin blood flow and temperature on adaptation


7.0 Abstract

Local passive heating of the forearm induces microvascular adaptations mediated, in part, by increases in skin blood flow. However, the impact of reflex-mediated increases in skin blood flow induced by core temperature elevation on microvascular adaptation, has not been assessed. The purpose of the current study was to examine the effect of repeated increases in core temperature, and consequent increases in forearm skin blood flow and temperature, on microvascular adaptation. Ten healthy young volunteers participated for 8 weeks in thrice weekly bouts of 30 mins lower limb heating (40°C). Throughout each lower limb heating session, a forearm cuff was placed on one arm and inflated to unilaterally restrict reflex-mediated blood flow responses. Core temperature, forearm skin blood flow, skin temperature and sweat rate increased during lower limb heating in the uncuffed arm ($P<0.001$), while responses in the cuffed arm were significantly attenuated. We observed an increase in skin blood flow to a local heating stimulus after 8 weeks of repeated leg heating bouts ($P<0.05$), an adaptation not evident in the cuffed arm. We also observed an increase in skin blood flow and sweat rate for a given core temperature during lower limb heating following the 8 weeks. Our findings suggest that forearm hyperaemia induces cutaneous microvascular adaptations indicative of enhanced vasodilator function.
7.1 Introduction

In an effort to assess the impact of repeated local heating of the skin on microvascular adaptation, Green et al. recently recruited subjects to undertake thrice weekly bouts of bilateral forearm heating across an 8 week intervention period (Green et al., 2010). A cuff was placed around one forearm during each heating session to determine the role of blood flow responses in local microvascular adaptation. The authors reported that repeated forearm heating increased forearm skin blood flow (SkBF) responses to a local heating protocol, an adaptation which was not apparent in the contralateral cuffed arm in which increases in SkBF were attenuated during bouts of forearm heating. This study suggested that improvements in microvascular function observed following repeated local heating (to 42°C) are partly mediated by the attendant episodic increases in SkBF. However, this experiment did not invoke large changes in core temperature (Tc) and therefore did not assess the impact of repeated thermoregulatory reflex-induced increases in cutaneous microvascular blood flow.

In the present study, subjects repeatedly sat for 30 mins in a custom-designed enclosed lower body heating bath (40°C) over 8 weeks. This allowed us to address the impact of exercise-independent Tc-induced increases in SkBF on intrinsic cutaneous adaptation. To this end, a cuff was also inflated on one forearm to unilaterally manipulate SkBF responses during the repeated bouts of lower limb heating. Using these within-subject, simultaneous assessment approaches, the following question was posed: Does repeated exposure to Tc elevation due to lower limb heating, and attendant increases in SkBF and skin temperature, induce adaptation in forearm cutaneous responses. We assessed the impact of repeated Tc elevation on the relationship between Tc and SkBF during lower limb heating, and in response to a localised heating stimulus.

7.2 Methods

Ethical Approval

This study complied with the Declaration of Helsinki and the Human Research Ethics Committee of the University of Western Australia approved the experimental protocol. All subjects provided written, informed consent before participating in the study.

Subject Characteristics

Ten young, healthy recreationally active (≤2 hrs of physical activity per week) males were recruited (25.8 ± 3.1 yrs, see Table 7.1). Subjects had no history of cardiovascular,
musculoskeletal or metabolic disease, did not smoke or take medication. Women were excluded from this study due to the effects of oestrogen on haemodynamic and vascular variables.

Table 7.1. Baseline subject characteristics

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<tr>
<td>Age (years)</td>
<td>25.8 ± 3.1</td>
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<tr>
<td>Height (m)</td>
<td>1.78 ± 0.09</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78.8 ± 8.2</td>
</tr>
<tr>
<td>BMI (kg.m$^{-2}$)</td>
<td>25.0 ± 1.8</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>93 ± 5</td>
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<tr>
<td>Heart rate (bpm)</td>
<td>62 ± 5</td>
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Values are mean ± SD

Study Design
All subjects underwent baseline assessments and were then required to attend the laboratory 3 times per week, for 8 weeks. During each of these sessions, subjects were seated in a custom-designed inflatable recovery bath (IC-iBody; iCoolsport, Queensland, Australia) and immersed up to their waist in warm water (40°C) for a period of 30 mins. The water bath temperature was maintained and continuously circulated via a heating pump (IC-Heat; iCoolsport, Queensland, Australia). A thick plastic sheet was placed over this bath to isolate the heating stimulus to the legs, leaving the upper body in ambient air. Finally, a pneumatic cuff was positioned and inflated to 80 mmHg around one forearm throughout each of the 30 min heating periods, to attenuate the increase in SkBF.

Experimental Procedures
Acute impact of leg heating on forearm skin blood flow, temperature and sweat rate responses at study entry
At study entry, to assess the efficacy of manipulation of our independent variable, SkBFs were determined using 7 Doppler array laser probes (Model 413; Periflux 5001 System, Perimed AB, Sweden) during the experimental condition described above. That is, SkBF was assessed across a 30 min period during isolated lower limb heating (40°C). The impact of forearm cuff placement was also assessed. Skin blood flow measures were collected simultaneously in both arms, effectively eliminating any impact of systemic haemodynamics. The data are presented in cutaneous vascular
conductance (CVC). Sweat rate (SR) was also measured throughout the lower limb heating protocol on the ventral sides of both forearms using the ventilated capsule method. Perspex sweat capsules (1.33 cm²) were positioned and attached to the skin by the adhesive Collodion. Dry nitrogen gas was supplied to each capsule at a flow rate of 600 mL/min and the humidity and temperature of the nitrogen gas leaving the capsules was measured by a capacitance hygrometer (HMP60, Vaisala, Helsinki, Finland).

**Impact of repeated lower limb heating on the relationship between core temperature and forearm skin blood flow responses**

To assess the impact of repeated leg heating on adaptations in forearm SkBF, temperature, SR, Tc and systemic haemodynamics, a sub-group of 8 subjects (25.1 ± 3.1 yrs) had measures collected throughout the leg heating protocol described above at weeks 0 and 8. Laser Doppler (Model 413; Periflux 5001 System, Perimed AB, Sweden) was used to record changes in SkBF throughout the 30 min leg immersions. Core temperature (RET-1, Physitemp Instruments, NJ, USA) was measured from rectal temperature probes throughout immersion. In this way it was possible to assess the impact of repeated episodic elevation in Tc on adaptations in forearm SkBF during an acute bout of lower limb heating undertaken at pre- and post-intervention.

**Impact of repeated lower limb heating on forearm skin blood flow responses to localised heating**

At weeks 0 and 8, all subjects attended an additional laboratory session having fasted for a minimum of 8 hours and abstained from alcohol, caffeine and vigorous exercise for at least 24 hours. These studies were conducted in a quiet, euthermic environment with subjects at rest and seated comfortably. The assessments were performed at the same time of day. They did not involve lower limb heating or cuffing or any intervention other than localised heating of the skin using heater discs, as described below.

Laser Doppler probe sites on each forearm were shaved and cleaned 24 hours prior to the laboratory attendance. Photographs were taken of the sites and measurements made from bony anthropometric landmarks at baseline so that similar placement sites were selected on each forearm for repeated measures at 8 weeks. Local heater discs (Perimed 455, Stockholm, Sweden) were attached to the forearms using double-sided adhesive rings. The 7 laser Doppler array probes were then fitted into the middle of the localised
heating discs. Room temperature was controlled and recorded throughout all assessments.

Once instrumented, the heater discs were increased to 33°C and remained at this temperature for a 20 min baseline period. The heater discs were then increased in increments of 0.5°C every 5 mins until 42°C was reached, so as to minimise any impact of axon reflexes (Minson et al., 2001) which are less NO dependent than the protocol adopted in this study (Black et al., 2008b). Finally, the heater discs remained at 42°C for a further 30 mins. Recent papers have established that SkBF in response to this gradual heating protocol is NO-mediated (Minson et al., 2002; Black et al., 2008b). Blood pressure was recorded every 5 mins at the ankle using a Dinamap automated monitor and later corrected for the hydrostatic column (Groothuis et al., 2008) and used to calculate CVC. All laser Doppler, room and Tc measurements were relayed and recorded in real time onto a laptop using the software program LabChart 7 (ADinstruments, Sydney, Australia).

