Effect of arterial blood perfusion pressure on vascular conductance and muscle blood flow at rest and exercise

by

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I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

The adaptations of vessel diameter represented by vascular conductance (VC), muscle blood flow (MBF) and oxygen delivery (DO_{2est}) were investigated during rest and exercise using the effects of gravity to manipulate muscle perfusion pressure (MPP) by placing the heart above (head-up tilt) and below (head-down tilt) the level of the muscle. This experimental paradigm was used to explore VC and MBF regulation and related control mechanisms during rest and exercise. Study 1 tested the repeatability of Doppler ultrasound measurements of muscle blood flow velocity (MBV), arterial diameter, MBF and VC. The adaptations in VC and MBF (Study 2) and changes in anterograde and retrograde MBV patterns (Study 3) were investigated during postural challenges at rest. Study 4, determined the peak VC and its fractional recruitment during transitions from rest to lower (LPO) and higher power output (HPO) calf muscle exercise in HDT and HUT. Study 5 investigated the combined effects of altered MPP and hypoxia during exercise. During rest-HDT, increases in VC compensated for the MPP reduction to maintain MBF, while in rest-HUT, MBF was reduced. Following the start of LPO and HPO exercises, MBF and VC responses were delayed in HDT and accelerated in HUT. During LPO, MBF steady-state was reduced in HUT compared to horizontal (HOR), while the greater increase in VC during HDT maintained MBF at a similar level as HUT. Post-exercise MBF recovered rapidly in all positions after LPO exercise but did not after HPO_{HDT}. During HPO_{HDT}, MBF was reduced despite the increase in VC, while in HPO_{HUT} MBF was similar to that in HPO_{HOR}. The hypoxic challenge added in exercise was met during LPO_{HDT} by increased VC to compensate reduced MPP and O_2 availability such that MBF maintained DO_{2est}. However, during HPO_{HDT} in hypoxia, VC reached maximal vasodilatory capacity, compromising MBF and DO_{2est}. Together, these findings indicate that LPO_{HDT} in normoxia or hypoxia VC increased to maintain MBF and DO_{2est}, but during HPO functional limitation for recruitment of VC constrained MBF and DO_2 in normoxia and hypoxia. Elevated muscle electromyograpic signals in HPO_{HDT} were consistent with challenged aerobic metabolism. MPP reduction in HDT caused slower adaptation of MBF limiting O_2 availability would result in a greater O_2 deficit that could contribute to an increase in the relative stress of the exercise challenge and advance the onset of muscle fatigue.

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Dedication

This thesis is dedicated to:

(Essa tese e dedicada a):

In memory of my dad Valerio Villar (1941-2008). He was a great man, wonderful husband and father. I always would be proud to be your son.

Em memoria de meu pai Valerio Villar (1941-2008). Ele foi um grande homem, maravilhoso marido e pai. Eu sempre terei orgulho de ter sido seu filho.

My wonderful mother Ruth. Thank you for everything. Your constant support since the beginning made me the man that I am today. You always are a source of inspiration and deep admiration. Mommy you are the best.

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Meus grandes irmaos Rogerio e Romulo. Voces dois sempre me fizeram sentir amado. Eu tenho muita sorte de ter irmaos como voces. Muito obrigado pelo amor, encouraja-mento, suporte e amizade. Voces sao os melhores.

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"Life is not about just what you want but how much you do to deserve it (Valrio Villar, 2008)"

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List of Abreviations

ΔP	Perfusion pressure gradient
$\dot{Q}$	Cardiac output
%VC	Vascular conductance expresses as percentage
$\%VC_{peak}$	percentage of peak vascular conductance
a-vO_2diff	arterial-venous oxygen content difference
ACh	acethylcoline
AD	Arterial diameter
AD_{pop}	Popliteal artery diameter
ADP	Adenosine diphosphate
AMPK	adenosine monophosphate-activated protein kinase
AO_2EX	Altered oxygen during exercise
ATP	Adenosine triphosphate
BP	Blood pressure
CaO_2	Arterial oxygen content
CO_2	carbon dioxide
CV	coefficient of variation
CvO_2	venous oxygen content

CVP	central venous pressure
DBP	Diastolic blood pressure
DO_{2est}	Estimated oxygen delivery
DO_2	Oxygen delivery
EDHF	endothelial-derived-hyperpolarization-factor
EMG	Electromyography
EMG_{index}	Electromyographyc index
EX	Transitons from rest to exercise
F_IO_2	Inspired oxygen fractions
FFT	Fast Fourier transformation
G	Gain of the response
GLH	Gastrocnemius lateral head
GMH	Gastrocnemius medial head
H_2O	water
H^+	Hydrogen ion
Hb	Hemoglobin
HDT	Head-down tilt
HOR	Horizontal
HPO	Higher power output
HPO_{HDT}	Higher power output in head-down tilt
HPO_{HOR}	Higher power output in horizontal
HPO_{HUT}	Higher power output in head-up tilt
HR	Heart rate

HUT	Head-up tilt
iEMG	Integrated EMG
K^+	potassium ion
LBNP	lower body negative pressure
LPO	Lower power output
LPO_{HDT}	Lower power output in head-down tilt
LPO_{HOR}	Lower power output in horizontal
LPO_{HUT}	Lower power output in head-up tilt
MAP	Mean arterial pressure
MBF	Muscle blood flow
MBF_{peak}	Peak muscle blood flow
MBF_{relax}	Muscle blood flow during relaxation between muscle contractions
MBV	Muscle blood flow velocity
MBV_{ant}	Anterograde muscle blood flow velocity
MBV_{net}	Muscle blood flow velocity
MBV_{peak}	Peak muscle blood flow velocity
MBV_{ret}	Retrograde muscle blood flow velocity
MPF	Mean power frequency
MPP	Muscle perfusion pressure
MPP_{peak}	Peak muscle perfusion pressure
MPP_{relax}	Muscle perfusion pressure during relaxation between muscle contractions
MVC	Maximal voluntary contraction
MVF	Maximal voluntary force

NIRS	near infrared spectroscopy
NMRS	nuclear magnetic resonance spectroscopy
NO	nitric oxide
O_2	Oxygen
O_2deficit	Deficit of oxygen
PaO_2	Arterial oxygen partial pressure
PCr	phosphocreatine
PG	prostaglandins
Pi	Inorganic phosphate
PO_2	Oxygen
SaO_2	Arterial oxygen saturation
SBP	Systolic blood pressure
SD	Standard Deviation
SS	Steady-state response
SV	Stroke volume
SVC	Systemic vascular conductance
$T_{63\%}$	Time to reach 63% of the steady state response
TMA	total muscle activity
TPR	Total peripheral resistance
VC	Vascular conductance
VC_{peak}	Peak vascular conductance
VC_{relax}	Vascular conductance during the relaxation between muscle contractions
VO_{2mus}	Muscle oxygen consumption
VO_2	oxygen consumption

Chapter 1

General Introduction

1.1 Introduction

The human body is constantly challenged by different types of activity increasing rapidly metabolic and cardiovascular demands. These increased demands of working muscle are met by the elevation in muscle blood flow (MBF) to match oxygen delivery (DO_2) to the metabolic demand. It is known that MBF is determined by the interaction between perfusion pressure gradient (ΔP) and vascular conductance (VC) expressed as MBF = VC $\times$ ΔP (Rowell, 1993, Hughson, Shoemaker, Tschakovsky, and Kowalchuk, 1996, Laughlin and Korzick, 2001, Tschakovsky and Sheriff, 2004). Therefore, the dynamic response of MBF can be challenged by manipulation of VC or ΔP. There are a number of approaches that can be used to manipulate ΔP, VC or MBF including: lower body negative pressure (Hughson, Cochrane, and Butler, 1993), multiple bouts of exercise (MacDonald, Naylor, Tschakovsky, and Hughson, 2001), short training programs (Shoemaker, Phillips, Green, and Hughson, 1996), Mourtzakis, Gonzalez-Alonso, Graham, and Saltin, 2004), changes in the physical adjustments of the body (Hughson et al., 1996) and modifications of inspired O_2 fractions (F_IO_2) (Calbet, 2000). For the purpose of this thesis, the last two approaches, changes in body position and hypoxia, were chosen to challenge MBF and VC responses during rest and exercise.

It is known that the human body is sensitive to postural challenges due to the effects of gravity inducing hydrostatic pressure changes and shifts in blood volume affecting MBF and VC (Rowell, 1993, Shoemaker and Hughson, 1999, Egana and Green, 2005). In fact, muscle perfusion pressure (MPP) can be enhanced or reduced if arterial blood pressure is itself increased or decreased by placing the heart above or below the level of the muscle (Hughson

et al., 1996). Consequently, compensatory adjustments in VC are required to respond to the alterations in MPP to modify MBF. Resting MBF is reduced despite the elevated MPP in the head-up tilt (HUT) or seated compared to supine position in healthy individuals (Delis, Nicolaides, and Stansby, 2000, Imadojemu et al., 2001, Groothuis, Boot, Houtman, van Langen, and Hopman, 2005) due to the decrease in VC via vasoconstriction through a complex interaction between local and central mechanisms (Laughlin and Korzick, 2001, Segal, 2005).

Since exercising muscles are sensitive to changes in limb or body position due to the effects of gravity (Egana and Green, 2005), positioning of the limb above and below the heart level promotes changes in the gravity-dependent distribution of arterial and venous pressures affecting the dynamic response of MBF at the onset and steady-state of exercise (Hughson et al., 1996, Walker et al., 2007, Nadland, Walloe, and Toska, 2009). At the onset of exercise, MBF increases rapidly to meet the metabolic demand (Laughlin and Korzick, 2001, Hughson, 2003) being dependent on the intensity and the relative duration of contraction and relaxation phases (Walloe and Wesche, 1988). The rapid increase in MBF observed at the exercise onset is controlled by the complex interaction between local mechanical factors related to muscle pump action increasing pressure gradient across the muscle (Laughlin and Korzick, 2001, Tschakovsky and Sheriff, 2004) and local vasodilator factors increasing VC (Tschakovsky and Hughson, 1999, Shoemaker and Hughson, 1999, Tschakovsky and Sheriff, 2004, Tschakovsky, Saunders, Webb, and O'Donnell, 2006) beginning with the first contraction (Shoemaker and Hughson, 1999). Since a large portion of the increase in pressure gradient across working muscles occurs early in exercise, further increases in MBF must be accomplished by increases in VC to match oxygen delivery (DO_2) to the metabolic demand (Laughlin and Korzick, 2001, Tschakovsky et al., 2006).

In submaximal steady-state exercise with small muscle mass, MBF determines DO_2 because there is no alteration in the arterial O_2 content (Walker et al., 2007). However, lower O_2 concentrations as in hypoxia cause a decrease in arterial O_2 content (CaO_2) resulting in a decrease in the O_2 availability unless compensated by an increase in MBF (Koskolou, Calbet, Radegran, and Roach, 1997, Gonzalez-Alonso, Richardson, and Saltin, 2001, Calbet et al., 2002). The combined effect of hypoxia and reduced MPP could challenge the ability to sufficiently increase DO_2 during submaximal exercise.

The overall purpose of this thesis was to investigate the effects of postural changes on MPP and the subsequent adaptations of lower limb VC and MBF at rest, during transitions from rest to exercise in different work rates and exercise in different work rates under altered F_IO_2. To characterize and investigate MBF and VC responses during postural challenges as proposed on this thesis, it was necessary that the measurement techniques had beat-by-beat resolution and high reliability (Shoemaker, Pozeg, and Hughson, 1996).

Doppler ultrasound is a non-invasive method that has the capability to produce continuous measurements on a beat-by-beat basis due to its high temporal resolution (Shoemaker et al., 1996, Radegran, 1997, Osada, 2004, Walther, Nottin, Dauzat, and Obert, 2006). Therefore, the use of Doppler ultrasound is an attractive tool to measure arterial diameter (AD) and muscle blood flow velocity (MBV) allowing determination of MBF (Leyk, Essfeld, Baum, and Stegemann, 1992, Leyk, Essfeld, Baum, and Stegemann, 1994, Tschakovsky, Shoemaker, and Hughson, 1995, Shoemaker et al., 1996, Hughson et al., 1996, Radegran, 1997,Osada, 2004, Walther et al., 2006). Although many studies have used Doppler ultrasound to quantify AD, MBV and MBF during rest and exercise there are few data available regarding its reproducibility for leg peripheral arteries (Walther et al., 2006). This issue of repeatability of the AD, MBV, MBF and VC responses at rest and exercise during body tilt using Doppler ultrasound was addressed in Chapter 2.

Resting limb blood flow is determined by the interactions between perfusion pressure gradient and VC (Rowell, 1993). In healthy individuals from supine to HUT, leg arterial perfusion pressure is increased but MBF is decreased due to the reduction in VC (Delis et al., 2000, Imadojemu et al., 2001, Groothuis et al., 2005). However, little is known about the adaptation of the lower limb VC and MBF responses when the lower limb is positioned above the heart level (head-down tilt, HDT). The main issue of Chapter 3 was to discuss the adaptations of lower limb resting VC and MBF during HUT and HDT postural challenges. The analysis of the results in this study was based only on the averaged MBV, MBF and VC over a beat and averaged over the last two minutes of testing representing the net arterial responses in each body position. However, when only mean values are presented, important information concerning to flow patterns are unexplored (Thijssen, Green, Steendijk, and Hopman, 2009). Therefore, this follow up study (Chapter 4) addressed as the main issue the changes in MBV waveforms and MBV patterns within a cardiac cycle under altered MPP caused by changes in body position.

The effects of gravity caused by changes in body position affect exercising muscle because of its sensitivity to perfusion pressure (Egana and Green, 2005). MBF adaptations at the onset and steady-state exercise have been challenged by positioning the limb above and/or below the heart level to alter arterial and venous pressures (Nadland et al., 2009, Walker et al., 2007, Lutjemeier et al., 2005, Shoemaker, Tschakovsky, and Hughson, 1998, Tschakovsky, Shoemaker, and Hughson, 1996, Hughson et al., 1996, Leyk et al., 1994). From supine to HUT (Nadland et al., 2009) or arm below versus above heart level (Hughson et al., 1996) the same level of MBF is achieved, but leg peak blood flow (Egana and Green, 2005) as well as mean leg blood flow in moderate and heavy exercises (Van Leeuwen, Barendsen, Lubbers, and de Pater, 1992) demonstrated to be lower in supine compared to upright position. The lower MBF in some experimental conditions may suggest that MBF

was compromised in supine compared to upright exercise (Eiken, 1988). The main issue of Chapter 5 was to investigate the peak and submaximal MBF and VC responses during exercise in three different body positions that affect the gravity-dependent distribution of arterial and venous pressures.

Manipulation of F_IO_2 via hypoxia resulted in compensatory adjustments in MBF to maintain DO_2 during submaximal exercise (Koskolou et al., 1997, Gonzalez-Alonso et al., 2001). Manipulations of limb position relative to the heart to alter arterial and venous pressure affected MBF and VC responses during submaximal exercise. The effects of altered F_IO_2 (hypoxia) in conjunction with altered arterial perfusion pressure on MBF, DO_2 and VC responses during exercise in humans are not known. This issue of the effects of altered F_IO_2 and altered arterial perfusion pressure on MBF, DO_2 and VC responses during exercise was addressed in Chapter 6.

1.2 Overview of Literature

1.2.1 Effects of altered arterial perfusion pressure on vascular conductance and muscle blood flow at rest

The hydrostatic component of the blood pressure is altered during changes in body position affecting central hemodynamics (Shoemaker and Hughson, 1999). During HDT, the alterations in regional hydrostatic pressure might reduce blood pressure as well as lower body venous pressure being related to the magnitude of the stimulus (Sheriff, Nadland, and Toska, 2007). This body position causes the translocation of venous blood volume from the lower extremities to the central region of the body (Bundgaard-Nielsen, Sorensen, Dalsgaard, Rasmussen, and Secher, 2009). The effective arterial pressure detected at the arterial baroreceptors in the carotid artery would increase as well as the stimulation of the cardiopulmonary baroreceptors. The combined effect of these mechanisms would result in reduction in sympathetic vasoconstrictor tone in the leg arteries (Nagaya, Wada, Nakamitsu, Sagawa, and Shiraki, 1995, Rowell, 1993). In the lower legs in HDT, MPP is reduced decreasing transmural pressure as well as wall tension would induce relaxation of the arteriolar resistance vessels through the myogenic response (Sheriff et al., 2007, Tschakovsky and Hughson, 2000). The gravity-assisted shift in venous blood volume would inhibit the veno-arteriolar reflex (Sheriff et al., 2007, Toska and Walloe, 2002, Tschakovsky and Hughson, 2000). These mechanisms might act to promote arteriolar vasodilation resulting in the increase of VC to compensate for the reduction in MPP to maintain resting MBF.

In the HUT position, there is a relatively greater arterial-venous pressure gradient driving flow to the lower limbs due to effects of gravity shifting blood from the central circulation into the abdominal region and the legs inducing venous pooling (Rowell, 1993, Toska and Walloe, 2002, Cui et al., 2003, Harms, van Lieshout, Jenstrup, Pott, and Secher, 2003, Groothuis et al., 2005, van Lieshout, Harms, Pott, Jenstrup, and Secher, 2005, Bundgaard-Nielsen et al., 2009). This would in turn reduce venous return lowering central venous pressure and stimulating the cardiopulmonary baroreflex response to enhance muscle sympathetic nerve activity (Imadojemu et al., 2001, Toska and Walloe, 2002, Cui et al., 2003). The reduction in SV and SBP combined with the positioning of the carotid arterial baroreceptors above the heart level would increase sympathetic vasoconstrictor activity as well as HR. The progressive pooling of blood early during HUT increases venous pressure (Matzen, Perko, Groth, Friedman, and Secher, 1991, Madsen et al., 1995, Cui et al., 2003) that would induce arteriolar vasoconstriction of the corresponding arteriole due to the veno-arteriolar reflex (Henriksen and Sejrsen, 1977, Tschakovsky and Hughson, 2000). The higher MPP

in HUT raises distending pressure on the vessel and increase transmural pressure as well as wall tension inducing vasoconstriction of the arteriolar resistance vessels via myogenic response (Imadojemu et al., 2001, Toska and Walloe, 2002). The combined effects of these mechanisms might act to promote vasoconstriction resulting in the decrease of VC due to the rise in vascular resistance in HUT (Harms et al., 2003) resulting in decreased resting MBF (Delis et al., 2000, Imadojemu et al., 2001, Groothuis et al., 2005).