Data Analysis

*Laser Doppler Protocol*

Skin perfusion unit (PU) data from the cuffed and uncuffed arms were averaged over a stable 30 sec period at the end of every 5 min interval to assess SkBF. Calibration of the probes was undertaken before the experiments using two generic points, 0 and 250 PU, in accordance with calibration guidelines using a zeroing disk and motility standard (Periflux System, Perimed AB, Sweden). Measurements in PU were converted to CVC which was calculated as PU/Dinamap mean arterial pressure (MAP). All core and room temperature readings were averaged at the end of each 5 min interval in °C.

*Statistical Analysis*

Skin CVC outcome data were compared within-subjects, across 2 study time points (0 and 8 weeks) using 2-factor ANOVA with planned comparisons performed on four temperature points; 34°C, 40-42°C and 42°C+30 mins. Post hoc t-tests were performed where significance was detected at \( P<0.05 \), using LSD tests to correct for multiple comparisons.
7.3 Results

Acute impact of lower limb heating on forearm skin blood flow, temperature and sweat rate responses at study entry

Resting SkBF was similar between forearms ($P=0.29$). Skin blood flow increased in both the uncuffed and cuffed arms during 30 mins of lower limb heating ($P<0.001$ and $P<0.001$, respectively; Figure 7.1A). Inflation of the cuff significantly reduced the increase in SkBF in the cuffed arm compared to the uncuffed ($P<0.001$), with differences at all time points ($P<0.05$) (Figure 7.1A). Similarly, skin temperatures in the uncuffed arm significantly increased across the 30 min lower limb heating period ($P<0.001$; Figure 7.1B). However, in the contralateral limb, cuff inflation abolished increases in skin temperature throughout the heating bout. Finally, SR increased in both the uncuffed and cuffed arms across the heating bout ($P<0.001$ and $P<0.001$, respectively; Figure 7.1C). However, inflation of the cuff significantly reduced the increase in SR in the cuffed arm compared to the uncuffed ($P<0.05$; Figure 7.1C) with differences at 10, 15, 20, 25 and 30 mins (all $P<0.05$).

Impact of repeated lower limb heating on the relationship between core temperature and forearm skin blood flow responses

Forearm SkBF and SRs did not significantly change across the 8 weeks in response to lower limb heating (Figure 7.2). However, resting Tc at week 8 was significantly lower compared to week 0 (37.2±0.3 vs 36.9±0.2, $P<0.001$). Similarly, Tc was significantly lower during lower limb heating at week 8 compared to week 0 ($P<0.001$) with differences at every 5 min interval ($P<0.05$) across the 30 mins (Figure 7.2C). When averaged change in SkBF and SR values from baseline at each time point were plotted against change in Tc across the 30 min session at weeks 0 and 8, a higher SkBF was evident for a given change in Tc (Figure 7.3A), however this response was not evident in SR (Figure 7.3B). Furthermore, when averaged absolute SkBF and SR values were compared against Tc at all time points between weeks 0 and 8, a leftwards shift in the threshold for SkBF and SR activation was evident (Figure 7.4).
Figure 7.1. Forearm cutaneous vascular conductance (CVC) (A), skin temperature (B) and sweat rate (C) in the cuffed (open squares) and uncuffed (solid squares) forearms at baseline and during 30 mins of lower limb heating (5 min intervals). Significantly different at $P<0.05$ from baseline ($\#$) or between the cuffed and uncuffed forearms (*). Data are mean ± SE.
Figure 7.2. Forearm cutaneous vascular conductance (CVC) (A), sweat rate (B) and core temperature (C) in response to 30 mins of lower limb heating at weeks 0 (open circle) and 8 (solid circle) in a subgroup of 8 subjects. Significantly different at $P<0.05$ from baseline ($^#$) or between weeks 0 and 8 (*). Data are mean ± SE.
Figure 7.3. Change in forearm cutaneous vascular conductance (CVC) (y axis) (A) and sweat rate (B) versus change in core temperature (x axis) during 30 mins of lower limb heating (5 min intervals) at weeks 0 (open circle) and 8 (solid circle). Data are derived from Figure 7.2.

Mean arterial pressure decreased during lower limb heating at week 0 (95.9±6.6 vs 85.4±7.5 mmHg, *P*<0.01, Figure 7.5A), primarily mediated by a drop in total peripheral resistance (*P*<0.05, Figure 7.5B). Cardiac output increased during the 30 min heating bout (*P*<0.001, Figure 7.5C) along with heart rate (*P*<0.001, Figure 7.5D). Stroke volume increased non-significantly by 10 mins, however returned to baseline levels by 30 mins (Figure 7.5E). There was a small but significant decrease in resting heart rate between weeks 0 and 8 (*P*<0.05). Similarly, a small increase in resting stroke volume was evident post-intervention (*P*<0.05). However, none of the systemic haemodynamic responses during 30 mins of leg heating differed between weeks 0 and 8 (Figure 7.5).
Figure 7.4. Absolute forearm cutaneous vascular conductance (CVC) (A) and sweat rate (B) against core temperature during 30 mins of lower limb heating (5 min intervals) at weeks 0 (open circle) and 8 (solid circle). Data are derived from Figure 7.2.

Impact of repeated lower limb heating on skin blood flow responses to a standardised local heating stimulus

Mean CVC values at 33°C were similar between weeks 0 and 8 in the cuffed arm (0.22±0.06 vs 0.29±0.09) and there was no significant difference in the response to incremental heating between weeks 0 and 8 in the cuffed arm ($P$=0.69; Figure 7.6A). In the uncuffed arm, mean CVC values at 33°C remained similar across weeks 0 and 8 (0.20±0.07 vs 0.23±0.11). SkBF responses to incremental heating between weeks 0 and 8 increased ($P$<0.05) (Figure 7.6B).
Figure 7.5. Change in mean arterial pressure (A), total peripheral resistance (B), cardiac output (C), heart rate (D) and stroke volume (E) during 30 mins of lower limb heating at weeks 0 (open circle) and 8 (solid circle). Significantly different at $P<0.05$ from baseline ($\hat{r}$) or between weeks 0 and 8 (*). Data are mean ± SE.
Figure 7.6. Forearm cutaneous vascular conductance (CVC) during the gradual heating protocol in the cuffed (A) and uncuffed (B) arms and at weeks 0 (open circle) and 8 (solid circle). *Significantly different from week 0 at $P<0.05$. Data are mean ± SE.
7.4 Discussion

In the present study, we have shown that 8 weeks of lower limb heating induces an increase in SkBF responsiveness to local heating. This adaptation was observed in the forearm that was exposed to increases in SkBF and skin temperature, however no adaptation was evident in the contralateral cuffed arm. This study reinforces the notion that local cutaneous microvascular adaptation is dependent upon hyperaemia. Following the intervention, we also observed higher SkBF and SR’s for a given Tc during lower limb heating, suggesting that repeated core heating is associated with central adaptation and possibly hypervolemia.

Our principal finding relates to the impact of repeated Tc elevation on responses to local heating-induced increases in SkBF. A previous study by Green et al. observed similar local cutaneous adaptations following 8 weeks of bilateral forearm heating (42°C) (Green et al., 2010). However, this study did not invoke large increases in Tc and therefore did not assess whether repeated thermoregulatory reflex-mediated increases in forearm SkBF can induce cutaneous microvascular adaptation. The current study therefore extends the findings of Green et al. by observing similar enhancement in cutaneous microvascular function in response to repeated systemic thermoregulatory reflex-mediated increases in forearm SkBF. As reported by Black et al. (Black et al., 2008b), the gradual local heating protocol employed in this study is known to induce a largely NO-dependent vasodilator response, therefore the increased microvascular function we observed in response to local heating is likely due to increased NO bioavailability post-intervention. No adaptation was observed when increases in SkBF during lower limb heating was attenuated by partial inflation of a cuff, consistent with Green et al., indicating the principal stimulus for cutaneous microvascular adaptation is increases in SkBF and/or skin temperature. It is well established that enhanced vascular function in larger conduit arteries following exercise training is due to increases in blood flow and shear stress. For example, Tinken et al. reported 8 weeks of handgrip exercise training increased brachial artery function (by flow-mediated dilation), however no adaptation was present when increases in flow and shear were attenuated during each exercise bout by inflation of a cuff just below the elbow (Tinken et al., 2010). A study by Hambrecht et al. reported that the exercise-induced increase in vascular function is due to shear-mediated upregulation of eNOS phosphorylation, subsequently increasing NO bioavailability (Hambrecht et al., 2003). The findings of the present study reinforce the suggestion blood flow and shear are important stimuli in
cutaneous microvascular adaptation, just as they are in upstream larger arteries. However, the cutaneous vasculature has complex control mechanisms, all of which may adapt to different stimuli, or combinations of stimuli, in distinct ways and further studies are required to truly determine the independent impacts of SkBF and skin temperature on cutaneous microvascular adaptation.

An additional finding of this study relates to the leftwards shift in the onset of activation of SkBF and SR responses during systemic (lower limb) heating, whereby higher values for both measures were observed for a given Tc post-intervention. It is generally accepted that acclimation results in increases in SkBF at an earlier Tc, and to a higher plateau level (Simmons et al., 2011b). Classic human integrative physiology studies furthermore suggest that whole-body heat exposure during brief periods of exercise training (referred to by some as “acclimation”), exaggerates this response (Roberts et al., 1977). A recent review (Simmons et al., 2011b) and paper (Ikegawa et al., 2011) attributed these effects on the SkBF/Tc relationship to changes in blood volume, although the latter study occurred across a brief timeframe and there is some evidence that blood volume changes are time-dependent (Sawka et al., 2000). Interestingly, the sensitivity, or rate of increase, of SkBF during lower limb heating observed in the current study increased post-intervention, whereas no such adaptation was observed in SR responses. These distinct responses suggest the current experimental protocol may have, to some degree, uncoupled changes in SkBF and SR. This may be a novel finding in terms of adaptation in the skin. The relative increase in SkBF for a given Tc that we observed is consistent with that previously observed (Fox et al., 1963; Simmons et al., 2011b) and strongly implies central blood volume expansion.

7.5 Conclusion
In summary, this study indicates that repeated thermoregulatory reflex-induced increases in forearm SkBF induced both local and central cutaneous microvascular adaptations. This experiment confirms our previous finding that repeated increases in skin blood flow are obligatory for inducing local cutaneous microvascular adaptation. We also observed higher SkBF and SRs for a given Tc during lower limb heating post-intervention, an adaptation likely to be central in origin (i.e. increased BV). In response to interventions that repeatedly increase Tc, it appears that adaptations in both microvascular function and blood volume may combine to enhance thermoregulatory capacity.
Chapter 8

Study 5

Cutaneous microvascular adaptation to repeated-passive core heating in humans: Isolating the impacts of skin blood flow and temperature


8.0 Abstract

Repeated increases in skin blood flow, induced by elevated core temperature, lead to forearm cutaneous microvascular adaptations which are partly mediated by repeated increases in cutaneous blood flow and/or skin temperature. In this study we aimed to examine the hypothesis that repeated exercise-independent increases in core temperature, and consequently skin blood flow, induce microvascular adaptations which are independent of changes in skin temperature. We recruited 9 healthy young volunteers to participate for 8 weeks in thrice weekly bouts of 30 mins lower limb heating (40°C) in a custom-designed and enclosed bath. Throughout each leg heating session both forearms were placed in their own water baths, maintained at a constant basal skin temperature (30°C). Finally, a forearm cuff was placed on one arm during each lower limb heating bout and inflated to unilaterally restrict reflex-mediated blood flow responses during core temperature elevation. Lower limb heating bouts induced
increases in core temperature \((P<0.001)\) and forearm skin blood flow \((P<0.001)\), with skin responses significantly attenuated in the cuffed forearm \((P<0.01)\). After 8 weeks of repeated exposure to lower limb heating, there was an upward and leftward shift in the relationship between changes in core temperature and skin blood flow during a bout of leg heating. In contrast, skin blood flow responses to a local heating protocol \textit{decreased} in the uncuffed \((P<0.05)\), but not in the cuffed arm, following 8 weeks. Our findings are consistent with a hypervolaemic impact of repeated increases in core temperature, but also indicate that repeated increases in core temperature and skin blood flow induce prolonged cutaneous microvascular transit time, possibly suggesting increased microvascular capillarity.

8.1 Introduction

We recently performed a series of experiments aimed at examining cutaneous microvascular adaptations in response to both local and systemic passive heating–induced changes in forearm skin blood flow (SkBF). In the first of these studies (Green \textit{et al.}, 2010), 8 weeks of bilateral forearm heating (30 min bouts, thrice weekly, 42°C) \textit{increased} local SkBF responses to a localised heating stimulus. This adaptation was not apparent in a cuffed forearm, suggesting an important mechanistic role for repeated episodic increases in SkBF and/or temperature. However, this study involved repeated increases in SkBF in response to a localised heating stimulus and core temperature (Tc) was not affected.

In an effort to further elucidate whether cutaneous microvascular adaptations occur in response to exercise-independent increases in Tc, we undertook a further experiment (summarised in the previous chapter) involving repeated Tc elevation induced by having subjects sit in a warm bath up to their waist (30 mins, thrice weekly, 40°C). As with the previous study, a pneumatic cuff was positioned around one arm during each heating bout to attenuate increases in SkBF. Following this, the uncuffed forearm exhibited \textit{increased} SkBF responses to local heating. As this change was not apparent in the cuffed limb, we again conclude that repeated episodic increases in SkBF contribute to cutaneous microvascular adaptation.

Taken together, the above findings suggest that repeated increases in SkBF may be obligatory for cutaneous microvascular adaptation. However, in the study summarised in Chapter 7, increases in skin temperature occur along with reflex-mediated increases
in SkBF. It is therefore possible that the local microvascular adaptation observed in the previous chapter may, in part, be explained by repeated local heating of the skin rather than to episodic effects of Tc elevation on SkBF. In the present study we therefore assessed the impact of repeated exercise-independent increases in Tc, which induced reflex-mediated increases in SkBF, on cutaneous microvascular adaptation. To address the distinct impact of repeated increases in SkBF, independent of attendant increases in skin temperature, both forearms were placed in thermostatically controlled water baths maintained at resting skin temperature (30°C) throughout each lower limb heating session. Finally, a pneumatic cuff was positioned around one forearm to attenuate increases in SkBF. In contrast to our recent experiment, this model allowed for the assessment of the impact of SkBF, independent of changes in skin temperature, on local cutaneous microvascular adaptation to repeated passive core heating.

8.2 Methods

Ethical Approval

This study complied with the Declaration of Helsinki and the Human Research Ethics Committee of the University of Western Australia approved the experimental protocol. All subjects provided written, informed consent before participating in the study.

Subject Characteristics

Nine young, healthy recreationally active (≤2 hrs of physical activity per week) males were recruited (24.3 ± 2.9 yrs, see Table 8.1). Subjects had no history of cardiovascular, musculoskeletal or metabolic disease, did not smoke or take medication. Women were excluded from this study due to the effects of oestrogen on haemodynamic and vascular variables.

<table>
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<tr>
<th>Table 8.1. Baseline subject characteristics</th>
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<tr>
<td>Age (years)</td>
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<td>Height (m)</td>
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<td>BMI (kg.m⁻²)</td>
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<td>Mean arterial pressure (mmHg)</td>
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<td>Heart rate (BPM)</td>
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Values are mean ± SD
Study Design

All subjects underwent baseline assessments and were then required to attend the laboratory 3 times per week, for 8 weeks, to undertake lower limb heating sessions. Specific details regarding the heating bout are provided in Chapter 7 (Page 125).

To eliminate the effect of lower limb heating and Tc-induced reflex increases in SkBF on forearm skin temperature, each forearm was immersed up to the elbows in thermostatically controlled euthermic water throughout each of the 30 min lower limb heating bouts. Pilot studies indicated that resting forearm skin temperatures averaged ~30°C in our laboratory, therefore these water baths were set and maintained at this basal temperature. Finally, a pneumatic cuff was positioned and inflated to 80 mmHg around one forearm throughout each of the 30 min heating periods, to attenuate the increase in SkBF. In this way, we controlled for the impact of skin temperature during core heating whilst manipulating SkBF bilaterally.