1.2.2 Muscle blood flow response during exercise

Humans have the extraordinary capacity to increase MBF by approximately 100 fold over resting values during exercise (Joyner and Wilkins, 2007), i.e., from 2-3 $mL \cdot 100g^{-1} \cdot min^{-1}$ at rest to greater than 300 $mL \cdot 100g^{-1} \cdot min^{-1}$ during maximal exercise (Rowell, 1993). During this transition from rest to exercise, metabolic and cardiovascular demands can increase rapidly due to muscle contraction generating a massive demand on the cardiovascular system (Laughlin and Korzick, 2001). This increase in MBF is proportional to the metabolic signal where changes in MBF amplitude are proportional to the increase in exercise intensity (Hughson, 2003). During constant load dynamic exercise, MBF increases to match oxygen delivery (DO_2) to the metabolic demand (Laughlin et al., 2006, Tschakovsky et al., 2006). MBF dynamics during forearm (Hughson et al., 1996) and leg exercise (Shoemaker et al., 1996, MacDonald, Shoemaker, Tschakovsky, and Hughson, 1998, Paterson, Kowalchuk, and Paterson, 2005, MacPhee, Shoemaker, Paterson, and Kowalchuk, 2005) have an impact on muscle oxygen consumption (VO_{2mus}) at the onset of exercise. Therefore, MBF regulation, O_2 availability and exercise intensity are important variables that need to be considered when changing from a resting to an exercising state (Hughson et al., 1996).

From rest to exercise MBF typically is characterized by a two phase adaptive dynamic response that increases very rapidly during muscular rhythmic contractions and reaches an initial plateau at $\sim$5 to 10 s of exercise (phase I). Following this onset of contractions, at $\sim$15 to 20 s, MBF shows a second but slower increase progressing toward a new steady state level, as known as phase II (MacDonald et al., 1998, Shoemaker and Hughson, 1999, Tschakovsky and Hughson, 1999, Hughson, 2003, Saunders and Tschakovsky, 2004, Tschakovsky et al., 2006, Clifford, 2007). Figure 1.1 demonstrates the dynamic response of MBF during moderate intensity plantar flexion exercise.

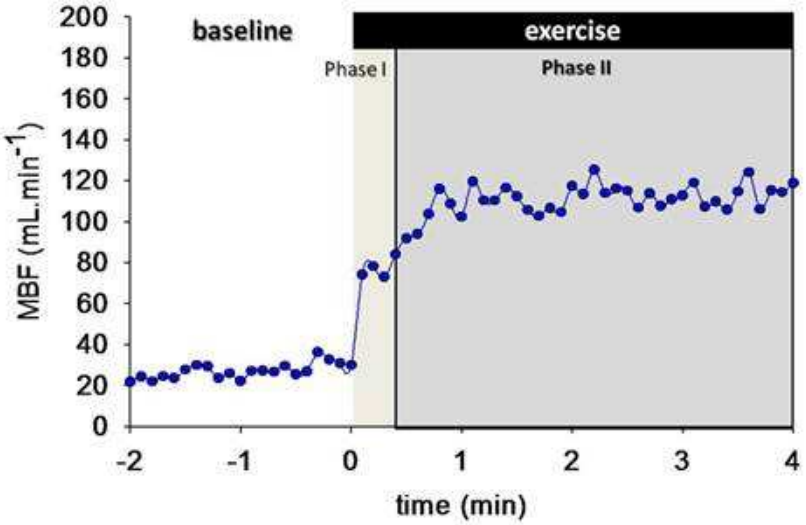

Figure 1.1: Illustration of the dynamic response of MBF during moderate intensity plantar flexion exercise (data from the current study displayed consistent with Shoemaker and Hughson, 1999 and Tschakovsky et al., 2006.)

The rate of increase in blood flow to the exercising muscle has an effect on steady-state conditions consequently the time course of the adaptive processes and control mechanisms responsible for the increase in MBF is critical during the transition from rest to exercise (Shoemaker and Hughson, 1999, Hughson et al., 1996). Therefore, the characterization of the time course and quantification of the dynamic response of MBF during the adaptive phase between rest and exercise offers a unique opportunity to obtain information about the potential underlying mechanisms and MBF regulators that is not possible during the steady-state phase of exercise (Tschakovsky et al., 2006, Hughson, 2003).

The adaptive dynamic response of MBF to an exercise challenge behaves in an approximately exponential manner; although, more complex models might be appropriate under conditions of very heavy or very light exercise. The exponential response can be described by three parameters characteristic of the dynamic response: (1) time delay (TD), representing the onset of stimulus to the onset of response; (2) time constant (τ), representing the rate of adaptation of the response, it is considered as the time required to reach 63% of the difference between baseline and a new steady state value; and (3) gain (G) representing the magnitude of the response, where total gain (TG) represents the total amplitude of the response (Hughson, 2003, Tschakovsky et al., 2006) (Figure 1.2).

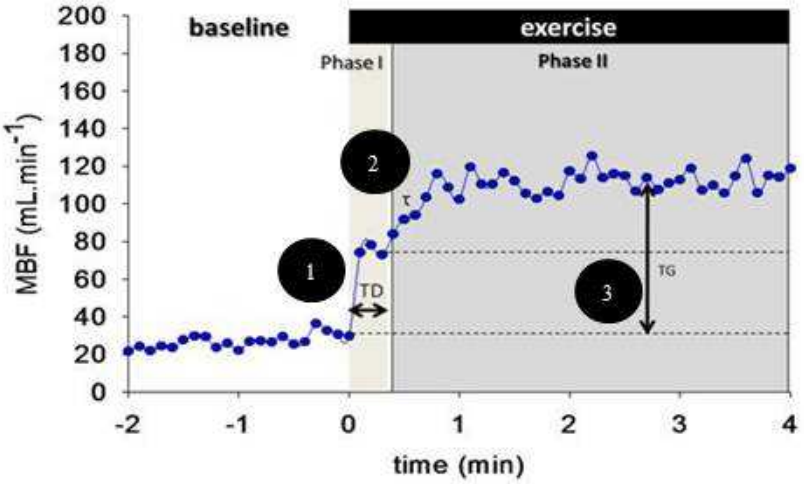

Figure 1.2: Illustration of the three parameters that are involved in a dynamic response of MBF: (1) time delay (TD), (2) time constant (τ) and (3) gain (TG) (data from the current study displayed consistent with Tschakovsky et al., 2006.)

1.2.3 Muscle blood flow response during phase I

MBF increases very rapidly in response to muscle contractions during the first 5 to 10 s of exercise (phase I). Since blood flow is the product between perfusion pressure gradient and VC, MBF distribution is dependent on the interaction between MPP and VC (Hughson et al., 1996, Laughlin and Korzick, 2001, Tschakovsky and Sheriff, 2004). There are many potential factors that might influence MBF at the onset of exercise acting to modify vascular tone (VC) and the pressure gradient across capillary beds (Hughson et al., 1996, Laughlin and Korzick, 2001, Tschakovsky and Sheriff, 2004)

The rapid increase in MBF is controlled by local mechanical factors related to muscle pump action increasing pressure gradient across the muscle (Tschakovsky and Sheriff, 2004, Laughlin and Korzick, 2001) and vasodilation increasing VC (Tschakovsky et al., 2006, Tschakovsky and Sheriff, 2004, Tschakovsky and Hughson, 1999, Shoemaker and Hughson, 1999) beginning with the first contraction (Shoemaker and Hughson, 1999). This early dilation is related to the production and release of the vasodilator substances during contraction to reduce vascular tone facilitating hyperemia during relaxation (Shoemaker and Hughson, 1999). Several candidates have been indicated as the main substance responsible for the early dilation at the onset of exercise, such as: muscle metabolites (ADP, Pi, CO_2, H^+, lactate) (Lash, 1996), potassium (K^+) (MacDonald et al., 2001), adenosine,

nitric oxide (NO), neural regulation and acetylcholine (ACh) (Welsh and Segal, 1998) as well as compression and deformation of the vessel wall considered as mechanical factors (Clifford, 2007). It appears that ACh might not play a role in the regulation of vascular tone in humans (Brock et al., 1998) because ACh had no effect on the magnitude of increase in blood flow suggesting no functional involvement of this substance (Shoemaker and Hughson, 1999). This feed forward mechanism probably cannot account for blood flow adaptation, at least in large muscle mass, owing to the physical distance between neuromuscular junction and the major portion of the muscle mass (Hughson, 2003). The increase in VC and MBF from rest to exercise during this adaptive phase I seems to be regulated by multiple and probably redundant mechanisms (Tschakovsky et al., 2006).

1.2.4 Muscle blood flow during phase II response

Following phase I, MBF continues to increase until 2-3 min of moderate intensity exercise. Despite the quick vasodilatory response at the onset of exercise in this phase, such response is not enough to achieve steady-state MBF. Since a large portion of the increase in pressure gradient across working muscles occurred early in exercise (phase I), further increases in MBF must be accomplished by increases in VC to meet the metabolic demand as well as steady state MBF requirements during a delayed phase II response (Shoemaker and Hughson, 1999). This phase is characterized by an exponential increase in MBF starting $\sim$15 to 20 s after the onset of exercise and it appears to be under much tighter feedback regulation than the phase I response (Shoemaker and Hughson, 1999, Tschakovsky and Hughson, 1999).

The metabolic and endothelial-derived regulatory factors probably are released proportionally to exercise intensity to provide feedback regulation of VC leading to an increase in MBF via vasodilation through a complex interaction between local and central mechanisms (Laughlin and Korzick, 2001, Segal, 2005). There are several control mechanisms as well as vasodilatory candidates identified to be involved in this phase. The MBF regulation represents the integration of multiple stimuli to obtain the required response where in the absence or reduction of the effect of one, others can completely compensate (Shoemaker and Hughson, 1999, Laughlin and Korzick, 2001, Hughson, 2003). Consequently, the dynamic response of MBF represents the net interactive effect of multiple mechanisms (Tschakovsky et al., 2006). The main control mechanisms likely involved in phase I and II response of MBF are presented in Figure 1.3.

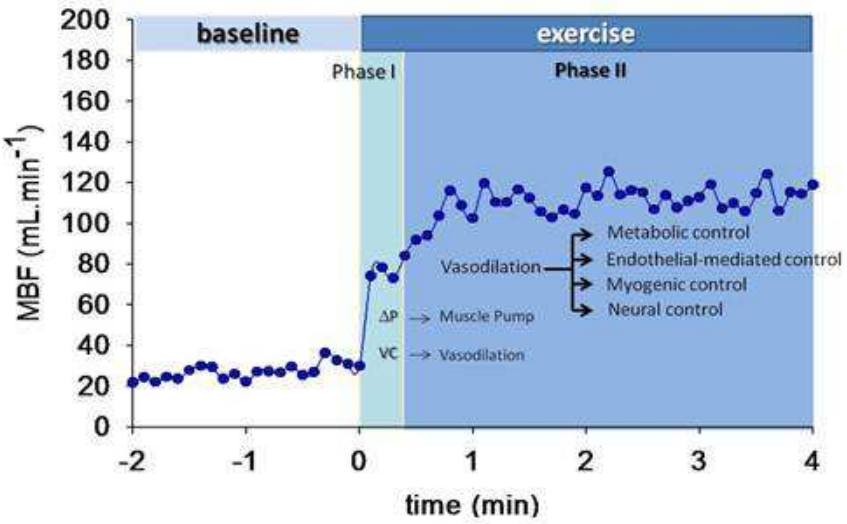

Figure 1.3: Illustration of possible mechanisms for control of muscle blood flow (MBF) through the popliteal artery in phase I and phase II following the start of exercise. During phase I, the main control mechanisms involved are: (1) changes in pressure gradient (ΔP) due to muscle pump action and (2) changes in vascular conductance (VC) due to vasodilation. In phase II, the main mechanism considered is vasodilation that is probably controlled by: metabolic, endothelial-mediated, myogenic and neural factors (adapted from Shoemaker and Hughson, 1999.)

1.2.5 Effects of altered arterial perfusion pressures on vascular conductance and muscle blood flow during exercise

Exercise hyperemia is affected by changes in body position through the interaction between changes in MPP and the mechanical effects of muscle contraction and muscle pump activation combined with local vasodilatory mechanisms that influence VC (Leyk et al., 1994, Tschakovsky et al., 1996, Shoemaker et al., 1998, Tschakovsky and Hughson, 1999, Shoemaker and Hughson, 1999, Tschakovsky and Sheriff, 2004, Tschakovsky et al., 2006, Walker et al., 2007). The increase in VC during exercise is probably a consequence of the combined effects of vasodilation and muscle pump activity increasing the perfusion pressure gradient across the working muscle enhancing the virtual conductance (Tschakovsky et al., 1996, Shoemaker et al., 1998, Shoemaker and Hughson, 1999, Nadland et al., 2009).

Positioning of the arm (Hughson et al., 1996, Walker et al., 2007) or legs (Leyk et al., 1994, Grassi et al., 1996, Radegran and Saltin, 1998, Nadland et al., 2009) below

heart level increases the rate of adaptation of MBF and VC toward a new steady-state level. The blunted VC response with the arm below heart level probably indicate that less accumulation of vasodilator metabolites is required as the muscle pump is more effective to increase MBF in conditions with elevated MPP (Folkow, Haglund, Jodal, and Lundgren, 1971, Laughlin and Joyner, 2003, Nadland et al., 2009). However, when the arm is placed above the heart level muscle pump no longer contribute to the increase in VC and MBF due to the minimized emptying effect of the veins and the lack of a hydrostatic column (Tschakovsky et al., 1996, Saunders and Tschakovsky, 2004). In this condition, local vasodilation occurs to increase VC in an attempt to compensate for the reduced MPP to maintain MBF (Hughson et al., 1996), but there is a possibility that MBF would be compromised under reduced MPP (Eiken, 1988).

1.2.6 Effects of hypoxia on vascular conductance, muscle blood flow and oxygen delivery during exercise

Hypoxia has been used to manipulate CaO_2 and PaO_2 to investigate MBF and DO_2 adjustments during exercise. Reductions of CaO_2 and PaO_2 via hypoxia resulted in compensatory adjustments in VC to increase MBF maintaining DO_2 during one or two-legged knee extension submaximal exercise in semi-supine position (Koskolou et al., 1997, Gonzalez-Alonso et al., 2001) or maintenance of MBF with reduction in DO_2 during alternated two-legged kicking submaximal exercise in sitting position (MacDonald, Tarnopolsky, and Hughson, 2000). The higher VC response in hypoxia compared to normoxia is probably due to local vasodilation in response to the reduced cellular PO_2 caused by the reductions of CaO_2, PaO_2 and O_2 availability (Calbet, 2000).

The red blood cells can sense the decrease in O_2 leading to release of ATP from the erythrocytes mediating vasodilatory responses relaxing the vascular smooth muscle via release of nitric oxide (NO), endothelium-derived-hyperpolarization-factor (EDHF) and/or release of NO from S-nitrosohemoglobin due to hemoglobin deoxygenation (Calbet, 2000, Stamler et al., 1997, Jia, Bonaventura, Bonaventura, and Stamler, 1996, Ellsworth, Forrester, Ellis, and Dietrich, 1995). In hypoxia, the accumulation of adenosine activating the adenosine-sensitive K^+ channels (Allen, Lamb, and Westerblad, 2008) , adenosine monophosphate-activated protein kinase (AMPK) stimulating the release of NO and PG (Fisslthaler and Fleming, 2009, Towler and Hardie, 2007) and release of metabolic vasodilators by muscle contractions (CO_2, H^+, lactate, Pi) could contribute to the vasodilation increasing VC (Calbet, 2000). The maintenance of DO_2 in hypoxia would be obtained by the compensatory adjustments in MBF due to the increase in VC.

1.3 Objectives of this thesis

The overall purpose of this thesis was to investigate the adaptations of lower limb VC and MBF during alterations in arterial perfusion pressure at rest, during transitions from rest to exercise and during exercise under altered F_IO_2. To do so, five studies were designed in an attempt to achieve such purpose.

The aim of the first study (Chapter 2) was to test data repeatability for popliteal MBV, popliteal arterial diameter (AD_{pop}), MBF and VC at rest, during the steady-state of exercise in different body positions using a custom-built tilt table associated with Doppler and echo Doppler ultrasound techniques. Previous studies have been conducted with manipulations of arm below and above heart level, or leg position below heart level and or changes from supine to upright posture, but not in head-down tilt with a focus on the lower legs. This study provided a critical basis for the subsequent studies which manipulated arterial perfusion pressure to affect limb VC and MBF at rest, during transitions from rest to exercise, and during exercise under altered F_IO_2 (hypoxia).

The aim of the second study (Chapter 3) was to investigate the adaptations of the lower limb VC and MBF during HUT and HDT postural challenges at rest. Previous studies reported the effect of HUT position in the cardiovascular system impacting VC and MBF. However, little is known about VC and MBF responses with the lower leg positioned above the heart level (HDT). This approach provided information on how VC and MBF adapted in conditions with reduced (HDT) and elevated (HUT) MPP at rest providing a basis for subsequent studies that investigated VC and MBF responses during exercise. The protocol applied in this study is illustrated in Figure 3.1.

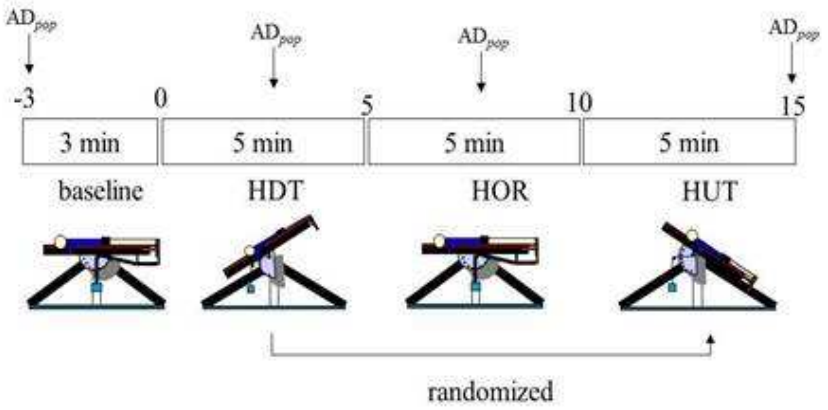

Figure 1.4: Resting protocol used to investigate the adaptations of the lower limb VC and resting MBF during HOR, HDT and HUT positions. Arrows indicate were AD_{pop} (popliteal arterial diameter) were taken.

The aim of the third study (Chapter 4) was to compare MBV and VC patterns during three different body positions that affected the gravity-dependent distribution of arterial and venous pressures. The effects of passive HDT and HUT on the anterograde muscle blood flow velocity (MBV_{ant}) and retrograde muscle blood flow velocity (MBV_{ret}) patterns are not well characterized. This analysis provided additional information on how VC and MBF adapts in conditions with reduced (HDT) and elevated (HUT) MPP at rest within cardiac cycles.