Experimental Procedures

Acute impact of lower limb heating on forearm skin blood flow responses at study entry

At study entry, to assess the efficacy of manipulation of our independent variable, SkBFs were determined using 7 Doppler array laser probes (Model 413; Periflux 5001 System, Perimed AB, Sweden) during the experimental condition described above. That is, SkBF was assessed across a 30 min period during isolated lower limb heating (40ºC) under circumstances where both forearms were immersed at 30ºC. The impact of forearm cuff placement during both studies was also assessed. As in the previous chapter, SkBF measures were collected simultaneously in both arms, effectively eliminating any impact of systemic haemodynamics. The data are presented in cutaneous vascular conductance (CVC).

Impact of repeated lower limb heating on core temperature and forearm skin blood flow responses

To assess the impact of repeated lower limb heating on adaptations in the relationship between Tc and SkBF, a sub-group of 6 subjects (23.3 ± 1.8 yrs) had measures collected throughout the lower limb heating protocol described above, with the difference that these assessments at week 0 and 8 were performed in the absence of the forearm temperature baths, with the arms in ambient air and at atrial level. Laser Doppler probes (Model 413; Periflux 5001 System, Perimed AB, Sweden) were used to record
changes in SkBF throughout the 30 min heating bout. Core temperature (RET-1, Physitemp Instruments, NJ, USA) was measured from rectal temperature probes throughout immersion.

**Impact of repeated leg heating on skin blood flow responses to localised heating**

Specific details relating to this technique are provided in Chapter 7 (Pages 126-127). Briefly, bilateral forearm SkBF responses to a gradual local heating protocol (from 33°C to 42°C) was assessed at study entry and following the intervention.

**Statistical Analysis**

Skin blood flow CVC outcome data were compared within-subjects, across 2 study time points (0 and 8 weeks) using 2-factor ANOVA with planned comparisons performed on four temperature points; 34°C, 40-42°C and 42°C+30 mins. Post hoc t-tests were performed where significance was detected at $P<0.05$, using LSD tests to correct for multiple comparisons.

### 8.3 Results

**Acute impact of lower limb heating on forearm skin blood flow responses at study entry**

Despite being bathed at a constant skin temperature of 30°C, forearm SkBFs increased significantly in both the uncuffed and cuffed forearms during 30 mins of lower limb heating at 40°C ($P<0.001$ and $P<0.001$, respectively; Figure 8.1A). However, inflation of the cuff around one forearm significantly attenuated the increase in SkBF compared to the uncuffed arm ($P<0.01$). Skin temperatures were maintained in both arms at 30°C throughout the heating session (Figure 8.1B), in contrast to the situation described in Chapter 7 (see Figure 7.1).
Figure 8.1. Forearm cutaneous vascular conductance (CVC) (A) and skin temperature (B) in the cuffed (open squares) and uncuffed (solid squares) forearms at baseline and during 30 mins of lower limb heating (5 min intervals) in a sub-group of 6 subjects. Significantly different at $P<0.05$ from baseline (*) or between the cuffed and uncuffed forearms (*). Data are mean ± SE.
Impact of repeated lower limb heating on the relationship between core temperature and forearm skin blood flow responses

During the first and last bout of 30 min heating (i.e. week 0 and 8), the forearms were in ambient air for both experiments so that the impact of repeated core heating on the relationship between Tc and SkBF could be assessed. Forearm SkBF did not change across the 8 weeks in response to lower limb heating ($P=0.75$; Figure 8.2A), whereas Tc responses to lower limb heating decreased after 8 weeks ($P<0.05$; Figure 8.2B). When averaged change in SkBF from baseline at all time points was plotted against change in Tc across the 30 min session at weeks 0 and 8, a higher SkBF was apparent for a given change in Tc (Figure 8.3). Furthermore, when absolute SkBF was plotted against Tc, there was a noticeable leftwards shift in the threshold for SkBF activation during lower limb heating post-intervention (Figure 8.4).

Impact of repeated lower limb heating on skin blood flow responses to a standardised local heating stimulus

Mean CVC values at 33°C were similar between weeks 0 and 8 in the cuffed arm (0.29±0.10 vs 0.29±0.11, $P=0.87$). Additionally, there was no significant difference between weeks 0 and 8 in responses to incremental heating in the cuffed arm ($P=0.72$; Figure 8.5A). In the uncuffed arm, mean CVC values at 33°C remained similar between weeks 0 and 8 (0.21±0.09 vs 0.17±0.08, $P=0.16$). However, a decrease in SkBF responsiveness to incremental heating throughout the local heating protocol was evident at week 8 compared to baseline ($P=0.05$; Figure 8.5B).
Figure 8.2. Forearm cutaneous vascular conductance (CVC) (A) and core temperature (B) responses to 30 mins of lower limb heating at weeks 0 (open circle) and 8 (solid circle) in a subgroup of 6 subjects. *Significantly different between weeks at $P<0.05$. †Significantly different between weeks at $P=0.05$. Data is mean ± SE.
Figure 8.3. Change in forearm cutaneous vascular conductance (CVC) (y axis) versus change in core temperature (x axis) during 30 mins of lower limb heating (5 min intervals) at weeks 0 (open circle) and 8 (solid circle). Data are derived from Figure 8.2.

Figure 8.4. Absolute forearm cutaneous vascular conductance (CVC) against core temperature during 30 mins of lower limb heating (5 min intervals) at weeks 0 (open circle) and 8 (solid circle). Data are derived from Figure 8.2.
Figure 8.5. Forearm cutaneous vascular conductance (CVC) during the gradual heating protocol in the cuffed (A) and uncuffed (B) arms and at weeks 0 (open circle) and 8 (solid circle). *Significantly different from week 0 at $P<0.05$. ‡$P=0.06$. Data is mean ± SE.
8.4 Discussion

In this experiment we have shown that 8 weeks of repeated increases in Tc, generated by isolated lower body heating, induces a decrease in SkBF responses to a localised heating stimulus. These localised adaptations were not evident in a cuffed arm that received lower blood flow during the lower limb heating bouts, suggesting that episodic Tc elevation induces local adaptation in the skin which is dependent upon microvascular hyperaemia. We also observed elevated SkBFs for a given Tc during leg heating. Taken together, these findings are consistent with the established hypervolaemic impact of repeated increases in Tc (Roberts et al., 1977; Convertino, 1991), but may also reflect prolonged cutaneous microvascular transit time following repeated elevation in Tc and SkBF, possibly due to increased microvascular capillarity. These data can be interpreted as suggesting that repeated Tc heating induces both central and local adaptations which enhance thermoregulatory capacity.

Our principal finding relates to the impact of repeated Tc elevation, in the absence of exercise or changes in skin temperature, on responses to local heating induced increases in SkBF. The decreases we observed in the uncuffed limb suggest lower SkBF at a given skin temperature during local disc heating. In contrast, 2 previous experiments elicited higher SkBF responses to local heating following 8 weeks of repeated forearm heating to 42ºC (Green et al., 2010) and lower limb heating to 40ºC (Chapter 7). The distinction between these experiments appears to be the skin temperature. In Green et al. al., forearm skin was heated to 42ºC and in Chapter 7 forearm skin temperatures rose by, on average, 4.1±1.9ºC during lower limb heating. In the current experiment, skin temperatures were clamped at 30ºC throughout each lower limb heating bout, in an experiment that was otherwise identical to that in the previous chapter. Consistent with Chapter 7, attenuation of SkBF responses during repeated Tc elevation (via cuff inflation) in the present experiment abolished the adaptation. Taken together, our findings therefore indicate that repeated episodic increases in SkBF and skin temperature may induce distinct adaptations in cutaneous microvessels.