In the fourth study (Chapter 5), the aim of the first experiment was to determine peak vascular conductance (VC_{peak}) in the lower limb by the sudden release of occlusion following intense, ischemic muscle contractions. The aim of the second experiment was to compare the impact of altered MPP on MBF and VC during transitions from rest to lower and higher power output plantar flexion exercise performed with manipulations of limb position relative to the heart. This approach determined the VC_{peak} and its fractional recruitment allowing investigation of the MBF regulation in different exercise challenges with altered perfusion pressure. The Figure 6.1 presents the illustration of the protocol used in the second experiment.

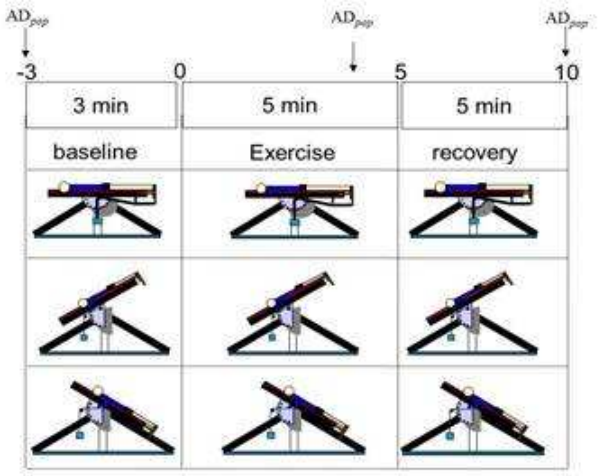

Figure 1.5: Exercising protocol used to test the impact of altered MPP on VC and MBF during transitions from rest to plantar flexion exercise in HOR, HDT and HUT positions. Arrows indicate were AD_{pop} (popliteal arterial diameter) were taken.

The main of the fifth study (Chapter 6) was to investigate MBF, DO_2 and VC responses during submaximal calf exercise in three different body positions that affect the gravity-dependent distribution of arterial and venous pressures under altered O_2 inspired fraction (normoxia,$F_IO_2 = 0.21$ and hypoxia, $F_IO_2 = 0.14$). The effects of hypoxia in conjunction with altered arterial perfusion pressure on MBF, DO_2 and VC responses during exercise in humans are not known. This approach provided further information about how VC and MBF adapt to the exercise challenge in conditions with reduced F_IO_2 associated with conditions under reduced MPP (HDT) and elevated MPP (HUT) eliciting some more details about blood flow regulation and control mechanisms. The Figure 1.6 presents the illustration of the protocol used in this study.

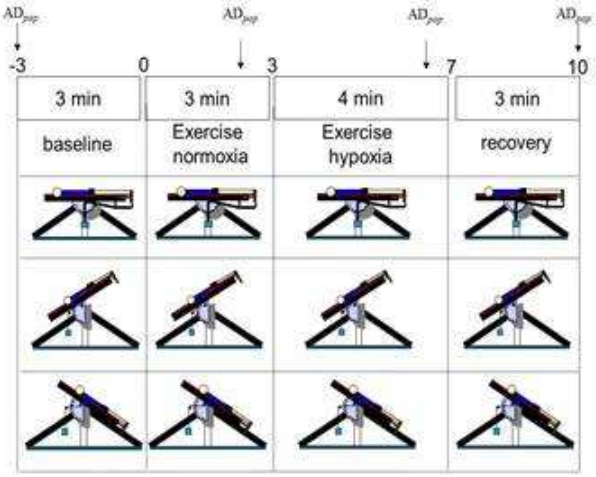

Figure 1.6: Exercising protocol used to investigate MBF, DO_2 and VC responses during submaximal calf exercise in HOR, HDT and HUT positions under altered O_2 inspired fraction (normoxia, $F_IO_2 = 0.21$ and hypoxia, $F_IO_2 = 0.14$). Arrows indicate were AD_{pop} (popliteal arterial diameter) were taken.

The final chapter of this thesis (Chapter 7) presented a summary of the studies as well as the implications of these findings and providing guidance for future investigations.

Chapter 2

Repeatability of the muscle blood flow response at rest and exercise during body tilt using Doppler ultrasound

2.1 Overview

This study tested the data repeatability for popliteal muscle blood flow velocity (MBV), popliteal arterial diameter (AD_{pop}), muscle blood flow (MBF) and vascular conductance (VC) at rest and exercise in different body positions and work rates (EX). Fifteen and eleven healthy volunteers participated in the two phases of the studies. Resting protocols were performed in horizontal (HOR), 35° head-down tilt (HDT) and 45° head-up tilt (HUT) for 5min in each body position on two separate days. EX was performed at lower and higher power outputs (repeated plantar flexion contractions at 20 and 30% maximal voluntary contraction, respectively) in HOR, 35°HDT and 45°HUT on two separate days. MBV was measured in the popliteal artery by Doppler ultrasound, AD_{pop} was measured by ultrasound imaging, MBF was calculated and VC was estimated by dividing MBF flow by muscle perfusion pressure (MPP) determined beat-by-beat from a finger cuff and corrected to the level of the mid-calf. All variables were not different and demonstrated good agreement between the two days of testing during both rest and exercise regardless of body position. In conclusion MBV, AD_{pop}, MBF, and VC were repeatable in all body positions during rest and exercise conditions. Therefore, these data support the utilization of Doppler ultrasound and echo Doppler as a reproducible method to measure MBV and AD_{pop} and consequently estimate MBF and VC in such conditions.

2.2 Introduction

Accurate, reliable and reproducible quantitative measurements of blood flow are essential in studies interested to explore the dynamic response of muscle blood flow during both rest and exercise. Especially during exercise, the major challenge is to obtain consistent measurements of arterial diameter and blood flow velocity due to muscle contraction and the variable velocity profile (Shoemaker et al., 1996). This problem is increased when different body positions are assumed because the velocity profile changes according to the alterations in posture and pressure gradients. Non-invasive ultrasound is a method that has the capability to measure arterial diameter, blood flow velocity to determine blood flow (Walther et al., 2006, Osada, 2004, Radegran, 1997, Shoemaker et al., 1996). The use of this method has been applied to measure femoral (Walther et al., 2006, Osada, 2004, Radegran, 1997, Stacey-Clear and Fish, 1984) brachial (Shoemaker et al., 1996) and popliteal (Labropoulos et al., 1998) arterial diameters and blood flow velocities and consequently estimation of the blood flow at rest.

Doppler ultrasound has the advantage to permit continuous measurements on a beat-by-beat basis due to its high temporal resolution allowing blood flow velocity measures during dynamic exercise (Walther et al., 2006, Osada, 2004, Radegran, 1997, Shoemaker et al., 1996). Therefore, this technique became an attractive tool to measure blood flow in different conduit arteries in different exercise models, such as, brachial artery during dynamic handgrip exercise (Shoemaker et al., 1996, Hughson et al., 1996, Tschakovsky et al., 1995), femoral artery during leg-extension exercise (Walther et al., 2006,Osada, 2004, Radegran, 1997) and plantar flexion exercise (Leyk et al., 1994, Leyk et al., 1992). Although many studies have used Doppler ultrasound to quantify arterial blood flow during exercise there are few data available regarding its repeatability for peripheral arteries in the lower limb (Walther et al., 2006). Therefore, the purpose of the current study was to test the data repeatability for popliteal muscle blood flow velocity, popliteal arterial diameter, muscle blood flow, and vascular conductance at rest and exercise at two work rates in different body positions.

2.3 Methods

2.3.1 Participants

Fifteen and eleven healthy, non-smoking, male volunteers participated in the two phases of the studies with experiments performed at rest and exercise in different body positions (EX). Participants' characteristics related to age, height, and body mass are described in the Table 2.1.

Table 2.1: Participants' characteristics related to age (years), height (cm) and body mass (Kg).

Conditions	Rest ($n = 15$)	EX ($n = 11$)
Age (years)	29.6 ± 2.7	28.0 ± 3.8
Height (cm)	177.1 ± 5.5	176.5 ± 5.3
Body mass (Kg)	80.4 ± 13.5	79.0 ± 6.9

Values are mean ± SD. Number of participants (n).

Participants received complete written and verbal details of the experimental procedures and potential risks involved prior to signing an information consent form approved by the Office of Research Ethics of the University of Waterloo. All participants were instructed to refrain from consuming alcohol, caffeinated beverages, engaging in vigorous exercise for 24 hours prior to testing, and from consuming a large meal within two hours of testing.

2.3.2 Experimental Design

Participants reported to the laboratory two times for the rest and four times for EX experimental conditions. For all tests, participants assumed a prone position on a custom built tilt table with their head supported by a massage table head piece. Shoulder blocks were adjusted accordingly and their arms were placed on the resting apparatus with the shoulders and elbows positioned at approximately 90°. Two belts, one located on the chest and other on the hips, were used to secure the participants during testing. Straps from these belts were attached in the tilt table to help to avoid the participants from sliding. In the resting condition, there was no tension placed on the foot and it was held in a natural

angle. Under exercise, the foot was secured to a foot plate with the angle of rotation at the ankle. Further details are provided in Chapter 3 and Chapter 4.

Following this procedure, ECG electrodes were applied on the skin surface in the back, 4 MHz pulsed Doppler ultrasound and 6-13 MHz echo Doppler ultrasound probes were placed on the skin over the popliteal artery. In the first day of a new section, once the participants were secured to the table, they were familiarized with a short trial which simulated the protocols with enough recovery time to ensure the return of the variables to the baseline levels. During familiarization, none of the participants reported any discomfort. Each participant performed four repeated randomized trials, two trials of each condition per day at rest, two repeated randomized trials, one trial of each condition per day for EX.

2.3.2.1 Resting Protocol

The resting protocol consisted of three different gravitational stress conditions. First, participants performed 3min of baseline in a HOR position at 0°. Then, the participant was tilted in approximately 3s to 35°HDT or 45°HUT and this body position was maintained for 5min. After this period of time, the table was rotated back (3s) to HOR (control condition) for 5min. Subsequently, subjects were tilted to the other position, HUT or HDT, for 5min. The order of these conditions was randomized and counterbalanced among the participants between HDT and HUT. In each body position, data were analysed between 3-5min after allowing for stabilization.

2.3.2.2 Exercise Protocol

The EX protocols were comprised of repeated plantar flexion exercise consisting of 1s to extend the footplate, 1s to return it to resting position, and 1s of rest (3s duty cycle) when the load was 20% or 30% of the maximal voluntary contraction (MVC). The work rates generated by these protocols were defined respectively as lower and higher power output. The angle of movement was $\sim$20° representing a displacement of 10cm. The foot plate movement was set in the rotational axis of the ankle to isolate contractions to the calf muscles. The four days of testing were performed two times per week separated by at least 48 hours. This break minimized any carry over and cumulative effect of the exercise protocols, as well as muscle adaptation to the stimulus.

The angles of tilting for HDT (35°) and HUT (45°) accomplished a reduction and increase in muscle perfusion pressure (MPP) estimated to the middle of the calf muscle of ~ -44mmHg and $\sim +55$mmHg, respectively. Changing the angle of the tilt table required

adjusting the load to achieve the same tension on the cable attached to the foot plate (for more details see Chapter 4.

For EX, each participant performed six different protocols; three tests per day on four different days randomized by blocks and counterbalanced among the participants. The specific sequence and recovery time provided between exercise bouts was designed to avoid muscle fatigue in both protocols. Each EX protocol consisted of 3min baseline in a body position followed by 5min of exercise. A more detailed description of the exercise protocol is provided in the Chapter 4.

All tests were conducted in a quiet room where room temperature was kept constant at 19.4 ± 0.9 °C to ensure minimal skin blood flow at rest and exercise protocols with humidity at 39.9 ± 4.8%, and barometric pressure at 730.8 ± 2.4 mmHg.

2.3.3 Data Acquisition

Muscle blood flow velocity (MBV) in the popliteal artery, heart rate (HR) and blood pressure (BP) were measured beat-by-beat recorded at 1000 Hz using a data acquisition system (Powerlab, ADInstruments, Colorado Springs, USA) and processed after testing with specific data analysis software (Chart, version 5.5, ADInstruments, Colorado Springs, USA). MBV was determined from the intensity weighted mean of a 4 MHz pulsed Doppler ultrasound probe (Neurovision Doppler Ultrasound, Model 500, Multigon Industries, Mt. Vernon, USA). The flat ultrasound probe was secured by surgical tape to the skin surface over the popliteal artery embedded at a 45° angle of insonation relative to the skin and adjusted according to the measured angle between the skin and the popliteal artery.

Prior to each trial the popliteal artery was investigated to establish proper alignment, and to determine the depth and gate to assure full insonation of the artery lumen without contamination from the venous signal. The probe location was marked on the skin to assist repositioning of the probe during the test and on subsequent tests. If it was necessary, the researcher manipulated the probe to preserve the best Doppler signal as determined by constant use of auditory and visual feedback. If the ultrasound signal quality was compromised during the last 2min of baseline or a tilt trial, it was excluded from the analysis. Two participants in HUT and one in HDT were excluded for the repeatability tests during exercise due to difficulties with the Doppler ultrasound signal resulting in loss of some data in those trials. Therefore, the data analysis for HUT and HDT were based on nine and ten rather than eleven participants, respectively.

Diameter of the popliteal artery (AD_{pop}) was measured with an 8-12 MHz echo Doppler ultrasound probe (Diagnostic Ultrasound System, Model M5, Shenzen Mindray Bio-medical

Electronics Co., Ltd., Shenzen, China). These popliteal artery images were recorded and stored for analysis. AD_{pop} measurements were performed in B-mode with the echo Doppler ultrasound probe positioned just proximal to the site of velocity measurements. AD_{pop} images at rest were taken as the average of three separate measurements during diastole at baseline (1.5min), 5.5 (HDT or HUT), 10.5 (HOR), and 15.5min (HUT or HDT). During exercise, AD_{pop} images were taken during diastole at baseline and at the end of recovery time and in the relaxation phase between contractions in the fourth minute of exercise for EX.

Arterial blood pressure was estimated from a photoplethysmograph cuff placed around the middle finger of the left hand (Finometer, Finapres Medical Systems, Arnhem, the Netherlands). Mean arterial pressure (MAP) was determined as the area under the curve between successive heart beats. Heart rate (HR) was continuously calculated from the RR-interval obtained from an electrocardiogram (Pilot 9200, Colin Medical Instruments Corp, San Antonio, Texas, USA).

2.3.4 Data Analysis

The MBV and the AD_{pop} cross-sectional area were used to calculate MBF ($mL \cdot min^{-1}$) as MBF = MBV$\times\pi r^2\times$60; where r is the vessel radius. Vascular conductance (VC) was estimated from MBF and MPP as VC ($mL \cdot min^{-1}$) = MBF/MPP. The MPP (mmHg) was estimated by MAP (mmHg) and the distance between subjects heart and the middle of the calf muscle for all body positions. For the HOR position, the distance between participants heart and the middle of the calf muscle is zero, thus, MPP was equal to MAP (i.e. 85mmHg + 0mmHg = 85mmHg). For the HDT position, MPP was equal to MAP subtracted from the vertical distance between participants heart and the middle of the calf muscle, converted in millimeters of mercury (1mmHg = 1.36cm of H_2O, due to the effects of gravity lowering arterial perfusion pressure (i.e. 85mmHg − 44mmHg, 60cm/1.36cm of H_2O = 41mmHg). For the HUT position, MPP was equal to MAP added from the vertical distance between participants heart and the middle of the calf muscle, converted in millimeters of mercury (1mmHg = 1.36cm of H_2O), due to the effects of gravity increasing arterial perfusion pressure (i.e. 85mmHg + 55mmHg, 75cm/1.36cmH_2O = 140mmHg).

Aberrant data from each trial were deleted and following this procedure data were averaged over 2min at rest and in the last 2min of exercise to generate a single value for HDT, HOR, and HUT positions. In HOR, the first minute of data were excluded to avoid carrying over effect of the tilting procedures.

2.3.5 Statistical Analysis

Order effect was analyzed with repeated measures one-way analysis of variance to detect possible statistical differences between trials in the same body position for MBV, AD_{pop}, MBF, and VC at rest. Day-to-day variability for the same variables and the same body positions during rest and exercise trials were determined with: (1) student t-test analysis to identify statistically significant differences between day 1 and day 2, with the level of significance set at $p < 0.05$; (2) calculation of coefficient of variation (CV), which is CV = (Standard deviation/mean)$\times$100, for each participant in each condition to measure variability between days and (3) Bland-Altman plots to access the level of agreement between the two days of testing. Data were presented as means $\pm$ standard deviation (SD).

2.4 Results

2.4.1 Resting Protocol

There were no statistical differences between the trials for all variables indicating no order effect was caused by the randomization procedures. All variables did not show statistical differences between two days of testing for all body positions (Figure 2.1A, 2.1B, 2.1C). The mean CV values were close regardless of the body position indicating that MBV, AD_{pop}, MBF and VC were consistent between two days of testing (Table 2.2).

Table 2.2: MBV, AD_{pop}, MBF and VC during baseline, HDT, HOR, and HUT with respect to coefficient of variation during the rest protocol.

Variables	days	Baseline	HDT	HOR	HUT
MBV ($cm \cdot s^{-1}$)	1	2.7 ± 0.8	2.4 ± 0.7	2.6 ± 0.8	1.6 ± 0.3
MBV ($cm \cdot s^{-1}$)	2	2.8 ± 1.0	2.5 ± 0.6	2.7 ± 0.9	1.5 ± 0.5
AD_{pop} (cm)	1	0.64 ± 0.06	0.64 ± 0.06	0.64 ± 0.06	0.64 ± 0.06
AD_{pop} (cm)	2	0.64 ± 0.06	0.64 ± 0.06	0.64 ± 0.06	0.64 ± 0.06
MBF ($mL \cdot min^{-1}$)	1	52.1 ± 15.4	45.8 ± 14.4	51.9 ± 17.5	29.9 ± 6.0
MBF ($mL \cdot min^{-1}$)	2	52.5 ± 20.6	46.9 ± 12.4	51.2 ± 18.0	30.1 ± 6.0
VC ($mL \cdot min^{-1} \cdot min^{-1}$)	1	0.60 ± 0.20	1.01 ± 0.38	0.54 ± 0.12	0.21 ± 0.05
VC ($mL \cdot min^{-1} \cdot min^{-1}$)	2	0.59 ± 0.23	0.99 ± 0.28	0.56 ± 0.19	0.21 ± 0.08

Values are mean ± SD of 15 participants in two days of testing. Muscle blood flow velocity (MBV), popliteal arterial diameter (AD_{pop}), muscle blood flow (MBF), vascular conductance (VC), horizontal (HOR), head-down tilt (HDT), head-up tilt (HUT) and coefficient of variation (CV).

The Bland-Altman analyses indicated that the 95% limits of agreement between the two days of testing showed no deviation of the mean value from 0, and relatively small variability about the mean for all body positions for MBV, AD_{pop}, MBF and VC (Figure 2.1D, 2.1E, 2.1F).

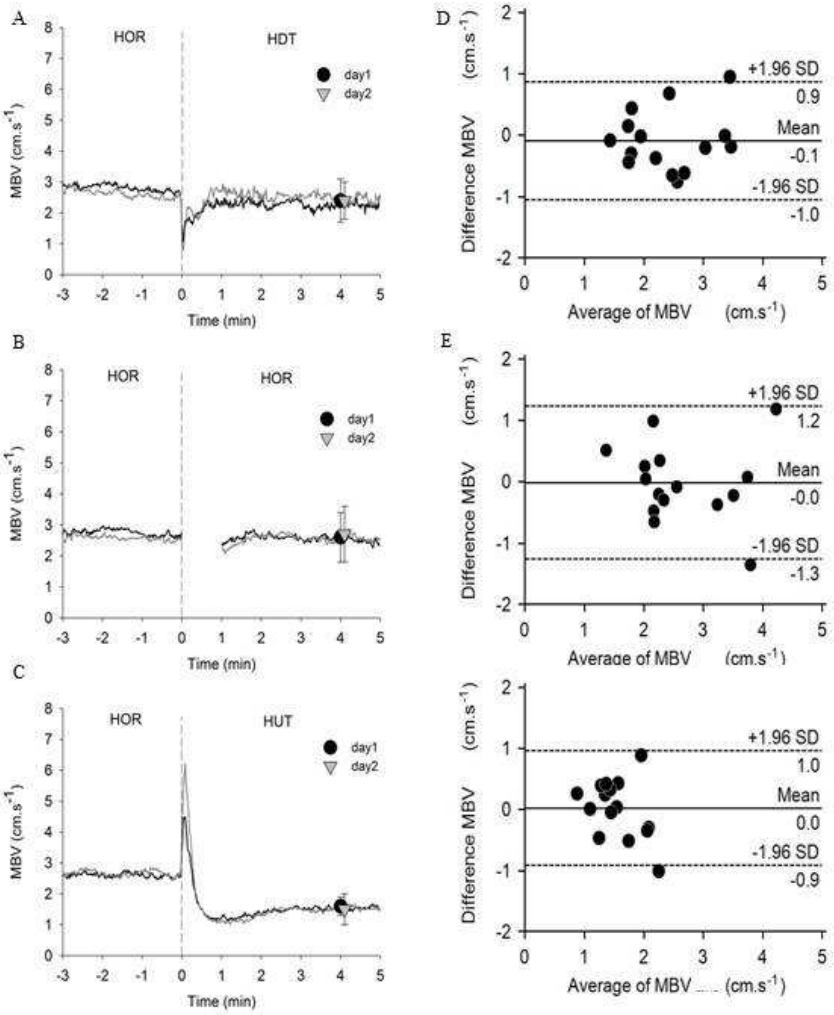

Figure 2.1: Muscle blood flow velocity (MBV) responses to changes in body position across two days of testing for HDT (A), HOR (B) and HUT (C). Lines indicate group response, symbols indicated the points at which repeatability were performed and dashed vertical lines indicate transition from baseline to HDT and baseline to HUT. Bland-Altman plots during HDT (D), HOR (E), and HUT (F) comparing two days of testing at rest (Difference MBV = MBVDay1 − MBVDay2, Average = (MBVDay1 − MBVDay2)/2). Solid horizontal lines show mean values and dashed horizontal lines represent 95% confidence limits. The symbols are offset for clarity.

2.4.2 Exercise Protocol

MBV, AD_{pop}, MBF and VC were not different between two days of testing during lower and higher power output exercise (Table 2.3). The mean CV values showed that MBV, AD_{pop}, MBF and VC were consistent between two days of testing regardless of the body position (Table 2.3). The Bland-Altman analyses for lower power output are presented in the Figure 2.2. The Bland-Altman analyses indicated that the 95% limits of agreement between the two days of testing ranged from -2.5 to 2.4 for HPO_{HDT}, -3.0 to 4.4 for HPO_{HOR} and -2.3 to 3.5 for HPO_{HUT}. These results indicate no deviation of the mean value from 0, and relatively small variability about the mean.

Table 2.3: MBV, AD_{pop}, MBF, VC and CV between two days of testing during lower and higher power output during plantar flexion averaged over the last two minutes of exercise.

Conditions	days	MBV ($cm \cdot s^{-1}$)	AD_{pop} (cm)	MBF ($mL \cdot min^{-1}$)	VC ($mL \cdot min^{-1} \cdot min^{-1}$)
LPO_{HOR} ($n = 11$)	1	8.7 ± 2.8	0.64 ± 0.04	175.1 ± 72.4	2.04 ± 0.83
LPO_{HOR} ($n = 11$)	2	9.6 ± 2.5	0.64 ± 0.04	187.8 ± 68.0	2.26 ± 0.84
CV (%)		7.6 ± 4.8	1.1 ± 0.9	7.5 ± 4.5	8.1 ± 4.7
LPO_{HDT} ($n = 10$)	1	7.8 ± 2.5	0.63 ± 0.05	142.0 ± 55.0	3.36 ± 0.92
LPO_{HDT} ($n = 10$)	2	8.0 ± 2.6	0.63 ± 0.04	158.2 ± 53.3	3.76 ± 1.10
CV (%)		4.3 ± 4.0	1.2 ± 0.8	5.0 ± 5.1	6.1 ± 5.9
LPO_{HUT} ($n = 9$)	1	6.4 ± 2.6	0.63 ± 0.04	126.4 ± 60.8	0.90 ± 1.10
LPO_{HUT} ($n = 9$)	2	5.6 ± 2.4	0.64 ± 0.04	110.0 ± 44.1	0.77 ± 1.30
CV (%)		7.8 ± 4.0	1.2 ± 0.7	8.0 ± 4.2	8.8 ± 5.3
HPO_{HOR} ($n = 11$)	1	12.7 ± 3.3	0.64 ± 0.05	257.3 ± 89.7	2.83 ± 1.1
HPO_{HOR} ($n = 11$)	2	12.0 ± 4.2	0.64 ± 0.05	236.0 ± 101.7	2.77 ± 1.3
CV (%)		6.6 ± 4.2	0.6 ± 0.7	7.1 ± 4.6	6.4 ± 4.4
HPO_{HDT} ($n = 10$)	1	8.7 ± 1.0	0.63 ± 0.05	161.0 ± 69.1	3.02 ± 1.10
HPO_{HDT} ($n = 10$)	2	8.8 ± 1.0	0.63 ± 0.04	164.7 ± 54.1	3.35 ± 1.27
CV (%)		6.1 ± 4.8	0.8 ± 0.9	5.6 ± 4.6	8.5 ± 5.9
HPO_{HUT} ($n = 9$)	1	11.8 ± 1.0	0.64 ± 0.04	227.6 ± 69.5	1.62 ± 0.52
HPO_{HUT} ($n = 9$)	2	11.7 ± 1.0	0.64 ± 0.05	229.4 ± 80.3	1.63 ± 0.61
CV (%)		9.0 ± 6.2	1.1 ± 0.3	9.7 ± 7.5	9.1 ± 7.7

Values are mean ± SD for two days of testing. Number of participants (n), muscle blood flow velocity (MBV), popliteal arterial diameter (AD_{pop}), muscle blood flow (MBF) and vascular conductance (VC), lower power output in horizontal (LPO_{HOR}), lower power output in head-down tilt (LPO_{HDT}), lower power output in head-up tilt (LPO_{HUT}), higher power output in horizontal (HPO_{HOR}), higher power output in head-down tilt (HPO_{HDT}), higher power output in head-up tilt (HPO_{HUT}) and coefficient of variation (CV).

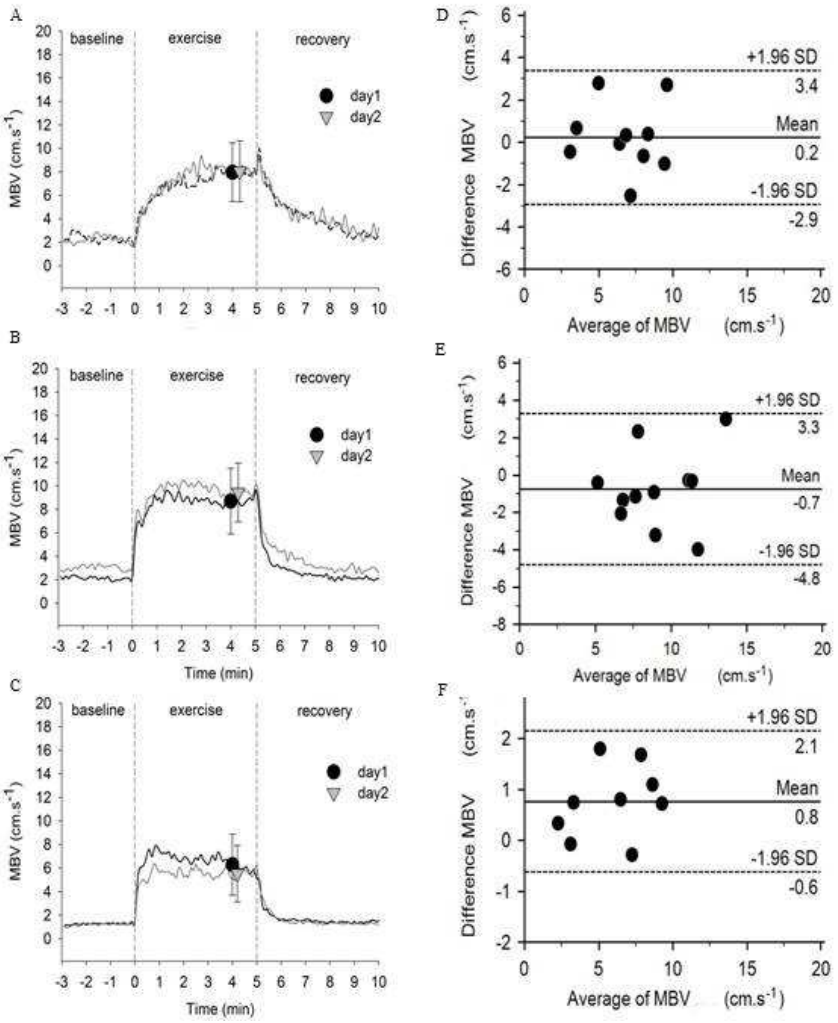

Figure 2.2: Muscle blood flow velocity (MBV) responses to dynamic plantar flexion exercise across two days of testing during lower power output: LPO_{HDT} (A), LPO_{HOR} (B) and LPO_{HUT} (C). Lines indicate group response, symbols indicate the points at which repeatability were performed and dashed vertical lines indicate the start and cessation of exercise. Data are the mean ± SD analyzed over the last two minutes of exercise. Bland-Altman plots during lower power output: LPO_{HDT} (D), LPO_{HOR} (E) and LPO_{HUT} (F) over the last two minutes of exercise (Difference MBV = MBVDay1 − MBVDay2, Average = (MBVDay1 − MBVDay2)/2). Solid horizontal lines show mean values and dashed horizontal lines represent 95% confidence limits. The symbols are offset for clarity.

2.5 Discussion

This is the first time that MBV, AD_{pop}, MBF and VC repeatability were tested at rest and during different work rates in HOR, HDT and HUT positions. The present study provided evidence that MBV and AD_{pop} measured by Doppler ultrasound techniques are repeatable during such body positions, consequently estimates of MBF and VC can be obtained by these methods. The ultrasound data proved to be reliable and suitable for studies testing the dynamic response of MBF and VC at rest and exercise with postural challenges.

2.5.1 Popliteal arterial diameter repeatability

AD_{pop} estimation by echo Doppler ultrasound technique showed to be highly repeatable between two days of testing during both rest and exercise for all body positions. In the current study, the mean CV between days for AD_{pop} during both rest and exercise was ∼2% (Table 2.2 and Table 2.3, respectively). The CV in the current study is in a similar range of previous studies between ∼2-4% for brachial (Shoemaker et al., 1996), ∼1-4% for axillary (Walther et al., 2006, Amundsen, Wisloff, Helgerud, Hoff, and Slordahl, 2002), ∼1.5-2% for femoral (Walther et al., 2006, Radegran, 1997) and ∼2-5% for popliteal (Labropoulos et al., 1998) arteries.

Measurement error in the estimation of vessel diameter could lead to large error in calculation of blood flow since flow is a function of the squared vessel radius (Walther et al., 2006, Radegran, 1997, Shoemaker et al., 1996). An error in the placement of the measurement calipers by the operator contributes for the variability in the diameter (Amundsen et al., 2002, Shoemaker et al., 1996). In the current study, the echo Doppler ultrasound used has a resolution of 0.1mm. Therefore, this error for diameter in the range of 0.64cm (6.4mm) would contribute to ∼1.6% of the variability. This variability is slightly lower than the range reported by Shoemaker et al., 1996 between ∼2.5-3% and similar to the range of Amundsen et al., 2002 between ∼1-2%.

A further contributor to the diameter measurement variability is the motion during muscle contraction (Shoemaker et al., 1996). Strapping the participants on the tilt table and the use of a small muscle mass with a short range of motion to exercise (plantar flexion) reduced the motion caused by calf muscle contractions. Despite this, movements of the popliteal artery could occur during contractions increasing variability. An experienced and well trained operator held the probe freely to follow the movement pattern of the artery to maintain optimal image. The same operator participated in all of the studies minimizing potential systematic errors due to technique. An average of the diameters

was taken to produce one data set. Taken together, all these procedures were used to minimize AD_{pop} measurements variability. Low coefficients of variation (∼2%) and good agreement between these two days of testing indicated that image AD_{pop} measurements were consistently optimal and were not affected by repositioning of the probe.

2.5.2 Muscle blood flow velocity repeatability

MBV did not show differences across two days of testing during both rest and exercise presenting greater variability than arterial diameter. In the current study, the CV for MBV ranged from ∼7-10% at rest (Table 2.2 and Figure 2.1) and from ∼4-9% during exercise (Table 2.3, Figure 2.2). This variability during both rest and exercise is within the range of the brachial blood velocity between ∼7-25% at rest and ∼5-31% during handgrip exercise (Walther et al., 2006), axillary between ∼13% at rest and ∼5-10% during elbow-flexion exercise (Walther et al., 2006, Amundsen et al., 2002), femoral between ∼14% at rest and ∼1-8% during knee-extension exercise (Walther et al., 2006, Radegran, 1997) and popliteal between ∼5-36% at rest (Labropoulos et al., 1998).

MBF was calculated from AD_{pop} and MBV, thus, CV values for MBF are dependent of these two variables. Since AD_{pop} presented very small variability (∼2%) the CV values for MBF were close to the MBV values during rest (Table 2.2) and exercise (Table 2.3). VC was calculated from MBF and MPP, thus, CV values for VC depends on the combined variability between MBF and MPP. Since MPP had small variation in the same body position, the CV values for VC were close to the MBF values during rest (Table 2.2) and exercise (Table 2.3).

The main sources of variability in velocity measurements are: (1) error in the angle of insonation (Walther et al., 2006, Amundsen et al., 2002, Walther et al., 2006, Gill, 1985); (2) improper alignment of the ultrasound beam with the artery (Radegran, 1997, Gill, 1985); (3) Doppler signal processing and (4) frequency estimation. Exercise contraction induced variability in the angle of insonation of ∼ 5% (Shoemaker et al., 1996) where an error of 3° when the angle of insonation was 55° would overestimate femoral and axillary blood velocities by 8% (Amundsen et al., 2002). In the current study, the flat Doppler ultrasound probe was fixed over the popliteal artery during each test in an attempt to maintain the angle of insonation constant and proper alignment between ultrasound beam and popliteal artery during both rest and exercise experiments. The pulsed ultrasound probe location was marked on the skin and the distance between probe and popliteal fossa was recorded to assist in the return of the probe to the same location during subsequent tests. Radegran, 1997 reported that vessel visualization and audiovisual blood velocity feedback with continuous velocity measurements provide for the most accurate results.

Further variability in velocity measurements are influenced by physiological sources, such as, variation due to muscle contraction-relaxation phases (Osada, 2004, Radegran, 1997, Shoemaker et al., 1996, Walloe and Wesche, 1988) and control of arterial perfusion pressure and downstream arteriolar tone (Radegran, 1997). Beat-by-beat blood flow velocity variability during dynamic exercise is affected by the transient variations in intramuscular pressure, cardiac cycle, and perfusion pressure during muscle contraction (Osada, 2004, Radegran, 1997, Walloe and Wesche, 1988). Blood flow stops or reverses momentarily and high intramuscular pressure is observed during muscle contraction phase. Subsequently, during the muscle relaxation phase, low intramuscular pressure allows a rapid and large increase in blood flow through the artery (Radegran, 1997, Shoemaker et al., 1996,Walloe and Wesche, 1988). The force (compression) produced during contractions could have an impact on the magnitude of the intramuscular pressure variations affecting perfusion pressure and consequently blood flow (Osada, 2004).

The muscle contraction-relaxation phases and blood flow velocity variability are closely related (Radegran, 1997). Therefore, if blood flow velocity variation is highly influenced by muscle contraction-relaxation phases, it is expected that averaging blood flow velocity data over longer periods of time (Radegran, 1997, Shoemaker et al., 1996) or higher number of contraction-relaxation cycles (Osada, 2004, Radegran, 1997) would reduce variability. Analysis of blood flow velocity over long durations (Radegran, 1997, Shoemaker et al., 1996) or over an increased number of contraction-relaxation cycles (Osada, 2004) diminished blood flow velocity variability. An additional source of blood flow velocity variability would be the cadence of the contraction-relaxation cycle. Shorter or longer durations could influence the timing of arterial perfusion pressure between the contraction and relaxation phases (Osada, 2004).

In the current study, attempts to attenuate the effects of the factors exposed previously were taken to reduce MBV variability. First of all, the security and stability of the participants were guaranteed by a strap system connecting them to the tilt table. More stability represented less unnecessary movements during muscle contractions, which improved the Doppler signal. An experienced and well trained operator performed all the tests. Room temperature was maintained sufficiently cool to diminish skin blood flow. The exercise-induced variability was improved by averaging MBV data in contraction relaxation cycles. Since duration of duty cycle influences MBV variability, the cadence used in this study, 60 beats per minute, 3s duty cycles (1s to extend the footplate, 1s to return it to resting position, and 1s of rest contraction) represented 20 contractions-relaxation cycles per minute, was chosen to permit sufficient time for full relaxation of the working muscle between contractions.

2.6 Conclusion

In conclusion MBV, AD_{pop}, MBF, and VC were repeatable regardless of the body position during rest and exercise in different body positions and workloads. These variables presented lower coefficient of variation and agreement over days of testing within a given body position, which are the requirements to ensure data repeatability. The data support the utilization of Doppler ultrasound and echo Doppler methods as a repeatable tool to measure conduit artery blood flow velocities and arterial diameters as well as for estimation of MBF and VC. The repeatability of these methods allows the measurement of the dynamic response of MBF and VC at rest and exercise.