Our observation of a decrease in SkBF during localised heating in the current study may conceivably relate to functional or structural microvascular adaptation. It is well established that SkBF is regulated by both neural and local mechanisms (Kellogg, 2006). For example, rapid local heating of the skin induces a transient peak in SkBF mediated by axon reflexes, a response followed by prolonged vasodilation mediated by the
release of NO (Minson *et al.*, 2001). The gradual local heating test we utilised in the present study is considered to be largely NO mediated (Black *et al.*, 2008b). In contrast, whole body heating elicits central thermoregulatory reflexes which increase cutaneous vasodilator outflow via sympathetic cholinergic nerves (Kellogg, 2006). There are several proposed mechanisms by which vasodilation is induced by this neural drive, including the co-transmitter concept whereby sweating and vasodilation are stimulated by the release of ACh and an as yet undetermined vasodilator substance (Kellogg, 2006; Wong, 2013). Recently, Wong reported that inhibition of sensory nerves and NO production reduced the reflex vasodilator response to whole body heating by ~80% and that NO is obligatory for full expression of reflex-mediated vasodilation in the skin (Wong, 2013). It was furthermore suggested that skin temperature-mediated activation of sensory nerves may play a distinct role in reflex vasodilation, compared to that associated with endothelium-dependent mechanisms. The present study adds to these important observations relating to the acute impacts of body heating by addressing the roles played by SkBF and temperature in response to repeated Tc elevation. Future studies, similar to those described above (Wong, 2013), involving blockade of specific transmitters will provide further mechanistic insight into the distinct impacts of repeated episodic changes in SkBF and skin temperature *in vivo*.

The explanation for the decrease we observed in SkBF response to local heating following the intervention is unclear. It is possible that, in the absence of changes in localised forearm skin temperature during our lower limb bath exposures, one or more of the many intrinsic vasodilators known to contribute to SkBF such as NO, EDHF, or prostacyclin was down-regulated. We believe this explanation somewhat unlikely, as repeated increases in blood flow and shear *per se* have not previously been associated with decreased expression of vasodilator substances in other arteries (Hambrecht *et al.*, 2003). Nonetheless, our study suggests that repeated Tc elevation, in the absence of changes in skin temperature or extrinsic skin heating, does not *up-regulate* reflex-mediated vasodilation. An alternate explanation for our finding invokes microvascular structural change. Although speculative, an increase in skin capillarisation following repeated Tc heating and blood flow (shear) stimulation might account for the decreases in SkBF we observed in response to local heating. Consequential prolongation in red cell transit time would enhance heat dissipation in the face of elevated Tc. By analogy, a similar prolongation in transit time due to increased capillarity has been proposed in skeletal muscle following exercise training (Krstrup *et al.*, 2004). Unfortunately, we
did not directly assess structural microvascular changes in the present study and future studies will therefore be required to elicit maximal CVC (i.e. local heating to 44°C or delivery of vasoactive substances via microdialysis) to confirm changes in skin capillarity, or otherwise address the question of changes in skin capillarisation in response to repeated heating stimuli. Whilst we are not aware of any previous direct evidence that skin capillarisation increases in response to either exercise training or repeated passive elevation in Tc, this possibility is consistent with our findings and previously reported changes in skeletal muscle (Krstrup et al., 2004) and worthy of future study.

The findings of the current study highlight the important role of skin temperature in determining the direction of the adaptive response to chronic Tc elevation. A possible explanation for the increase in SkBF responses to local heating that we observed in Chapter 7, and also in our previous forearm heating experiment (Green et al., 2010), may relate to the bioavailability of vasodilators in response to the significant increases in skin temperature. It is known that increases in temperature enhance the expression of heat shock proteins (HSP), and there is a known association between HSP90 and eNOS (Garcia-Cardeña et al., 1998; Shah et al., 1999). Indeed, Shastry et al. reported that inhibition of HSP90 (by geldanamycin) reduced SkBF in response to local heating and acetylcholine, highlighting the essential role of HSP90 in full eNOS activation (Shastry & Joyner, 2002). In this context, it is possible to rationalise the previous findings (Chapter 7) as reflective of heating-induced expression of HSP90 (or other HSPs) and subsequent activation of eNOS, thereby enhancing functional vasodilator capacity. Such a cascade may not be apparent in the current study, because of the lack of increase in skin temperature during the lower limb heating bouts. We also cannot discount the possibility that clamping skin temperatures to 30°C altered the association between eNOS and HSPs, or other vasodilators, in such a way that SkBF responses to local heating were reduced due to functional down-regulation. However, it seems unlikely that preventing activation of vasodilator pathways by maintaining skin temperature at euthermic levels would actively down-regulate microvascular function. For this reason, and because repeated shear stress has previously been associated with structural arterial remodelling (Langille & O'Donnell, 1986; Brown, 2003), we suggest that increased capillarisation may have resulted from the repeated episodic hyperaemia induced in the present study.
Apart from the adaptations referred to above and pertaining to localised skin vasodilation, SkBF and Tc changes *during* lower limb heating bouts were also measured before and after the intervention period. Reassuringly, our data pertaining to changes in Tc and changes in forearm SkBF during leg heating are consistent with those from Chapter 7; a noticeable upward shift occurred in the relationship between change in SkBF and change in Tc. Whether the adaptation is of central or peripheral origin remains unclear, but previous literature has reported similar changes in the Tc/SkBF relationship following exercise training, which were related to changes in blood volume (Ikegawa *et al.*, 2011; Simmons *et al.*, 2011b). It is tempting to speculate, therefore, that central adaptations which increase blood volume combined with peripheral adaptations in the skin which enhance microvascular structure and function, enhance thermoregulatory capacity following repeated exposure to increased Tc in humans.

One potential limitation of the present study relates to the placement of the laser Doppler probes before and after the intervention. It is possible that differential placement may have affected the changes we observed. However we believe the findings of this study are robust and internally consistent for several reasons. Each subject’s initial baseline probe placement was recorded using distances from anatomical landmarks and a photograph was taken to ensure follow-up placement was as close as possible to the baseline site. Furthermore, the study design was within-subjects and between arms and truly random effects should have similarly impacted both limbs. A further limitation of this study was that maximal SkBF following the local heating protocol was not induced. Elicitation of maximal SkBF by either local heating to 44°C or infusion of vasoactive substances via microdialysis, such as sodium nitroprusside, may have provided further evidence for structural adaptation in the cutaneous vasculature.

8.5 Conclusion

In summary, this experiment suggests that repetitive increases in SkBF induce distinct adaptations to those associated with repeated increases in blood flow and skin temperature. Whilst episodic increases in SkBF induce microvascular changes consistent with prolonged red blood cell transit time, attendant increases in skin temperature may be required to fully manifest functional adaptation and enhanced red cell flux.
Chapter 9

General Discussion

9.0 Reiteration of Aims and Objectives

The global aim of this thesis was to examine the impacts of manipulation of blood flow and shear stress on conduit and microvascular adaptation in humans.

The thesis was presented in two sections. The aim of the first section was a) to investigate the acute effects of changes in conduit blood flow and shear stress, mediated by distinct exercise modalities, on arterial diameter change and b) to assess whether repeated episodic increases in blood flow and shear stress, induced by either localised bilateral forearm heating or systemic passive heating, both exercise-independent stimuli, would modify brachial artery function and/or structure. The aim of the second section was to investigate whether repeated episodic increases in SkBF, induced by systemic heating, independent of exercise, would modulate cutaneous microvascular function and to determine the role of skin temperature in the adaptive response.
9.1 General Discussion

Cardiovascular diseases (CVD) are the leading cause of global death, responsible for 17.3 million deaths in 2008 (WHO, 2011). It is now well established that endothelial dysfunction is an integral and early atherogenic event (Davignon & Ganz, 2004). Numerous studies have revealed that exercise training improves both macro- and microvascular endothelial function (Black et al., 2008b; Tinken et al., 2008b; Tinken et al., 2010), thereby potentially contributing to reductions in cardiovascular risk (Maiorana et al., 2003; Green, 2009; Joyner & Green, 2009). One possible explanation for improvement in endothelial function in response to exercise training relates to repeated increases in exercise-induced shear stress (Tinken et al., 2008b; Tinken et al., 2010), but the mechanisms responsible for improvements in vascular health remain unclear, due to the difficulty in eliminating confounding factors which are apparent during exercise such as neural and humoral activity. If shear stress is truly relevant, then repeated increases in shear stress, which occur independently of an exercise stimulus, should also be associated with arterial modification. This notion forms the underlying theme of the chapters included in this thesis.

9.1.1 Section One – The contribution of shear stress on conduit artery adaptation in vivo

The findings of Chapter 3 indicated that brachial and radial arterial diameters responded to acute interventions that manipulated conduit artery shear, whether that shear was induced by exercise-dependent or –independent means. These findings confirm that shear stress, which may be responsible for inducing vascular adaptation, is in evidence during the application of passive stimuli such as forearm heating. Furthermore, when forearm heating-induced shear stress responses were attenuated by cuff placement, no arterial diameter changes were apparent. Hence, passive heating induces acute changes in conduit artery diameter which are shear stress mediated. This finding informs previous experiments relating to the role of shear stress as a stimulus during exercise.