Chapter 3

Adaptations of lower limb vascular conductance and resting blood flow during head-up and head-down postural challenge

3.1 Overview

This study tested the hypothesis that central and local reflex mechanisms affecting vascular conductance through the popliteal artery completely compensated for the reduction in arterial perfusion pressure to maintain muscle blood flow during head-down tilt (HDT) but not in head-up tilt (HUT). Resting measurements were made on 15 healthy men, all non-smokers, on two separate days during horizontal (HOR) baseline, HDT or HUT applied in random order. In each body position, the leg and foot were suspended so that there was no muscle tension as with normal standing or HUT with weight bearing. Popliteal artery blood flow velocity and diameter were measured by ultrasound and muscle blood flow (MBF) was calculated. Muscle perfusion pressure (MPP) was corrected to mid-calf from measured finger cuff pressure, and vascular conductance (VC) was estimated by dividing MBF by MPP. The MPP in HDT (48.4 ± 2.0 mmHg) was approximately 100 mmHg less than in HUT (145.0 ± 2.1 mmHg). MBF was similar between HOR (51.6 ± 17.8 $mL \cdot min^{-1}$) and HDT (46.6 ± 13.5 $mL \cdot min^{-1}$), but was lower in HUT (30.0 ± 8.7 $mL \cdot min^{-1}$). VC was different between HDT (1.0 ± 0.34 $mL \cdot min^{-1} \cdot mmHg^{-1}$), HOR (0.55 ± 0.16 $mL \cdot min^{-1} \cdot mmHg^{-1}$) and HUT (0.21 ± 0.06 $mL \cdot min^{-1} \cdot mmHg^{-1}$) postures, but the relatively greater reduction during HUT resulted in lower MBF which could not be attributed to an elevated muscle tension that occurs with normal standing. These results suggested that the baroreflex, venoarteriolar reflex and/or myogenic responses contributed to a disproportionate reduction in VC during HUT at rest decreasing MBF, but in HDT reduced activation of the arterial and cardiopulmonary baroreflexes, myogenic vasodilation and inhibition of the veno-arteriolar reflex probably contributed to maintain MBF as the same level as the control condition (HOR).

3.2 Introduction

Resting limb blood flow is determined by the interactions between perfusion pressure gradient and vascular conductance (Rowell, 1993). Even though arterial perfusion pressure is elevated in the head-up (HUT) or seated position, leg blood flow is reduced 33% or more compared to the supine position in healthy subjects (Groothuis et al., 2005, Imadojemu et al., 2001, Delis et al., 2000) and in patients with spinal cord injury (Groothuis et al., 2005). The mechanisms accounting for the reduction in blood flow in the upright posture probably include elevated sympathetic vasoconstriction due to activation of the cardiopulmonary and arterial baroreflexes (Bundgaard-Nielsen et al., 2009, Imadojemu et al., 2001, Ng, Johnson, Callister, and Seals, 1995, van Lieshout et al., 2005, Cui et al., 2003, Harms et al., 2003, Toska and Walloe, 2002, Matzen et al., 1991) an increase in myogenic response with elevated arterial pressure (Groothuis et al., 2005, Imadojemu et al., 2001) and an activation of the veno-arteriolar reflex as the venous pressure is also elevated by the gravitational gradient (Groothuis et al., 2005, Tschakovsky and Hughson, 2000, Henriksen and Sejrsen, 1977).

Little is known about blood flow when the lower limb is positioned above the heart level. During a transient, 20s, period in head-down tilt (HDT), it appeared that femoral blood flow returned close to the supine baseline values (Sheriff et al., 2007), but their data did not provide evidence that blood flow and vascular conductance stabilized in this body position. Blood flow to the arm in the above heart position has been reported to be either not different (Hughson et al., 1996, Hildebrandt, Herrmann, and Stegemann, 1993) or elevated (Walker et al., 2007, Saunders and Tschakovsky, 2004, Tschakovsky and Hughson, 2000) when compared to below the heart. A single study reported arm blood flow to be lower in both above and below heart positions when compared to the arm at heart level (Hildebrandt et al., 1993). The results from the arm appear to contrast with observations from the leg and do not provide a basis for prediction of leg vascular responses in HDT.

The purpose of the current study was to compare the central cardiac and peripheral vascular responses in three different body positions that affected the gravity-dependent distribution of arterial and venous pressures. To avoid changes in intramuscular pressure due to loading, the body was supported and the ankle was maintained in a constant, relaxed state. We hypothesized that central cardiac and local reflex mechanisms affecting vascular conductance would completely compensate for the reduction in arterial perfusion pressure to maintain muscle blood flow during HDT but, consistent with previous reports (Groothuis et al., 2005, Imadojemu et al., 2001, Delis et al., 2000), that muscle blood flow would be reduced in HUT.

3.3 Methods

3.3.1 Participants

Fifteen healthy men, all non-smokers (29.6 ± 2.7 years, height 177.1 ± 5.5 cm, and body mass 80.4 ± 13.5 Kg) volunteered for this study. Participants received complete written and verbal details of the experimental procedures and potential risks involved prior to signing an information and consent form approved by the Office of Research Ethics of the University of Waterloo. All participants were instructed to refrain from consuming alcohol, caffeinated beverages and engaging in vigorous exercise for 24 hours prior to testing, and from consuming a large meal within two hours of testing.

3.3.2 Experimental Design

Participants reported to the laboratory on two separate occasions. They assumed a prone position with their face supported by a massage table head piece. Shoulder blocks were adjusted accordingly and the arms were placed on the resting apparatus with the shoulders and elbows positioned at approximately 90°. Two belts, one located on the chest and other on the hips, were used to secure the participants during testing. Straps from these belts were attached in the tilt table to help to keep the participants from sliding.

Following this procedure, ECG electrodes were applied on the skin surface in the back, 4 MHz pulsed Doppler ultrasound and 6-13 MHz echo Doppler ultrasound probes were placed on the skin over the popliteal artery. Once the participants were secured to the table, they were familiarized with the tilt protocols then rested for approximately 30min before starting the testing. None of the participants reported any discomfort during familiarization. Each participant performed four repeated randomized trials, two trials of each condition per day.

The protocol consisted of three different gravitational stress conditions. First, participants performed 3min of baseline in a horizontal (HOR) position at 0°. Then, the participant was tilted in approximately 3s to 35°HDT or 45°HUT and this body position was maintained for 5min. After this period of time, the table was rotated back (3s) to HOR (control condition) for 5min. Subsequently, subjects were tilted to the other position, HUT or HDT, for 5min. The order of these conditions was randomized and counterbalanced among the participants between HDT and HUT (Figure 3.1).

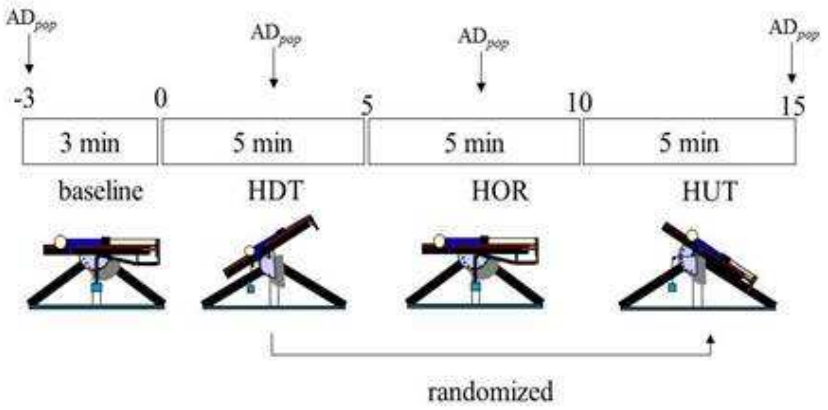

Figure 3.1: Resting protocol used to investigate the adaptations of the lower limb VC and resting MBF during HOR, HDT and HUT positions. Arrows indicate were AD_{pop} (popliteal arterial diameter) were taken.

The measurements were obtained in a quiet room, temperature was kept constant at 21.1 ± 0.4 C, humidity at 34.0 ± 9.3% with barometric pressure at 733.4 ± 3.0 mmHg.

3.3.3 Data Acquisition

Muscle blood flow velocity (MBV) in the popliteal artery, heart rate (HR), cardiac output ($\dot{Q}$) and blood pressure (BP) were measured beat-by-beat. MBV was determined from the intensity weighted mean of a 4 MHz pulsed Doppler ultrasound probe (Neurovision Doppler Ultrasound, Model 500, Multigon Industries, Mt. Vernon, USA). The flat ultrasound probe was secured by surgical tape to the skin surface over the popliteal artery embedded at a 45° angle of insonation relative to the skin and adjusted according to the measured angle between the skin and the popliteal artery.

Prior to each trial the popliteal artery was investigated to establish proper alignment, and to determine the depth and gate to assure full insonation of the artery lumen without contamination from the venous signal. The probe location was marked on the skin to assist repositioning of the probe during the test and on subsequent tests. During the test, a researcher stood up beside the participants to follow the tilt table movement up or down. If it was necessary, the researcher manipulated the probe to preserve the best Doppler signal as determined by constant use of auditory and visual feedback.

Diameter of the popliteal artery (AD_{pop}) was collected using a 6-13 MHz echo Doppler

ultrasound probe (Sonosite Titan, Sonosite, Bothell, WA, USA). These AD_{pop} images were recorded and stored into a mini DV digital video cassette tape (Hitachi Maxell Ltd., model DVM60SE, Osaka, Japan) for analysis. AD_{pop} measurements were performed in B-mode with the echo Doppler ultrasound probe positioned a few centimeters proximal to the place of velocity measurements. AD_{pop} images were taken during diastole and each diameter was selected from the single frozen screen image performed by the same operator measured four separate times at baseline (1.5min), 5.5 (HDT or HUT), 10.5 (HOR), and 15.5 minutes (HDT or HUT).

Systolic (SBP) and diastolic blood pressure (DBP) were determined by the inflation of a photoplethysmograph cuff around the middle finger of the left hand (Finometer, Finapres Medical Systems, Arnhem, the Netherlands). Mean arterial pressure (MAP) was provided by the integration of the area under the curve. HR was continuously calculated from the RR-interval obtained from an electrocardiogram (ECG, Pilot 9200, Colin Medical Instruments Corp, San Antonio, Texas, USA). $\dot{Q}$ as estimated from the finger arterial pulse wave with the Modelflow algorithm that incorporates age, sex, height, and weight as factors to estimate stroke volume (SV) (Langewouters, Wesseling, and Goedhard, 1984). MBV, SBP, DBP, MAP and HR were continuously collected at 1000 Hz using a data acquisition system (Powerlab, Chart, version 5.5, ADInstruments, Colorado Springs, USA).

3.3.4 Data Analysis

MBV, SBP, DBP, MAP, HR, and $\dot{Q}$ were analysed as beat-by-beat data (Chart, version 5.5, ADInstruments, Colorado Springs, USA). Aberrant data from each trial were deleted then time aligned and linearly interpolated to produce second-by-second values (Matlab, version 7.0, The Mathworks, Natick, Massachusetts, USA). Data were averaged to obtain one single value for each second and then averaged over the last two minutes of testing (3-5min).

MBF was estimated based on the MBV and AD_{pop} cross-sectional area as MBF = MBV$\times\pi r^2\times$60; where r is the vessel radius. Stroke volume (SV in mL) was calculated from $\dot{Q}$ (L·min^{-1}) as SV = ($\dot{Q}$/HR)$\times$1000. Systemic vascular conductance (SVC in L·min^{-1}·mmHg^{-1}) was calculated as SVC = ($\dot{Q}$/MAP)$\times$1000, with total peripheral resistance (TPR, mmHg·L·min^{-1}) = MAP/$\dot{Q}$. MPP was estimated by MAP (mmHg) and the distance between subjects heart and middle of the calf muscle (cm) in HOR, HDT, and HUT converted in millimeters of mercury (mmHg, 1 mmHg = 1.36 cm of H_2O) as demonstrated elsewhere (see 2.3.4 Data Analysis, page 22). Vascular conductance (VC, mL·min^{-1}·mmHg^{-1}) was determined by MBF (mL·min^{-1}) divided by MPP (mmHg) as VC = MBF/MPP.

3.3.5 Statistical Analysis

Variables were averaged over the last two minutes of testing for each position. Data underwent to a one way analysis of variance for repeated measures. If statistical differences were not detected between the trials, data for each trial was averaged for each condition to obtain a single value. Subsequently, a two-way analysis of variance for repeated measures was performed with main effects of selected times (baseline and average of the last two minutes) and body positions (HOR, HDT, and HUT) for the dependent variables. The Student-Newman Keuls post-hoc test was performed on significant main effects and interactions. The level of significance was set at $p < 0.05$. Data are presented as means $\pm$ standard deviation (SD).

3.4 Results

There were no significant differences in any of the measured peripheral vascular variables during the baseline periods prior to the HOR, HDT and HUT positions. The two-way repeated measures ANOVA indicated a main effect of time and body position and an interaction between time and body position for MPP, MBV and MBF ($p < 0.05$) and a main effect of body position and interaction between time and body position for VC ($p < 0.05$). The post-hoc analysis indicated that following tilt, MPP was $\sim$47% lower in the HDT compared to HOR position, and $\sim$60% higher in HUT than HOR position ($p < 0.05$, Table 3.1). AD_{pop} was not different between HOR, HDT, and HUT positions (Table 3.1). The post-hoc analysis indicated that following tilting, MBV was not different between HOR and HDT positions, but was $\sim$38% lower in HUT compared to both HOR and HDT positions ($p < 0.05$, Table 3.1). As a consequence of the reduction in MBV, the calculated MBF was also reduced in HUT ($p < 0.05$, Table 3.1, Figure 3.2A). The post-hoc analysis indicated that VC increased $\sim$82% in HDT position compared to HOR and reduced $\sim$62% in HUT compared to HOR position (Table 3.1, Figure 3.2B).

Table 3.1: MPP, AD_{pop}, MBV, MBF and VC values at baseline and averaged data from 3 to 5min following tilting during HOR, HDT, and HUT conditions.

Variables	time points	HOR	HDT	HUT
MPP (mmHg)	baseline	89.8 ± 6.9	90.2 ± 6.6	90.5 ± 6.1
MPP (mmHg)	avg3-5min	90.7 ± 5.7	48.4 ± 7.9^{ac}	145.0 ± 8.1^{abc}
AD_{pop} (cm)	baseline	0.64 ± 0.06	0.64 ± 0.06	0.64 ± 0.06
AD_{pop} (cm)	avg3-5min	0.64 ± 0.06	0.64 ± 0.06	0.64 ± 0.06
MBV ($cm \cdot s^{-1}$)	baseline	2.6 ± 0.7	2.7 ± 0.9	2.7 ± 0.8
MBV ($cm \cdot s^{-1}$)	avg3-5min	2.7 ± 0.8	2.4 ± 0.7	1.6 ± 0.4^{abc}
MBF ($mL \cdot min^{-1}$)	baseline	53.2 ± 17.7	52.3 ± 18.6	53.2 ± 18.1
MBF ($mL \cdot min^{-1}$)	avg3-5min	51.6 ± 17.8	46.6 ± 13.5	30.0 ± 8.7^{abc}
VC ($mL \cdot min^{-1} \cdot min^{-1}$)	baseline	0.60 ± 0.21	0.60 ± 0.22	0.54 ± 0.12
VC ($mL \cdot min^{-1} \cdot min^{-1}$)	avg3-5min	0.55 ± 0.16	1.00 ± 0.34^{ac}	0.58 ± 0.19^{abc}

Values are mean ± SD of 15 participants. Muscle perfusion pressure (MPP), popliteal arterial diameter (AD_{pop}), muscle blood flow velocity (MBV), muscle blood flow (MBF), vascular conductance (VC), horizontal (HOR), head-down tilt (HDT) and head-up tilt (HUT). Statistically significant differences compared to: [a]HOR, [b]HDT and [c]baseline in the same condition, $p<0.05$.

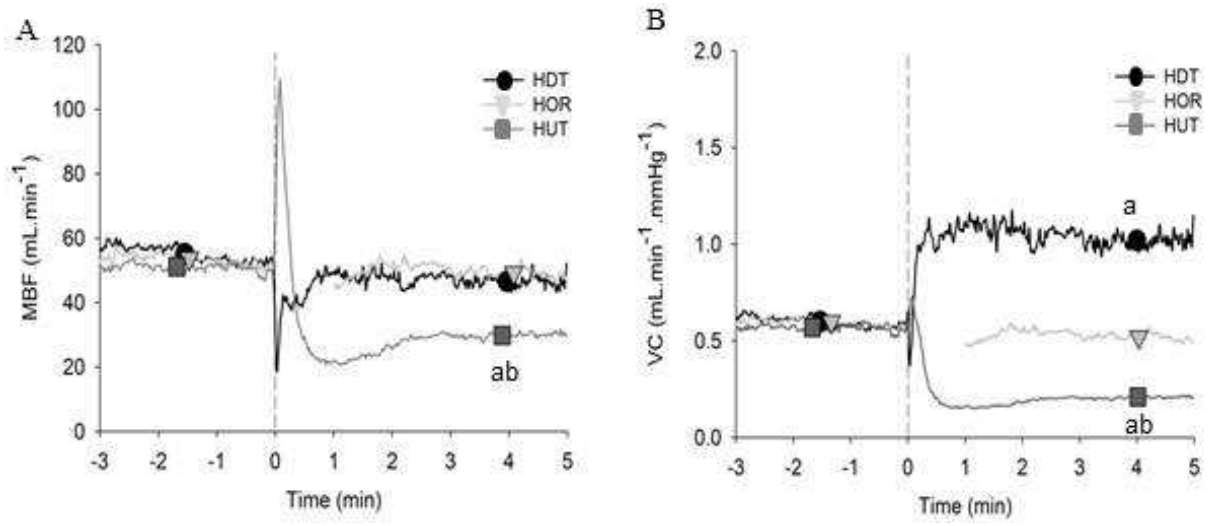

Figure 3.2: Comparison of MBF (A) and VC (B) responses during HOR, HDT and HUT positions in steady-state at rest. Lines indicate the group responses, symbols indicate the points at which statistical comparisons were performed (mean, $n = 15$) and dashed vertical lines indicate transitions from baseline to HDT and baseline to HUT. SD values were omitted and are found in Table 3.1. Statistically significant differences compared to: [a]HOR and [b]HDT, p<0.05. The symbols were offset for clarity.

Central cardiovascular responses were not different during baseline measurements, but were affected by tilting. The two-way repeated measures ANOVA indicated a main effect of body position and an interaction between time and body position for SBP and MAP and a main effect of time for DBP ($p < 0.05$). The post-hoc analysis indicated that there was a small but significant increase in SBP (∼4%) during HDT and a decrease in HUT (∼6%) compared to HOR position ($p < 0.05$, Table 3.2). Following tilting, DBP values were not different between all body positions, but increased slightly during HDT (∼5%), while MAP increased slightly during HUT both with respect to their baselines ($p < 0.05$, Table 3.2).

The two-way repeated measures ANOVA indicated a main effect of time and body position for $\dot{Q}$ ($p < 0.05$). The post-hoc analysis indicated that there were no significant changes in $\dot{Q}$ with tilt but slightly decreased during HDT with respect to its baseline (∼5%). A main effect of time and body position and an interaction between time and body position was identified by the two-way repeated measures ANOVA for HR, SV, SVC and TPR ($p < 0.05$). The post-hoc analysis indicated that following tilting, HR decreased ∼5% during HDT and increased ∼11% in HUT position compared to HOR position ($p <$

0.05, Table 3.2). SV was not different between HOR and HDT positions but was ∼12% reduced during HUT compared to HOR ($p < 0.05$, Table 3.2). SVC and TPR values were not different between HOR and HUT positions, but SVC decreased ∼7% and TPR rose ∼9% during HDT with respect to their baseline values ($p < 0.05$, Table 3.2).

The transient responses of MBF with the onset of tilt were primarily a consequence of changes in MPP. In HUT, MBF increased to 109.4 ± 35.3 $mL \cdot min^{-1}$ compared to 53.2 $mL \cdot min^{-1}$ in HOR ($p < 0.05$), while in the transition to HDT, MBF decreased to 18.5 ± 5.7 $mL \cdot min^{-1}$ compared to 52.3 $mL \cdot min^{-1}$ in HOR ($p < 0.05$). Changes in VC are not reported in the transition because of simultaneous, unknown effects on arterial and venous pressures affecting calculations.

Table 3.2: SBP, DBP, MAP, $\dot{Q}$, HR, SV, SVC and TPR values at baseline and averaged data from 3 to 5min following tilting during HOR, HDT, and HUT conditions.

Variables	time points	HOR	HDT	HUT
SBP (mmHg)	baseline	122.5 ± 7.9	123.0 ± 7.3	122.5 ± 7.1
SBP (mmHg)	avg3-5min	123.1 ± 6.3	128.3 ± 8.2^{ac}	116.1 ± 6.7^{abc}
DBP (mmHg)	baseline	71.1 ± 6.1	71.2 ± 6.0	71.9 ± 5.6
DBP (mmHg)	avg3-5min	72.1 ± 5.4	74.5 ± 5.3^{c}	73.1 ± 5.9
MAP (mmHg)	baseline	89.8 ± 6.9	90.2 ± 6.6	90.2 ± 6.1
MAP (mmHg)	avg3-5min	90.7 ± 5.7	92.6 ± 6.7^{ac}	89.4 ± 6.2^{b}
$\dot{Q}$ ($L \cdot min^{-1}$)	baseline	6.2 ± 1.0	6.2 ± 1.0	6.1 ± 0.8
$\dot{Q}$ ($L \cdot min^{-1}$)	avg3-5min	6.1 ± 0.9	5.9 ± 1.1	5.9 ± 0.4
HR (bpm)	baseline	63.2 ± 6.7	63.1 ± 6.8	63.5 ± 7.1
HR (bpm)	avg3-5min	63.5 ± 7.2	60.4 ± 6.0^{ac}	70.6 ± 9.3^{abc}
SV (mL)	baseline	99.1 ± 12.8	99.5 ± 13.5	96.0 ± 12.3
SV (mL)	avg3-5min	96.3 ± 13.1	98.2 ± 14.2^{ac}	84.7 ± 11.4^{abc}
SVC ($mmHg^{-1} \cdot mL \cdot min^{-1}$)	baseline	69.7 ± 12.8	69.4 ± 10.4	67.4 ± 10.0
SVC ($mmHg^{-1} \cdot mL \cdot min^{-1}$)	avg3-5min	67.2 ± 10.1	64.2 ± 11.4^{ac}	67.4 ± 10.0^{b}
TPR ($L \cdot min^{-1} \cdot mmHg^{-1}$)	baseline	14.6 ± 2.2	14.7 ± 2.3	15.1 ± 2.2
TPR ($L \cdot min^{-1} \cdot mmHg^{-1}$)	avg3-5min	15.2 ± 2.3	16.0 ± 2.7^{ac}	15.3 ± 2.0^{b}

Values are mean ± SD of 15 participants. Systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), cardiac output ($\dot{Q}$), heart rate (HR), stroke volume (SV), systemic vascular conductance (SVC) and total peripheral resistance (TPR). Statistically significant differences compared to: [a]HOR, [b]HDT and [c]baseline in the same condition, $p<0.05$.

3.5 Discussion

The novel finding during the HDT in the present study was that the 82% increase in VC completely compensated the 42 mmHg reduction in MPP in the lower leg such that resting MBF through the popliteal artery was maintained in this body position. In contrast, higher arterial and venous pressure in the HUT position was associated with a ∼62% reduction in VC and as a consequence resting MBF through the popliteal artery was diminished ∼38%. These results were consistent with the hypotheses as predicted based on observations from the forearm (Hughson et al., 1996) and previous investigations from the leg (Groothuis et al., 2005, Delis et al., 2000).

3.5.1 Local vascular responses in the transition from horizontal to head-down tilt

As anticipated the gravitational stress promoted by the transition from HOR to HDT position caused an immediate decrease in MPP from ∼90 mmHg to ∼48 mmHg. Despite this considerable drop in MPP that induced a brief reduction in MBF, steady-state MBF remained unchanged in HDT compared to HOR (Figure 3.2). These results contrast with those of Sheriff et al., 2007. However, these researchers reported data from only the first 20s in 15°HDT when femoral blood flow velocity and femoral blood flow dropped transiently. The results of the current study showed that if sufficient time is allowed for the response to stabilize in the new HDT position MBF recovered to the same level as in HOR and higher than HUT position.

Sheriff et al., 2007 reported a transient fall in femoral vascular conductance during 20s in 15°HDT. The recovery of MBF during sustained HDT was a consequence of a ∼82% increase in VC compared to the HOR position. There was only a very small increase in MAP during HDT (∼3%) and therefore the decline in MPP was primarily the effect of the gravitational gradient with the altered body position. The augmented VC response, which was similar to that observed with the arm above heart level (Tschakovsky and Hughson, 2000), indicated that vasodilation occurred at the level of resistance vessels (arterioles) and microvasculature to compensate for the reduction in MPP during HDT. Resting AD_{pop} measurements were not different between body positions (Table 3.1) indicating no detectable vasodilatory response of the feed artery.

The mechanisms accounting for the increase in VC by vasodilation in the leg during HDT were related to effects on gravitational gradients and shifts in blood volume. Postural change from HOR to HDT induced a hydrostatic pressure gradient that caused the

translocation of venous blood volume from the lower extremities to the central region of the body (Bundgaard-Nielsen et al., 2009) as well as an increase effective arterial pressure detected at the arterial baroreceptors in the carotid artery. The combined effects of these two changes in stimulation of the cardiopulmonary and arterial baroreflexes would be a reduction in sympathetic vasoconstrictor tone in the leg arteries (Nagaya et al., 1995, Rowell, 1993). In HDT, the activation of the aortic and carotid arterial baroreceptors afferent would be altered by the changes in the dimensions of the vessel walls contributing to the decrease in the responsiveness of the baroreflex resulting in decreased sympathetic activity mediated by decreased vasomotor efferent activity (Charkoudian et al., 2004). As a consequence, there was vascular smooth muscles relaxation increasing arterial diameters as well as increased activity of the parasympathetic vagal efferent resulting in the reduction of the HR.

The reduced MPP in the lower legs during HDT (Sheriff et al., 2007, Tschakovsky and Hughson, 2000) decreased transmural pressure and wall tension probably inducing relaxation of the arteriolar resistance vessels through the myogenic response (Sheriff et al., 2007, Tschakovsky and Hughson, 2000, Rowell, 1993). The gravity-assisted shift in venous blood volume probably inhibited the veno-arteriolar reflex (Sheriff et al., 2007, Tschakovsky and Hughson, 2000). The combined effects of reduced sympathetic vasoconstrictor tone (inhibition of arterial and cardiopulmonary baroreflex), myogenic relaxation and inhibition of the veno-arteriolar reflex vasoconstrictor response is an increase in vasodilation and consequently VC (Sheriff et al., 2007, Tschakovsky and Hughson, 2000, Madsen et al., 1995) that fully compensated for the reduced MPP in the legs to maintain resting MBF.

3.5.2 Local vascular responses in the transition from horizontal to head-up tilt

The initial transition from HOR to HUT caused a brief elevation in MBF, but in spite of the sustained increase in MPP from ∼90 mmHg to ∼145 mmHg in the HUT position, MBF was reduced ∼ 38% during resting steady-state condition in HUT compared to the HOR position (Figure 3.2A). The MBF responses in the current study are in line with those measured previously in transitions from horizontal to sitting (Delis et al., 2000) and from horizontal to 40°HUT (Imadojemu et al., 2001). In these previous studies (Imadojemu et al., 2001, Delis et al., 2000), it was not specified whether there was weight bearing on the leg which could increase muscle tension compressing the capillaries increasing vascular resistance. In the present study, there was no change in muscle tension with changes in body position, so the vascular responses could be attributed to local changes in arterial and venous pressure, and to central reflex mechanisms.

The elevated MPP was expected to induce myogenic vasoconstriction that would contribute to the reduced VC with HUT in the current study and in previous investigations (Sheriff et al., 2007, Groothuis et al., 2005, Harms et al., 2003, Toska and Walloe, 2002, Imadojemu et al., 2001). There was no detectable constriction of the feed artery as indicated by the measurement of AD_{pop} (Table 3.1) a finding consistent with other investigations (Groothuis et al., 2005, Delis et al., 2000, Tschakovsky and Hughson, 2000).

A sympathetic nervous system vasoconstrictor response contributed to the reduction in VC in the HUT position. HUT shifted blood from the central circulation into the abdominal region and the legs inducing venous pooling (Bundgaard-Nielsen et al., 2009, van Lieshout et al., 2005, Groothuis et al., 2005, Cui et al., 2003, Harms et al., 2003, Toska and Walloe, 2002, Rowell, 1993). This would in turn reduce venous return lowering central venous pressure and stimulating the cardiopulmonary baroreflex response to enhance muscle sympathetic nerve activity (Cui et al., 2003, Toska and Walloe, 2002, Imadojemu et al., 2001). Further, HUT was associated with a reduction in SV and a decrease in SBP which, combined with the positioning of the carotid arterial baroreceptors above the heart level, would have increased sympathetic vasoconstrictor activity and increased HR. In this position, aortic and carotid baroreceptors afferent activity was inhibited decreasing the impulses to the brain resulting in increased sympathetic activity mediated by increased vasomotor efferent activity. As a result there was vasoconstriction of the vascular smooth muscle reducing arterial diameter as well as decreased activity of parasympathetic efferent (vagus) resulting in elevation of the HR.

Venous pressure increased with the progressive pooling of blood early during HUT (Cui et al., 2003, Madsen et al., 1995, Matzen et al., 1991) increasing arteriolar vasoconstriction of the corresponding arteriole due to the veno-arteriolar reflex (Tschakovsky and Hughson, 2000, Henriksen and Sejrsen, 1977). The combined effects of the elevated sympathetic vasoconstrictor activity (arterial and cardiopulmonary baroreflex), the veno-arteriolar reflex and the myogenic response caused vasoconstriction of the leg arteries contributing to the reduction in VC decreasing MBF. Enhanced sympathetic vasoconstriction would have prevented further reduction in central blood volume acted to preserve SV and $\dot{Q}$ (Rowell, 1993).

3.5.3 Central cardiovascular responses

In contrast to the marked vascular responses of the lower legs in the current study, the central circulatory effects resulting from changes in body position were relatively minor. In HDT, there were small increases in SBP and MAP along with a small reduction in

HR as seen previously (Bundgaard-Nielsen et al., 2009, Harms et al., 2003). $\dot{Q}$ and SV were not different in HDT probably because the angle of tilt was not sufficient to induce marked changes in venous filling pressure from the HOR position (Bundgaard-Nielsen et al., 2009, van Lieshout et al., 2005, Harms et al., 2003). The small decrease in SVC was consistent with the estimated results based on the data provided by Bundgaard-Nielsen et al., 2009from supine to 45° and 70°HDT, but for previous investigations with only 20°HDT there were no differences between SVC (van Lieshout et al., 2005, Harms et al., 2003). Thus, in HDT when there was a marked increase in VC of the lower leg, there was a slight overall reduction in SVC which reflected regional differences in vascular responses induced by the altered gravitational effects.

In the HUT position, the anticipated reduction in SV was observed (Bundgaard-Nielsen et al., 2009, van Lieshout et al., 2005, Groothuis et al., 2005, Cui et al., 2003, Harms et al., 2003, Toska and Walloe, 2002) along with small decreases in SBP and MAP which were consistent with some (van Lieshout et al., 2005, Harms et al., 2003, Imadojemu et al., 2001), but not all investigations of HUT (Bundgaard-Nielsen et al., 2009, Groothuis et al., 2005, Toska and Walloe, 2002). HR was elevated in HUT, probably as a consequence of arterial baroreflex withdrawal of vagal activity, thus there was no overall change in during HUT (Sheriff et al., 2007, Groothuis et al., 2005, Harms et al., 2003); although some studies have reported reduced (Bundgaard-Nielsen et al., 2009, van Lieshout et al., 2005, Cui et al., 2003, Toska and Walloe, 2002). Overall, SVC was not different in HUT and HOR (Table 3.2) which reflects again regional differences in vascular responses with tilt as the lower leg VC was reduced during HUT. Unchanged SVC was shown with HUT to 45° by Groothuis et al., 2005. However, previous investigations using higher angles of tilting up to 70° demonstrated a reduced SVC suggesting an augmented vasoconstrictor response during HUT position (Bundgaard-Nielsen et al., 2009, van Lieshout et al., 2005, Cui et al., 2003, Harms et al., 2003, Toska and Walloe, 2002).

3.5.4 Methodological considerations and study limitations

In the current study, subjects were positioned prone (face-down) to enable easy access to the popliteal artery. Participants were suspended on the tilt table by a chest belt and hip belt as well as held during HDT by shoulder blocks, the feet were in a relaxed state. These procedures ensured that the leg did not support any weight in any position and both leg and foot were in the same position eliminating any change in the loading of the calf muscles. The positioning prevented any muscle activity that might change metabolic demand or development of tension in the muscle that could have altered the external pressures on capillaries and venules as experienced during standing (van Dijk et

al., 2005, Modin, 2003). Therefore, intravascular pressures were modified only by gravity and vascular responses occurred in response to these changes and to reflex mechanisms to regulate MBF.

In the current study, venous pressure was not measured; however, our approach was similar to that of Sheriff et al., 2007 who referred to this as the virtual conductance. VC was reported only during the steady-state in each posture so transient changes in venous pressure were not included in any calculations considered in this study. Likewise, CVP was not estimated thus calculations of SVC or TPR were limited to estimates based solely on MAP.

Central cardiovascular responses were estimated from the Modelflow algorithm analysis of the finger arterial pressure wave. Recent evidence suggested that this estimate of SV and $\dot{Q}$ might be high under conditions of HUT (Dyson, Shoemaker, Arbeille, and Hughson, 2010). This could mean in the current study that the drop in $\dot{Q}$ from HOR to HUT was under-estimated by this method.

3.6 Conclusion

In conclusion, resting MBF through popliteal artery was maintained during HDT position probably due to combined effects of myogenic vasodilatory response, inhibition of the veno-arteriolar reflex constrictor response and decreased baroreflex activity causing vasodilation and consequent increase in VC. Conversely in HUT, VC was decreased when MPP was higher so that lower leg MBF was actually reduced compared to both HOR and HDT, probably due to the combined effects of increased arterial and cardiopulmonary baroreflex activation of the sympathetic nervous system, vasoconstriction induced by the veno-arteriolar reflex and/or myogenic responses. The results have implications for muscle oxygen delivery and the potential for meeting the demands of physical activity under conditions where MPP is varied as a function of body position. Under resting conditions, maintained MBF in the HDT position kept oxygen delivery constant, but during the challenge of exercise it is possible that there will be limitations in the ability to recruit increases in VC so that oxygen delivery could be compromised in the face of reduced perfusion pressure. In contrast during HUT, resting MBF was reduced even though perfusion pressure was increased. This probably means there is a greater reserve in the upright position to increase VC during exercise and allow for greater oxygen delivery as a consequence of the elevated arterial perfusion pressure.

Chapter 4

The changes in anterograde and retrograde muscle blood flow velocity patterns during postural challenge

4.1 Overview

This study tested the hypothesis that anterograde and retrograde blood flow velocities (MBV_{ant} and MBV_{ret}) in the lower legs present different patterns during three different body positions that affected the gravity-dependent distribution of arterial and venous pressures impacting net muscle blood flow velocity (MBV_{net}) and vascular conductance (VC). Resting measurements were made on 15 healthy men on two separate days during horizontal (HOR), head down-tilt (HDT) or head-up tilt (HUT) applied in random order. Popliteal artery blood flow velocity (MBV) and diameter (AD_{pop}) were measured by ultrasound and muscle blood flow (MBF) was calculated. Muscle perfusion pressure (MPP) was corrected to mid-calf from measured finger cuff pressure, and vascular conductance (VC) was estimated by dividing MBF by MPP. In six participants, popliteal artery longitudinal view and MBV profile images were measured by colour Doppler ultrasound imaging. MBV_{ant} and MBV_{ret} were provided by the mean velocity within each cardiac cycle. MBV_{ant} and MBV_{ret} were greater in HDT (8.3 $cm{\cdot}s^{-1}$ and -5.9 $cm{\cdot}s^{-1}$) and lower in HUT (1.8 $cm{\cdot}s^{-1}$ and -0.6 $cm{\cdot}s^{-1}$) compared to HOR (3.6 $cm{\cdot}s^{-1}$ and -1.5 $cm{\cdot}s^{-1}$). MBV_{net} was maintained despite reduction in perfusion pressure during HDT (2.4 $cm{\cdot}s^{-1}$) compared to HOR (2.1 $cm{\cdot}s^{-1}$) but it was lower under elevated perfusion pressure (1.5 $cm{\cdot}s^{-1}$), while VC was greater in HDT but lower in HUT. In conclusion, the same MBV_{net} was attained in HOR and HDT despite differences in MBV_{ant} and MBV_{ret}. However, in HUT, the decreased MBV_{net} was accompanied by reductions in MBV_{ant} and MBV_{ret}. The mechanisms accounting for these responses probably involved changes in vascular conductance and arterial compliance.