In Chapter 4, repeated passive forearm heating was shown to induce changes in brachial artery function and structure. These arterial adaptations are similar to those previously reported in subjects following forearm handgrip training (Tinken et al., 2010). That the brachial artery adaptations in response to local heating were related to shear stress was
confirmed by the absence of change in the contralateral limb, which was heated but had shear stress attenuated by cuff inflation during all sessions. This study reveals the novel finding that repeated localised forearm heating induces conduit artery adaptations which are shear stress mediated. This study did not, however, indicate whether core body heating, and consequent reflex-induced increases in forearm blood flow, are similarly capable of inducing conduit artery adaptations. The study described in Chapter 5 was designed to address this question.

The aim of Chapter 5 was to repeatedly increase Tc whilst assessing the impact of the consequent episodic, reflex-induced, increases in blood flow to the forearm. An experiment was therefore devised involving heating which was isolated to the lower limbs, with the upper body maintained under ambient conditions. Repeated lower limb heating had a systemic impact on vascular function, as evidenced by a significant increase in brachial FMD responses. However, no adaptation in brachial structure was apparent. These findings are generally consistent with those of Birk et al., who examined the impact of 8 weeks of leg cycling exercise on brachial artery characteristics (Birk et al., 2012). Taken together, these studies suggest the existence of a threshold below which shear-induced structural adaptation may not occur.

In summary, the data presented in the Chapters of Section 1 of this thesis indicate that:

1. Passive heating increases shear stress to levels associated with acute changes in brachial and radial artery function
2. Increases in shear stress, independent of exercise, can induce acute and chronic vascular adaptations
3. The role of shear stress in these responses was proven by the fact that no adaptation was evident when increases in shear were attenuated by partial inflation of a pneumatic cuff
4. Repeated core heating has beneficial effects on systemic conduit artery function

9.1.2 Section Two – Microvascular adaptation to exercise-independent stimuli in vivo

The chapters above indicate that brachial artery adaptations can occur in response to local or systemic passive heating and that increases in shear stress represent an
important stimulus. It seems plausible that similar adaptations might occur in downstream microvessels. In Section 2 of this thesis, the role of increases in SkBF and shear as mediators of microvascular adaptation were examined.

The aim of Chapter 7 was to determine whether repeated increases in forearm SkBF, induced by lower limb heating, would result in cutaneous microvascular adaptation. A pneumatic cuff was placed around one forearm and inflated to 80 mmHg throughout each lower limb heating bout. Repeated lower limb heating induced a significant increase in SkBF responses to a gradual local heating protocol, performed at rest, in the uncuffed arm. In contrast, no change was evident in the cuffed arm. These findings are consistent with those of Green et al. following 8 weeks of forearm bilateral heating (Green et al., 2010). However, skin temperatures increased significantly in the uncuffed arm during lower limb heating and local heating of the skin is a potent stimulus for the release of NO (Minson et al., 2000; Black et al., 2008b). This experiment was therefore unable to distinguish between repeated increases in blood flow and shear stress versus repeated increases in skin temperature, as the mechanisms responsible for cutaneous microvascular adaptation in this experiment. Chapter 8 was designed to address this question.

In Chapter 8, we repeated the lower limb heating intervention used in Chapter 7, with the modification that forearm skin temperatures were clamped at 30°C throughout each lower limb heating bout by immersion in their own thermostatically controlled water baths. In this instance, a significant decrease in the forearm SkBF response to gradual local heating was observed in the uncuffed arm, whilst no change was evident in the cuffed arm. The results of Chapters 7 and 8 indicate that increases in skin temperature, when applied in the presence of increases in SkBF, may transmute the adaptive cutaneous microvascular response which is evident if flow changes occur in the absence of a rise in skin temperature. Although speculative, this reduction in SkBF responses during local heating in Chapter 8 is interpreted as indicative of prolongation in the transit time of blood flow through the cutaneous circulation. Such a change is consistent with observations in skeletal muscle following exercise training when increases in capillarisation occur (Krstrup et al., 2004).
The results of Chapters 7 and 8 may therefore indicate that, when skin temperature is allowed to increase with flow, SkBF in response to local heating may be enhanced due to the heat-induced upregulation of HSP90 which augments eNOS, leading to significantly greater levels of NO and functional adaptation.

The Chapters summarised in Section 2 of this thesis, pertaining to cutaneous microvascular adaptation, indicate that:

1. Repeated increases in SkBF, independent of exercise, can induce local microvascular adaptation. The direction of this adaptive response is, however, dependent upon whether changes in skin temperature accompany those in microvascular perfusion
2. As no adaptations were observed in the cuffed arms in Chapters 7 or 8, increases in SkBF represent an obligatory stimulus for microvascular adaptation
3. Repeated core heating has beneficial effects on systemic cutaneous microvascular function

9.2 Mechanisms of vascular adaptation

9.2.1 Time-course of brachial adaptation

The importance of the endothelium in mediating functional and structural vascular adaptations is well established. For example, Langille and O'Donnell demonstrated a significant reduction in common carotid artery diameter in rabbits following 2 weeks of reduced flow, induced by ligation (Langille & O'Donnell, 1986). This response was endothelium-dependent. Shear stress is a known stimulus for the release of endothelium-derived NO. A study by Hambrecht et al. concluded that the exercise-induced increase in vascular function is due to the effect of repeated increases in arterial shear stress on eNOS expression (Hambrecht et al., 2003). This finding was recently reinforced by human studies in which shear stress was manipulated during exercise (Tinken et al., 2010).

As originally suggested by Laughlin (Laughlin, 1995), arterial functional improvement may be transient and superseded by structural remodelling of the artery. Tinken et al. recently verified this biphasic concept in humans (Tinken et al., 2008b). The authors reported that running and cycling exercise enhanced brachial and popliteal FMDs
following 4 weeks, before returning to baseline values by week 8, and being superseded by structural remodelling in both arteries. The findings in Chapter 4 are consistent with this pattern. However, increases in brachial structure following lower limb heating were not observed in Chapter 5. Interestingly, Birk et al. reported similar functional, but not structural, improvements in the brachial artery following leg cycling exercise training (Birk et al., 2012). The difference in conduit artery adaptation observed between Chapters 4 and 5 may therefore be due to inherent differences between localised forearm stimuli (handgrip training or forearm heating) versus more remote lower limb interventions. One possibility is that lower limb stimuli induce shear stress responses in the upper limbs which differ in magnitude and/or pattern, as has previously been reported (Green et al., 2005). A point of difference between these Section 1 experiments was that, in Chapter 4, increases in brachial shear were induced by local heating of the forearms to 42°C, whereas in Chapter 5 brachial shear was dependent upon the degree of rise in Tc. Future experiments, performed in identical subjects, which characterise the shear stress stimuli associated with each intervention, will be required to address this question pertaining to stimulus magnitude and threshold effects.

9.2.2 Cutaneous adaptation
Cutaneous microvascular adaptations to exercise, and in particular passive heating, have not been comprehensively studied. Black et al. concluded that exercise training enhanced skin microvascular NO-mediated function (Black et al., 2008b) and a subsequent study by Green et al., involving 8 weeks of bilateral forearm heating, concluded that increases in SkBF are obligatory for cutaneous adaptation (Green et al., 2010). However this latter study did not induce increases in Tc or induce thermoregulatory reflexes, and it was not known whether these stimuli are also capable of inducing microvascular adaptation.