4.2 Introduction

In the previous study (Chapter 3), it was demonstrated that resting muscle blood flow velocity (MBV) and muscle blood flow (MBF) were not different between head-down tilt (HDT) and horizontal (HOR) position, but MBV and MBF were reduced in head-up tilt (HUT). These responses indicated that MBV, MBF and vascular conductance (VC) responses were influenced by alterations in muscle perfusion pressure (MPP) with consequent changes in VC. These previous results were based only on the averaged MBV, MBF and VC over a beat and averaged over the last two minutes of testing representing the net arterial responses in each body position. However, when only mean values are present important information concerning to flow patterns are unexplored (Thijssen et al., 2009).

In Chapter 3, it was noticed that the MBV waveform was quite different between body positions, thus, it was decided to investigate the MBV waveforms and patterns within a cardiac cycle under altered MPP. Anterograde and retrograde flow velocity waveforms and patterns (MBV_{ant} and MBV_{ret}) in a peripheral artery affect the shear patterns (Padilla et al., 2010) being dependent on the interplay between perfusion pressure and local peripheral vascular resistance or conductance (Baccelli et al., 1985) and downstream compliance (Zamir, Goswami, Salzer, and Shoemaker, 2007). Previous studies demonstrated that changes in perfusion pressure and local peripheral vascular resistance modified blood flow velocity waveform and profile in brachial human artery (Baccelli et al., 1985) or in femoral, popliteal, posterior tibial and pedal arteries during supine position (Risoe and Wille, 1978) or in popliteal artery from supine to orthostatic position (Modin, 2003). Nevertheless, Zamir et al., 2007 demonstrated that not only changes in vascular resistance, but also changes in compliance were involved in vascular tone control. However, the effects of passive HDT and HUT on the MBV_{ant} and MBV_{ret} patterns are not well characterized.

The purpose of the current study was to compare MBV patterns during three different body positions that affected the gravity-dependent distribution of arterial and venous pressures. We hypothesized that MBV_{ant} and MBV_{ret} in the lower leg during HOR, HDT and HUT positions would present different patterns and waveforms due to the effects of gravity on perfusion pressure, changes in VC and arterial compliance. We also hypothesized that in HDT position, less distension of the arterioles would increase arterial compliance and that associated with an increase in VC there would be an increase in MBV_{ant} with corresponding changes in MBV_{ret} to maintain MBV_{net}. Likewise, in HUT, the higher distending pressure in the arterioles would decrease arterial compliance that combined with the reduction of VC would not only reduce, but also change the ratio of MBV_{ant} to MBV_{ret} with consequent decrease in MBV_{net}.

4.3 Methods

4.3.1 Participants

Fifteen healthy men, all non-smokers (29.6 ± 2.7 years, height 177.1 ± 5.5 cm, and body mass 80.4 ± 13.5 Kg) volunteered for this study. Participants received complete written and verbal details of the experimental procedures and potential risks involved prior to signing an information and consent form approved by the Office of Research Ethics of the University of Waterloo. All participants were instructed to refrain from consuming alcohol, caffeinated beverages and engaging in vigorous exercise for 24 hours prior to testing, and from consuming a large meal within two hours of testing.

4.3.2 Resting Protocol

The experimental design used in the current study was previously described elsewhere (2.3.2 Experimental design, page 19-20). The resting protocol consisted of three different gravitational stress conditions. First, participants performed 3min of baseline in HOR position at 0°. Then, the participant was tilted in approximately 3s to 35°HDT or 45°HUT and this body position was maintained for 5min. After this period of time, the table was rotated back (3s) to HOR (control condition) for 5min. Subsequently, subjects were tilted to the other position, HUT or HDT, for 5min. The order of these conditions was randomized and counterbalanced among the participants between HDT and HUT (see, Figure 3.1, page 37). The measurements were obtained in a quiet room, temperature was kept constant at 21.1 ± 0.4 °C, humidity at 34.0 ± 9.3% with barometric pressure at 733.4 ± 3.0 mmHg.

4.3.3 Data Acquisition

Muscle blood flow velocity (MBV) in the popliteal artery, heart rate (HR), cardiac output ($\dot{Q}$) and blood pressure (BP) were measured beat-by-beat. MBV was determined from the intensity weighted mean of a 4 MHz pulsed Doppler ultrasound probe (Neurovision Doppler Ultrasound, Model 500, Multigon Industries, Mt. Vernon, USA). The flat ultrasound probe was secured by surgical tape to the skin surface over the popliteal artery embedded at a 45° angle of insonation relative to the skin and adjusted according to the measured angle between the skin and the popliteal artery.

Prior to each trial the popliteal artery was investigated by the echo Doppler ultrasound to establish proper alignment, and to determine the depth and gate to assure full insonation of the artery lumen without contamination from the venous signal. The probe location was marked on the skin to assist repositioning of the probe during the test and on subsequent tests. During the test, a researcher stood up beside the participants to follow the tilt table movement up or down. If it was necessary, the researcher manipulated the probe to preserve the best Doppler signal as determined by constant use of auditory and visual feedback.

The colour Doppler ultrasound imaging measurements were collected using a 8-12 MHz echo Doppler ultrasound probe (Diagnostic Ultrasound system, Mindray, Model M5, Shenzen Mindray Bio-medical Electronics Co., Ltd., Shenzen, China). These popliteal artery (AD_{pop}) images were recorded and stored for analysis. The echo Doppler ultrasound probe was positioned just proximal to the site of velocity measurements. The AD_{pop} images from the longitudinal view were taken in M-mode and MBV profile images were taken in B-imaging with Doppler and colour during systole and diastole.

Arterial pressure was determined by the inflation of a photoplethysmograph cuff around the middle finger of the left hand (Finometer, Finapres Medical Systems, Arnhem, the Netherlands). Mean arterial pressure (MAP) was provided by the average within each cardiac cycle. HR was continuously calculated from the RR-interval obtained from an electrocardiogram (ECG, Pilot 9200, Colin Medical Instruments Corp, San Antonio, Texas, USA).MBV, SBP, DBP, MAP and HR was continuously collected at 1000 Hz using a data acquisition system (Powerlab, ADInstruments, Colorado Springs, USA) connected to the computer to record data for analysis (Chart, version 5.5, ADInstruments, Colorado Springs, USA).

4.3.4 Data Analysis

MBV waveform was separated into MBV_{ant} and MBV_{ret} within the cardiac cycles for each body position (Matlab, version 7.0, The Mathworks, Natick, Massachusetts, USA). The MBV_{ant}, MBV_{ret} and MBV_{net} were determined as the mean velocity within each cardiac cycle. Following this, these data were averaged over the last minute of testing for each body position. VC was taken as the reciprocal of vascular resistance calculated as MBF divided by MPP as VC = MBF/MPP as previously demonstrated elsewhere (Chapter 2, page 22).

4.3.5 Statistical Analysis

Variables were averaged over the last minute of testing for each position. The data underwent a two-way analysis of variance for repeated measures with main effects of body position (HOR, HDT and HUT) and flow velocities for MBV_{ant}, MBV_{ret}, MBV_{net} and VC as dependent variables. The Student-Newman-Keuls post-hoc test was performed on significant main effects and interactions. The level of significance was set at $p < 0.05$.

4.4 Results

4.4.1 Muscle blood flow velocity patterns in different body positions

The MBV_{ant} and MBV_{ret} within a cardiac cycle were greater in HDT and smaller in HUT position than in the HOR position (Figure 4.1).

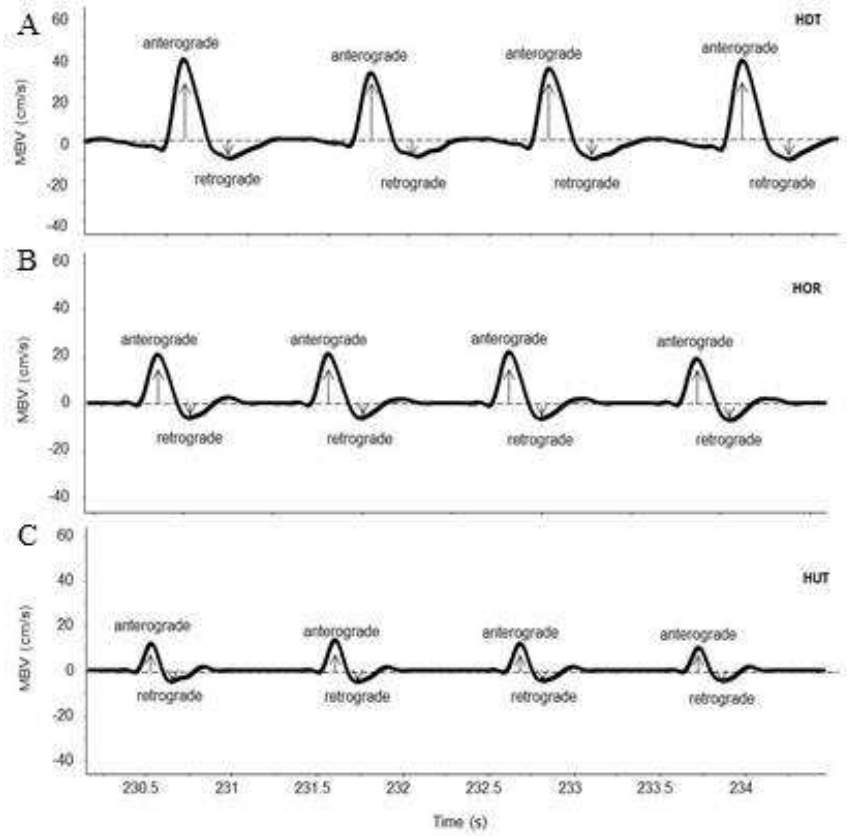

Figure 4.1: Illustration of MBV profile taken from a single participant during rest in different body positions: (A) head-down tilt (HDT); (B) horizontal (HOR) and (C) head-up tilt (HUT) using a non-imaging Doppler ultrasound. During the cardiac cycle, there are anterograde (positive) and retrograde (negative) flow patterns related to the vascular resistance and downstream compliance.

Longitudinal images of the popliteal artery from the colour Doppler ultrasound imaging demonstrated that during the systolic phase of the cardiac cycle there was an anterograde flow velocity (positive, in red) followed by retrograde flow (negative, in blue) during the diastolic phase of the cardiac cycle (Figure 4.2A, 4.2B, 4.2C, 4.2D). The velocity waveforms obtained in the same conditions were similar between Doppler ultrasound imaging (Mindray) which shows the placement of the ultrasound gate in the popliteal artery, and the MBV from the non-imaging Doppler ultrasound system (Multigon) with anterograde and retrograde velocities during systole and diastole during horizontal (HOR) (Figure 4.2E, 4.2G) and HDT (Figure 4.2F, H).

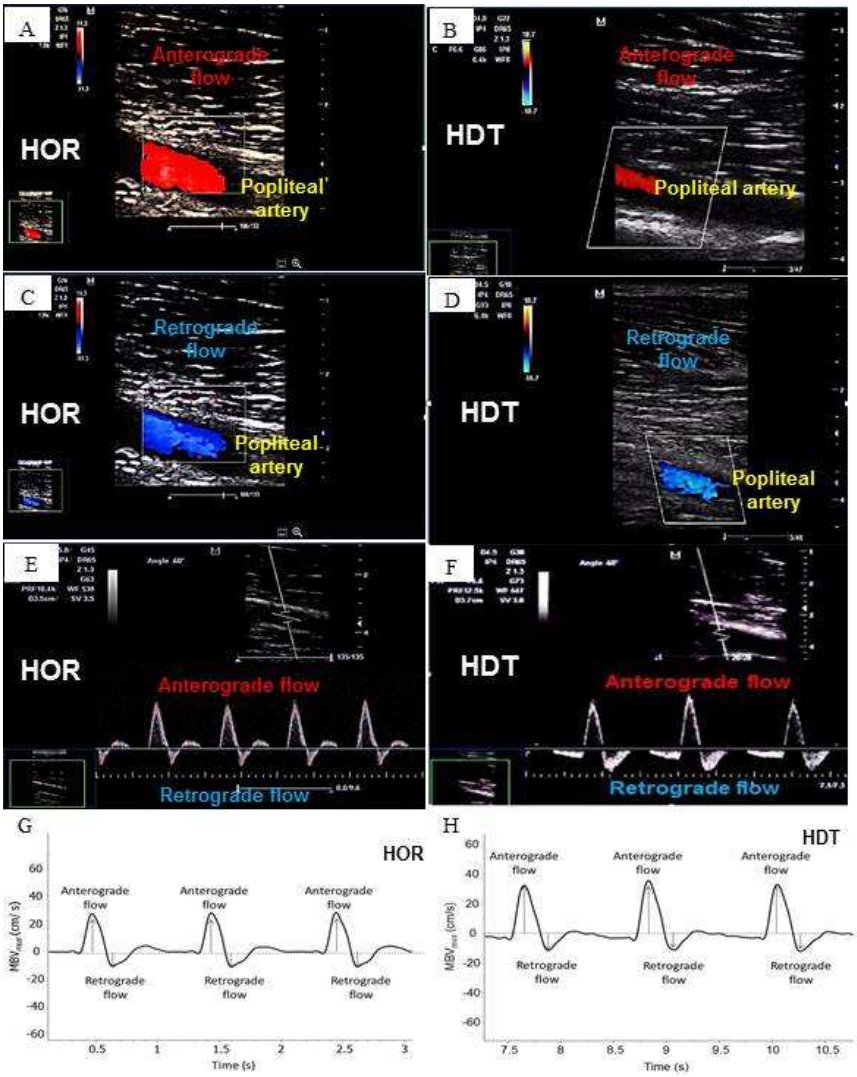

Figure 4.2: Illustration of the Doppler ultrasound imaging of the anterograde (red) and retrograde flow (blue) in the popliteal artery during HOR and HDT taken from a single participant. Longitudinal views during anterograde flow in HOR (A) and HDT (B); longitudinal views during retrograde flow in HOR (C) and HDT (D); flow velocity profiles using colour Doppler ultrasound imaging in HOR (E) and HDT (F); flow velocity profiles using non-imaging Doppler ultrasound in HOR (G) and HDT (H).

4.4.2 Muscle blood flow velocity patterns in different body positions

The two-way repeated measures ANOVA indicated a main effect of flow velocities and an interaction between flow velocities and body position ($p < 0.05$). The post-hoc analysis showed that in the last minute of tilt in each body position, MBV_{ant} was greater than MBV_{ret} ($p < 0.05$) which ensured net forward flow velocity for all body positions (Table 4.1). The MBV_{ant} and MBVret were greater in HDT but were smaller in HUT position compared to HOR ($p < 0.05$, Table 4.1). Despite the differences in MBV patterns, the MBV_{net} was not different between HDT and HOR but it was lower in HUT compared to HOR and HDT positions ($p < 0.05$, Table 4.1).

Table 4.1: MBV_{ant}, MBV_{ret}, MBV_{net}, MPP, AD_{pop} and VC averaged in the last minute of tilting during HOR, HDT, and HUT conditions.

Variables	HOR	HDT	HUT
MBV_{ant} ($cm \cdot s^{-1}$)	3.6 ± 1.2^{c}	8.3 ± 2.5^{ac}	1.8 ± 0.7^{abc}
MBV_{ret} ($cm \cdot s^{-1}$)	-1.5 ± 0.5	-5.9 ± 2.1^{a}	-0.6 ± 0.2^{ab}
MBV_{net} ($cm \cdot s^{-1}$)	2.1 ± 0.9	2.4 ± 1.2	1.2 ± 0.5^{ab}
MPP (mmHg)	90.7 ± 5.7	48.4 ± 7.9^{a}	145.0 ± 8.1^{ab}
AD_{pop} (cm)	0.64 ± 0.06	0.64 ± 0.06	0.64 ± 0.06
VC ($mL \cdot min^{-1} \cdot min^{-1}$)	0.55 ± 0.16	1.00 ± 0.34^{a}	0.21 ± 0.06^{ab}

Values are mean ± SD of 15 participants. Anterograde muscle blood flow velocity (MBV_{ant}), retrograde muscle blood flow velocity (MBV_{ret}), net muscle blood flow velocity (MBV_{net}), muscle perfusion pressure (MPP), popliteal arterial diameter (AD_{pop}), vascular conductance (VC). Statistically significant differences compared to: [a]HOR, [b]HDT and [c]MBV_{ret}, $p<0.05$.

4.5 Discussion

The patterns of MBV_{ant}, MBV_{ret}, and MBV waveforms within a cardiac cycle were quite different between body positions. The primary novel finding of the present study was that the same average MBV_{net} in the popliteal artery was attained in the HOR and HDT positions with different absolute values of MBV_{ant} and MBV_{ret} in the two body positions. In HDT, the greater increase in both MBV_{ant} and MBV_{ret} was probably due to the increase in arterial compliance and VC that compensated for the lower MPP to maintain MBV_{net} at the same level as HOR position. Conversely, lower MBV_{net} in the popliteal artery was observed in the HUT due to the reductions of MBV_{ant} and MBV_{ret} probably as the result of lower arterial compliance and VC changing the ratio between MBV_{ant} and MBV_{ret} compared to the other two body positions.

4.5.1 Muscle blood flow velocity waveforms in different body positions

The popliteal artery MBV waveforms were different between all body positions consisting of a response with three components. The first component was the highest forward flow magnitude (anterograde flow) during systole that was higher in HDT and lower in HUT position. This phase was followed by a second reverse component (retrograde flow) during diastole that was higher in HDT and lower in HUT position. After that, a third component containing a second forward flow (anterograde flow) during late diastole of less magnitude was observed in HOR and HUT (HOR > HUT), but not in HDT before blood flow velocity fell close to zero (Figure 4.1). The highest MBV_{ant} and MBV_{ret} occur in the centre of the artery lumen (Baccelli et al., 1985, Blackshear, Phillips, and Strandness, 1979) which both diminished progressively from this point to the proximity of artery wall (Baccelli et al., 1985) within a cardiac cycle for all body positions (Figure 4.2A, 4.2B, 4.2C, 4.2D). The characteristics of MBV waveforms for HOR and HUT positions are consistent with the descriptions for brachial (Baccelli et al., 1985), femoral (Blackshear et al., 1979, Risoe and Wille, 1978), popliteal (Risoe and Wille, 1978) and posterior tibial arteries (Risoe and Wille, 1978).