Chapters 7 and 8 were therefore the first studies to examine microvascular adaption in response to an exercise-independent, systemic stimulus, and to assess the role of SkBF. Although a speculative suggestion, the decrease in SkBF to local heating observed following the intervention in Chapter 8 may be due to increased cutaneous capillarisation. Brown has previously suggested that, whilst vascular adaptation in larger coronary and resistance arteries and arterioles occurs in response to physical
stimuli, such as shear, (Brown, 2003) (Figure 9.1) capillary beds adapt by increasing in density. This paradigm suggests “an unchanged capillary supply but a larger and more profuse arterial supply, which may assist by increasing arterial flow capacity and hence oxygen transport” in muscle (Brown, 2003). The corollary in the skin in response to local heating would infer prolongation of cutaneous microvascular transit time. The decreased SkBF responsiveness to local heating observed in the uncuffed arm in Chapter 8 may be explained by this adaptive response in the cutaneous microvessels. In swine, increased capillarisation begins with vascular budding from pre-existing vessels (White et al., 1998). The mechanisms which stimulate this are not fully understood, but our findings suggest that they are not dependent upon large increases in skin temperature and that they are not apparent if repetitive increases in SkBF are diminished by cuff placement. The findings of Chapter 8 are integral to rationalising the results of the preceding study. If repeated increases in SkBF are coupled with increased skin temperatures, then the adaptations may potentially be two-fold, involving increased capillarisation as well as the functional microvascular upregulation, possibly via interaction between HSP90 and eNOS. This results in greater NO release, thereby explaining the increased SkBF responsiveness to local heating.

9.3 Physiological Implications
These findings have implications for thermoregulatory capacity. Increased capillarisation, resulting in prolongation of the transit time of blood through the cutaneous vasculature, would facilitate greater heat loss, thereby enhancing thermoregulatory efficiency. This, in turn, has potential implications for exercise performance, as it might delay the competition for blood flow between the skin and the working muscles. Numerous studies have also reported exercise training and passive heating induce an increase in plasma and blood volume (Convertino et al., 1980; Convertino, 1991). Absolute SkBFs during lower limb heating were similar between weeks 0 and 8 in both Chapters 7 and 8, however Tc’s were significantly lower during heat exposure at week 8. These results indicate that SkBF was higher for a given Tc, post-intervention. Since this occurred in both studies and was apparent in both forearms, a central or systemic mechanism is likely and Ikegawa et al. (Ikegawa et al., 2011) have previously suggested that increases in blood volume contribute to acclimation benefits. If blood volume increases as a result of isolated lower body heating and
absolute SkBFs did not change, proportionately less blood is being sent to the skin post-intervention. In an exercise setting this means more blood being re-directed to the working muscles. Despite the distinct adaptations in the local cutaneous microvasculature between Chapters 7 and 8, the adaptation in the SkBF/Tc relationship following both interventions were similar. Hence, central mechanisms such as enhanced blood volume would be expected to contribute to improved function and possibly performance post-heating, and local skin adaptations may also contribute.

**Figure 9.1.** A schematic demonstrating the adaptive response to exercise-induced stimuli at the different segments of the coronary vascular tree. Modified from (Brown, 2003).

### 9.4 Clinical Implications

The studies within this thesis utilised exercise-independent modalities and reported beneficial vascular adaptations in both conduit arteries and cutaneous microvessels. These results therefore have implications for clinical and ageing populations where exercise training is contraindicated due to associated increases in physical stress on joints and the musculoskeletal system. Passive heating may provide these groups an alternate modality in which vascular health can be maintained or improved.
9.5 Future Directions

9.5.1 Conduit arteries
- Future studies should be performed utilising similar exercise-independent modalities (e.g. baths, spas and saunas) in different population groups who are at higher cardiovascular risk such as diabetics, heart failure patients and the elderly.
- The magnitude of shear stress required to induce functional and structural adaptations should be further examined as the findings of Chapter 5 suggests the potential existence of a shear stress threshold necessary for vascular adaptation.

9.5.2 Cutaneous microvessels
- The importance of skin temperature in determining the adaptive response in cutaneous microvessels should be further examined, in particular following exercise training interventions in contrasting environmental conditions. If the influence of changes in skin temperature on microvascular adaptation is confirmed, future studies should focus on identifying and designing the most effective and efficient modalities and environments to induce the beneficial adaptations.
- Future studies should utilise the subcutaneous infusion of vasoactive substances via microdialysis to examine the contribution of nitric oxide and other mediators to cutaneous vasodilation. This would assist in elucidating the mechanisms responsible for the distinct adaptive responses in cutaneous microvascular function.
- Future repeat studies of Chapters 7 and 8 should also induce maximal cutaneous vasodilation by infusion of sodium nitroprusside via microdialysis or local heating to 44°C in order to confirm any structural adaptations. Alternatively, new techniques should be adopted to assess skin capillarity in humans in response to exercise and repeated heating.
The suggestion in Chapter 7 that adaptive responses to repeated core temperature elevation may differ in terms of skin blood flow and sweating requires further elucidation.

9.6 Summary

The Chapters contained within this thesis provide evidence for a systemic effect of changes in shear stress on vascular adaptation. Exercise-independent modalities were used to modulate and examine the effects of shear stress, to inform previous studies of exercise training. The primary finding highlights the plasticity of the vasculature and the complexity of the adaptive responses that occur at distinct levels of the arterial tree. The complex nature of the microvascular control mechanisms, compared to those acting at the conduit level, provides some basis for understanding the differences we observed. Nonetheless, we found that repeated increases in blood flow, and shear stress, act as important triggers for adaptation in the function and health of arteries at both levels of the circulation in humans. Future studies should investigate these observations regarding the permissive impact of increases in skin blood flow, which clearly modulate cutaneous adaptive responses to body heating. This thesis cumulatively promotes the theory that the cardiovascular benefit of exercise can be mechanistically explained by repeated increases in blood flow and shear stress, a primary stimulus for vascular adaptation in vivo. It also implies that cardiovascular health benefits can accrue in response to passive or exercise-independent interventions in humans.
References


References


References


References


Other publications by the candidate during PhD tenure


The effects of shear stress on endothelial function and structure in humans

— Subject Information Sheet —

Purpose
The purpose of the proposed study is to determine whether changes in blood flow through large arteries cause changes in the function and size of the artery in the short and long term.

PROCEDURES
Subject will initially be required to attend four preliminary sessions at the School of Sports Science, Exercise and Health at the University of Western Australia. Subsequently, subjects will be required to attend the laboratory at SSEH three times per week, for a period of 8 weeks.

Preliminary assessments:

- Day 1: Non-invasive ultrasonography of the brachial artery in the arm will involve placing a blood pressure cuff around the forearm and inflating it for five (5) minutes to a high pressure, then images of the artery are taken for analysis using an ultrasound probe similar to that utilised for fetal scanning. This will be followed by a period of 30 minutes, during which you will ride on a stationary bicycle with both arms immersed in warm water while ultrasound measures are repeated.

- Day 2: On a separate day, the procedures above will be repeated, with the difference that the water temperature used to immerse the forearms will be cooler.

- Day 3: The above procedures will be again be repeated, but with the difference that, in place of the cuff on the forearm, a spray of glycercly trinitrate (GTN 400 micrograms) will be administered beneath the tongue. This is a drug which causes arteries to widen. After the period of cycling with forearm water immersion, a repeat GTN spray is administered. Measures will then be taken of skin blood flow by placing small Doppler probes on the forearm and heating them to 42C.

- Day 4: The GTN procedures described above will be repeated, with the difference that the water temperature will be cooler. Measures will then be taken of peak forearm blood flow, by inflating a cuff on the arm while hand grip exercise is undertaken.

Longitudinal Assessments:

- Following completion of the measures above, you will be required to attend the laboratory for 3 sessions of 1 hour per week, for 8 weeks.

- Each of these sessions will involve a period of bilateral forearm heating for a period of 30 minutes. This procedure is painless and safe.

- Every 2 weeks during this 8 week period, measures of artery function will be collected using ultrasound and cuff inflation, with and without hand grip exercise. A dose of GTN will also be administered every 2 weeks and skin blood flow measures will be repeated.
During one of the above sessions, skin temperatures will be measured at five sites on the body by taping small flat sensors to the skin and internal body temperature will be measured by placing a thin probe, a few mm thick, in the lower rectum 6cm beyond the anal sphincter. This does not cause any discomfort and you are able to place and remove the probe yourself in privacy. The re-useable probe will be covered with a protective sheath and will be sterilised according to strict hygiene procedures following each use. The placement and positioning of this temperature probe does not cause discomfort.

**RISKS**
The ultrasound tests are safe and use a machine similar to that involved in fetal scanning. One part of this test involves inflating a cuff around the forearm for 5 minute, during which time some hand grip exercise is performed. This procedure can cause mild discomfort and a pins and needles like sensation. This resolves immediately upon cuff deflation. If you experience any pain or severe discomfort, please tell the researcher, who will take immediate remedial action.

One of the tests requires the use of sublingual glyceryl trinitrate (GTN). This drug causes arteries to widen and it may elicit a headache or nausea. This occurs in less than 5% of cases. These symptoms usually resolve quickly and without any medical treatment, but the headache can be safely managed using paracetamol. **You should tell the researcher if you are taking any medications whatsoever, including Viagra-type drugs, which may interact with the GTN.**

**BENEFITS**
The benefits to society involve an increased understanding of the role of physical activity in cardiovascular disease development and the causes and preventions of artery disease in humans.

**CONFIDENTIALITY**
The subjects confidentially will be maintained throughout the study. Subjects will be randomly assigned a number to de-identify their data. Data collected, which includes images and video of the ultrasonography, will be securely stored on a password-protected laptop. Only research personnel will have access to the passwords for the laptop.

**SUBJECT RIGHTS**
Participation in this research is voluntary and you are free to withdraw from the study at any time without prejudice. You can withdraw for any reason and you do not need to justify your decision. If you withdraw from the study and are a patient recruited from one of the affiliated clinics your treatment will not be prejudiced or affected in any way. If you do withdraw we may wish to retain the data that we have recorded from you but only if you agree, otherwise your records will be destroyed.

Your participation in this study does not prejudice any right to compensation that you may have under statute of common law.

If you have any questions concerning the research at any time please feel free to ask the researcher who has contacted you about your concerns. Further information regarding this study may be obtained from Howard Carter 0406795986, Andrew Govus 0417094922, Matt Fitzsimons 0439516160, Louise Naylor, 6488 2361, or Daniel Green, 6488 2361.
The Human Research Ethics Committee at the University of Western Australia requires that all participants are informed that, if they have any complaint regarding the manner, in which a research project is conducted, it may be given to the researcher or, alternatively to the Secretary, Human Research Ethics Committee, Registrar’s Office, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009 (telephone number 6488-3703). All study participants will be provided with a copy of the Information Sheet and Consent Form for their personal records.

Thank you for considering participating in this research project.
The effects of shear stress on endothelial function and structure in humans

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Purpose
The purpose of the proposed study is to determine whether changes in blood flow through large arteries cause changes in the function and size of the artery in the short and long term.

PROCEDURES
Subject will initially be required to attend four preliminary sessions at the School of Sports Science, Exercise and Health at the University of Western Australia. Subsequently, subjects will be required to attend the laboratory at SSEH three times per week, for a period of 8 weeks.

Preliminary assessments:

- **Cerebrovascular assessment, day 1:** On this day, we will image the blood vessels in your brain, by placing a head frame, which resembles a bicycle helmet, over your head. This frame is then used to fix two Doppler ultrasound probes above and in front of the ears. Following the resting measures, blood flow velocities are measured in response to increasing blood concentrations of carbon dioxide (CO$_2$). This is achieved by placing a face mask, resembling a scuba mask, over your nose and mouth for 4 minutes after switching from room air to mixtures of gas which contain higher levels of CO$_2$ (e.g. 4% CO$_2$ + 21% O$_2$, balance N$_2$; or 8% CO$_2$ + 21% O$_2$, balance N$_2$). In another test, you will be asked to voluntarily hyperventilate for a period of time. We will also measure blood flow in the brain during blood pressure changes. To do this, you will lie with your lower body (to the level of your waist) inside a large cylindrical tube, and we will carefully increase the levels of negative pressure (suction) around your legs. The purpose of this procedure is to decrease arterial blood pressure by causing temporary blood pooling in the legs. Finally, brain blood flows will be measured during a series of trials involving voluntary eye movements (tracking), and during other tasks include memory tests and handgrip and stationary cycle exercises. These assessments will take approximately 2 hours to complete.

- **Macro- and micro-vascular assessment, day 2:** Non-invasive ultrasonography of the brachial artery in the arm will involve placing a blood pressure cuff around the forearm and inflating it for five (5) minutes to a high pressure, then images of the artery are taken for analysis using an ultrasound probe similar to that utilised for fetal scanning. The above procedures will again be repeated, but with the difference that, in place of the cuff on the forearm, a spray of glyceryl trinitrate (GTN 400 micrograms) will be administered beneath the tongue. This is a drug which causes arteries to widen. Measures will then be taken of peak
forearm blood flow, by inflating a cuff on the arm while hand grip exercise is undertaken.

Skin blood flow will be measured by placing small Doppler probes on the forearm and heating them to 42°C. These assessments will take approximately 2 hours to complete each.

- **Acute lower body heating assessment, day 3:** Measures of skin temperatures at five sites on the body using small flat sensors and internal body temperature using a thin probe, a few mm thick, in the lower rectum 6cm beyond the anal sphincter will be measured along with cerebral, brachial and skin blood flows before, during and after a 30 min bout of lower body heating. Sweat rate production will also be measured during this time by attaching perspex capsules to the skin at five sites. *The rectal probe does not cause any discomfort and you are able to place and remove the probe yourself in privacy. The re-useable probe will be covered with a protective sheath and will be sterilised according to strict hygiene procedures following each use.*

**Longitudinal Assessments:**

- Following completion of the measures above, you will be required to attend the laboratory for 3 sessions of 1 hour per week, for 8 weeks.
- Each of these sessions will involve a period of bilateral lower body (up to the waist) heating for a period of 30 minutes. This procedure is painless and safe.
- Every 2 weeks during this 8 week period, measures of brain blood flow, and brachial artery (in the arm) function will be collected using ultrasound and cuff inflation, with and without hand grip exercise. A dose of GTN will also be administered every 2 weeks and skin blood flow measures will be repeated.
- During your last lower body heating session at week 8, the acute measures performed during your first immersion (Preliminary assessments, day 3) will be repeated which includes measures of skin and internal body temperature, cerebral, brachial and skin blood flows as well as sweat rate production.

**Risks**

The ultrasound tests are safe and use a machine similar to that involved in fetal scanning. One part of this test involves inflating a cuff around the forearm or upper thigh for 5 minute, during which time some hand grip exercise is performed. This procedure can cause mild discomfort and a pins and needles like sensation. This resolves immediately upon cuff deflation. If you experience any pain or severe discomfort, please tell the researcher, who will take immediate remedial action.

There are no known side effects of the brain Doppler assessment and no radiation involved. All techniques used in this study are commonly used in clinical physiology, with no known adverse side-effects. If you experience any discomfort at any time, the researcher will stop the testing immediately.
The carbon dioxide reactivity protocols are routine in clinical physiology and are not associated with any pain or discomfort, although you may experience some sensation to breathe more heavily and deeply. You will be able to switch to breathing room air at any time and supplemental oxygen is also available in the unlikely event that any serious sensation of breathlessness occurs. Another of the tests requires the use of sublingual glyceryl trinitrate (GTN). This drug causes arteries to widen and it may elicit a headache or nausea. This occurs in less than 5% of cases. These symptoms usually resolve quickly and without any medical treatment, but the headache can be safely managed using paracetamol. You should tell the researcher if you are taking any medications whatsoever, including Viagra-type drugs, which may interact with the GTN.

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The effects of shear stress on endothelial function and structure in humans

— Consent Form —

I ___________________________ have read the information provided and any questions I have asked have been answered to my satisfaction. I agree to participate in this activity, realising that I may withdraw at any time without reason and without prejudice, or without prejudice to my future medical treatment. I understand that all information provided is treated as strictly confidential and will not be released by the investigator unless required to by law. I have been advised as to what data is being collected, what the purpose is, and what will be done with the data upon completion of the research.

I agree that research data gathered for the study may be published provided my name or other identifying information is not used.

__________________________                    __________________
Participant                                             Date

The Human Research Ethics Committee at the University of Western Australia requires that all participants are informed that, if they have any complaint regarding the manner, in which a research project is conducted, it may be given to the researcher or, alternatively to the Secretary, Human Research Ethics Committee, Registrar’s Office, University of Western Australia, 35 Stirling Highway, Crawley, WA 6009 (telephone number 6488-3703). All study participants will be provided with a copy of the Information Sheet and Consent Form for their personal records.