4.5.2 Patterns of the muscle blood flow velocity and vascular conductance in different body positions

4.5.2.1 Head-down tilt

The MBV_{ant} and MBV_{ret} presented an increase of ∼131% and ∼293%, respectively, and VC increased 82% when the legs were positioned above the heart level (HDT) in response to the ∼47% reduction in MPP (Table 4.1). Regardless of the larger relative increase in the MBV_{ret}, the absolute MBV_{ant} surpassed the MBV_{ret} resulting in forward MBV_{net} within the cardiac cycles during HDT being sufficiently great to maintain the MBV_{net} values similar to the HOR position (Table 4.1). Despite similar MBV_{net} both body positions presented marked differences in MBV_{ant} and MBV_{ret} as previously reported by Thijssen et al., 2009, Baccelli et al., 1985 and Risoe and Wille, 1978 during supine position. In the current study, the results indicated that MBV_{ant} and MBV_{ret} were directly related to the VC and inversely related to the perfusion pressure and local peripheral vascular resistance demonstrating the importance of local vascular resistance for the shape of the velocity pattern in the artery (Risoe and Wille, 1978). Baccelli et al., 1985 demonstrated that reduction of perfusion pressure by progressive inflation of an arm cuff decreased both anterograde and retrograde peak brachial artery flow velocities in supine position. In their study, MBV_{ant} was inversely related to local vascular resistance while MBV_{ret} was directly related to local vascular resistance. Zamir et al., 2007 reported that after wrist cuff inflation, vascular resistance in the brachial artery increased with no changes in arterial compliance but with elevation of the arm above heart level arterial compliance increased with no changes in resistance indicating that changes in vasomotor tone involved not only changes in arterial diameter, but also changes in arterial stiffness. Grassi et al., 1995 showed inverse relationship between radial compliance and muscle sympathetic nerve activity.

The mechanisms accounting for the greater MBV_{ant} and MBV_{ret} during HDT position probably involves the increase in arterial compliance (Zamir et al., 2007, Grassi et al., 1995) and increase in VC (Thijssen et al., 2009, Baccelli et al., 1985, Risoe and Wille, 1978). In HDT, the lower MPP diminished distending and transmural pressures leading to a reduction in the stretch of the arterioles probably increasing arterial compliance consequently enhanced arterial inflow would be expected. The relaxation of the vascular smooth muscle during HDT causing vasodilation increasing arterial compliance is in agreement with the higher arterial compliance with the arm above heart level (Zamir et al., 2007) and with greater compliance under lower muscle sympathetic nerve activity (Grassi et al., 1995). The distending pressure on the vessel wall reduces transmural pressure across the arterioles inducing vascular smooth muscle relaxation via myogenic reflex (Toska and

Walloe, 2002, Imadojemu et al., 2001). The venous emptying induces the unloading of the veno-arteriolar reflex response (Sheriff et al., 2007, Tschakovsky and Hughson, 2000). The arterial baroreflex activity is probably reduced which would decrease the sympathetic vasoconstrictor tone in the lower leg arteries (Nagaya et al., 1995). Together, these effects induced vasodilation with consequent increase in VC and arterial compliance.

In HDT, the higher MBV_{ant} associated with greater arterial compliance and VC would increase the net storage of blood in the elastic arteries during systole. As a consequence, during diastole, greater volume of blood would be available in these elastic arteries during relaxation increasing MBV. However, during diastole in HDT, the reduction in pressure gradient combined with increases in resistance to flow caused by the effects of gravity would not sustain forward flow resulting in greater reverse flow (MBV_{ret}). Despite the greater increase in MBV_{ret}, the combined effects of increased arterial compliance and VC enhanced MBV_{ant} sufficiently to maintain MBV_{net} in this position at the same level as HOR despite the reduction in MPP.

4.5.2.2 Head-up tilt

The MBV_{ant} and MBV_{ret} decreased ∼50% and ∼60%, respectively, when the legs were positioned below the heart level (HUT) in response to the ∼60% increase in MPP (Table 4.1). Modin, 2003 demonstrated from supine to standing with weight bearing that MBV_{ant} was reduced and MBV_{ret} decreased until it disappeared in the popliteal artery. In the present study, both MBV_{ant} and MBV_{ret} were reduced but MBV_{ret} was not abolished as described by Modin, 2003. Although standing-up and passive HUT are comparable, they are not physiological identical forms of postural challenge (Spodick and Lance, 1977). During the passive HUT in this study, the legs did not support any weight eliminating any change in the loading of the calf muscles, preventing muscle activity to increase metabolic demand and development of muscle tension that could have altered the external pressures on the capillaries and venules. The muscle reflexes activation associated with the development of muscle tension probably augment external pressures on the capillaries and venules during weight bearing could reduce VC, but also could increase VC due to the metabolites produced by muscle contraction. This could be the reason for the differences between MBV_{ret} and VC responses in both studies.

The MBV_{ant} and MBV_{ret} demonstrated marked changes between body positions affecting MBV_{net}. The MBV_{ant} decreased ∼7% less than MBV_{ret} resulting in forward MBV_{net} within the cardiac cycles during HUT, despite this, MBV_{net} decreased ∼38% compared to HOR and HDT positions (Table 4.1). The increase in MPP and local vascular resistance

were associated with decreases in MBV_{ant} and MBV_{ret} indicating an inversely relationship (Risoe and Wille, 1978).

The mechanisms accounting for the lower MBV_{ant} and MBV_{ret} during HUT position probably involves the decrease in arterial compliance and VC (Grassi et al., 1995). In HUT, the increase in distending and transmural pressures associated with greater volume of blood in the lower legs would increase the distending force in the arteries. As a consequence of the increased distending pressure within the arterioles, the stretch of the arterioles would increase resulting in decrease of arterial compliance (Zamir et al., 2007). During HUT, the combined effects of myogenic vasoconstriction (Groothuis et al., 2005, Toska and Walloe, 2002, Imadojemu et al., 2001), activation of the veno-arteriolar reflex (Groothuis et al., 2005, Tschakovsky and Hughson, 2000, Henriksen and Sejrsen, 1977) and the activation of the arterial and cardiopulmonary baroreflexes to elevate sympathetic vasoconstriction in the arteries of the legs (Cui et al., 2003, Toska and Walloe, 2002, Imadojemu et al., 2001) reduced VC. The combination of these effects probably increased the contractile state of the vascular smooth muscle in the lower legs decreasing arterial diameter and arterial compliance (Grassi et al., 1995).

During HUT, elevated MPP probably caused the decrease in arterial compliance and arterioles diameter (as indicated by the decrease in VC) resulting in a reduction of forward flow (MBV_{ant}) due to higher resistance in these arterioles. During diastole, the lower forward flow (MBV_{ant}) could not be sustained due to the reduction in the pressure gradient as a consequence of the drop in diastolic pressure resulting in reverse flow (MBV_{ret}). The combined effects of reduced arterial compliance and increased vasoconstriction diminishing vascular conductance would result in a decrease in MBV_{ant} and MBV_{ret} resulting in lower MBV_{net} in conditions with elevated MPP.

4.5.3 Limitations

The anatomical location of the popliteal vein related to the popliteal artery could affect the signal of the Doppler ultrasound due to the proximity between them. As a result MBV_{ret} could be overestimated if negative flow velocity from the popliteal vein is present. In this scenario, the gate to assure full insonation of the artery lumen could be insonating part of the vein flow (Figure 4.3B, 4.3D). It was taken a great care to avoid vein in the Doppler signal (Figure 4.3A, 4.3C). Prior to each trial the popliteal artery was investigated to establish proper alignment, and to determine the depth and gate to assure full insonation of the artery lumen without the vein. The intensity of the Doppler spectrum was constantly verified by audio and visual feedback. Therefore, it is believed that these procedures

ensured a reliable set of data and did not affect the interpretation of the results in the current study.

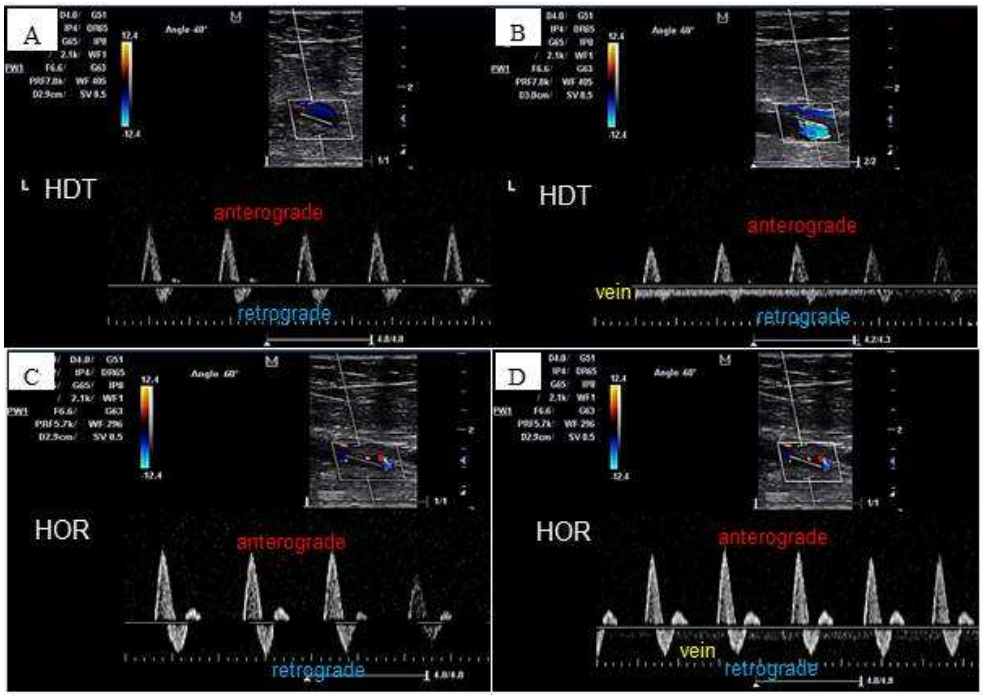

Figure 4.3: Illustration of the Colour Doppler ultrasound images of the flow velocity profiles consisting of anterograde flow (positive) and retrograde flow (negative) in the popliteal artery during HOR and HDT without vein (A and C, respectively) and with the presence of the vein (B and D, respectively).

The colour Doppler ultrasound imaging alias occurs if velocity exceeds the limit of the colour scale as a result equipment interprets high velocity in one direction as lower velocity in the other direction (Lunt, 1999). The highest flow velocities patterns were located in the centre of the popliteal artery due to the laminar flow characteristics regardless of body position. Consequently, MBV need to be analysed carefully to account for this alias effect on the colour Doppler ultrasound imaging signal especially in the centre of the artery. Despite this limitation, colour Doppler ultrasound imaging was able to demonstrate that MBV patterns and waveforms were different between body positions being confirmed by the comparison between the waveforms with non-imaging Doppler ultrasound system (Multigon) (Figure 4.2E, 4.2F, 4.2G, 4.2H).

In the current study, VC was calculated dividing MBF and MPP (VC = MBF/MPP), but the arterial compliance component was not determined. Therefore, it is uncertain the contribution of arterial compliance for the differences between MBV_{ant} and MBV_{ret} between body positions. Despite this the results of the current study are in line of those reported by Grassi et al., 1995 and Zamir et al., 2007. Zamir et al., 2007 determined arterial compliance in the brachial artery with the arm above the heart level. In their model not only resistance and compliance were include, but also viscoelasticity of the vessel and inductance representing the effects of fluid and vessel wall inertia (RCKL model). Both studies suggested that not only changes in vessel diameter (vasodilation or vasoconstriction), but also changes in vessel stiffness (compliance) should be considered to analyse changes in vasomotor tone. Even though, important information about the role of vascular compliance in vasomotor control in skeletal muscle was obtained with RCKL model, it represents a simplification of the physiological system Zamir et al., 2007, thus data need to be analysed with caution.

4.6 Conclusion

In conclusion, patterns of MBV_{ant} and MBV_{ret} and MBV waveforms within a cardiac cycle were different between body positions due to the effects of gravity on MPP and its consequences to VC and arterial compliance. Similar average MBV_{net} in the popliteal artery was attained in the HOR and HDT positions with different absolute values of MBV_{ant} and MBV_{ret}. A greater forward (MBV_{ant}) and reverse (MBV_{ret}) flow was observed in the lower legs during HDT position probably due to the increase in arterial compliance and VC that compensated for the lower MPP. In contrast, during HUT, the average MBV_{net} in the popliteal artery was lower presenting smaller forward (MBV_{ant}) and reverse (MBV_{ret}) flows compared to HOR and HDT positions probably due to the decrease in arterial compliance and VC.

Chapter 5

Effect of altered arterial perfusion pressure on vascular conductance and muscle blood flow responses during exercise in humans

5.1 Overview

The hypothesis that vascular conductance fully compensates for the effects of altered arterial perfusion pressure on muscle blood flow consequently oxygen delivery during exercise was tested. Eleven healthy volunteers exercised at lower and higher power outputs (repeated plantar flexion contractions at 20−30% maximal voluntary contraction, respectively) in horizontal (HOR), 35° head-down tilt (HDT) and 45° head-up tilt (HUT) on separate days. Muscle blood flow velocity (MBV) was measured in the popliteal artery by Doppler ultrasound, popliteal arterial diameter (AD_{pop}) was measured by ultrasound imaging, muscle blood flow (MBF) was calculated, vascular conductance (VC) was estimated by dividing MBF flow by muscle perfusion pressure (MPP) determined beat-by-beat from a finger cuff) and arterial oxygen saturation (SaO_2%) was measured by a finger pulse oximeter. The rate of increase in MBF and VC assessed from the time to reach 63% of the response ($T_{63\%}$), gain (G) and exercise responses were compared between body positions. $T_{63\%}$ for MBF and VC during both the lower and higher power output exercises was faster during HUT and slower during HDT compared to the HOR condition. In the lower power output exercise, MBF was higher in HOR than HDT and HUT with no differences between HDT and HUT. The higher VC in HDT during the lower power output compensated for the reduction in MPP. However, during exercise of higher power output exercise, MBF was lower during HDT than HOR and HUT, but VC was higher during HDT than HOR and HUT indicating that VC was not able to fully restore MBF during HDT. VC computed only during relaxation phase of the duty cycle (VC_{relax}) showed to be close to the maximal vasodilatory capacity during higher power output exercise in HDT indicating that the ability to recruit increases in VC was limited, thus MBF and subsequently delivery of O_2 were compromised in the face of reduced arterial perfusion pressure.

5.2 Introduction

Exercising muscles are sensitive to changes in limb or body position due to the effects of gravity (Egana and Green, 2005). Exercise performed with the limb positioned above and/or below the heart level to alter arterial and venous pressure has been used to challenge muscle blood flow (MBF) adaptations at the onset of exercise (Shoemaker et al., 1998, Tschakovsky et al., 1996, Leyk et al., 1994), steady-state (Lutjemeier et al., 2005) or both (Nadland et al., 2009, Walker et al., 2007, Hughson et al., 1996). Different body positions affect exercise hyperemia by the interaction between changes in muscle perfusion pressure (MPP) and the mechanical effects of muscle contraction and muscle pump activation combined with local vasodilatory mechanisms that influence vascular conductance (VC) (Walker et al., 2007, Tschakovsky et al., 2006, Tschakovsky and Sheriff, 2004, Tschakovsky and Hughson, 1999, Shoemaker and Hughson, 1999, Shoemaker et al., 1998, Tschakovsky et al., 1996, Leyk et al., 1994).

Limb position is an important determinant of the dynamic response of blood flow following the onset of exercise. Positioning of the arm (Walker et al., 2007, Hughson et al., 1996) or legs (Nadland et al., 2009, Radegran and Saltin, 1998, Grassi et al., 1996, Leyk et al., 1994) below, relative to at, heart level can increase the rate of adaptation of blood flow toward the steady-state because of the contribution of the muscle pump (Nadland et al., 2009, Laughlin and Joyner, 2003, Folkow et al., 1971). When the arm is placed above the heart, the role of the muscle pump is eliminated (Saunders and Tschakovsky, 2004, Tschakovsky et al., 1996). Vasodilation due to accumulation of metabolites near the resistance vessels of contracting muscles contributes as well to the increase in blood flow (Shoemaker and Hughson, 1999). During low and moderate exercise intensities, MBF reached the same level whether comparing supine with HUT (Nagaya et al., 1995) or arm below versus above heart level (Hughson et al., 1996). However, MBF_{peak} to the leg differs between upright and supine postures (Van Leeuwen et al., 1992) as well as mean MBF (estimated during relaxation) in moderate and heavy exercise intensities (Egana and Green, 2005). The supine cycling work performance was increased by lower body negative pressure (LBNP) and was further enhanced in upright cycling suggesting that MBF was compromised in supine position (Eiken, 1988).

The purpose of the current study was to investigate the peak and submaximal MBF and VC responses during exercise in three different body positions that affect the gravity-dependent distribution of arterial and venous pressures. The aim of the first experiment was to determine peak vascular conductance (VC_{peak}) in the lower limb by the sudden release of occlusion following intense, ischemic muscle contractions. It was hypothesized that VC_{peak} response in the lower limb represents the maximal vasodilator capacity and

it would not differ between HOR, 35°HDT and 45°HUT positions despite the differences in MPP. The aim of the second experiment was to compare the impact of altered MPP on MBF and VC during transitions from rest to lower and higher power output plantar flexion exercise performed with manipulations of limb position relative to the heart. It was hypothesized that following the onset of exercise the rate of increase in MBF and VC would be faster during HUT than HOR and both would be faster than the corresponding response during HDT in both lower and higher power outputs. It is also hypothesized that the adaptations in VC would counteract the changes in perfusion pressure so that MBF and consequently the delivery of oxygen (DO_2) to the working muscle would be maintained regardless of body position during lower power output exercise. However, during the higher power output exercise the ability to recruit VC would be limited in HDT thus compromising MBF and DO_2.

5.3 Methods

5.3.1 Participants

Eleven healthy, non-smoking male, volunteers participated in the two phases of the studies. The average physical characteristics were: age 28.0 ± 3.8 years, height 176.5 ± 5.3 cm and body mass 79.0 ± 6.9 Kg. Their maximal isometric voluntary contraction force was 60.0 ± 3.5 Kg during plantar flexion exercise in the prone horizontal position. Participants received complete written and verbal details of the experimental procedures and potential risks involved prior to signing an information consent form approved by the Office of Research Ethics of the University of Waterloo. All participants were instructed to refrain from consuming alcohol, caffeinated beverages, engaging in vigorous exercise for 24 hours prior to testing, and from consuming a large meal within two hours of testing.

5.3.2 Experimental Design

Participants reported to the laboratory five times, four times for the transitions from rest to exercise (EX) and once for measurement of peak vascular conductance (VC_{peak}). After arrival in the laboratory, they assumed a prone position with their head supported by a massage table head piece. Shoulder blocks were adjusted accordingly and their arms were placed with the shoulders and elbows positioned at approximately 90°. Two belts, one located on the chest and other on the hips, were used to secure the participants

during testing. Straps from these belts were attached to the tilt table to help to avoid the participants from sliding. A footplate was connected to the tilt table allowing plantar flexion exercise to be performed. Participants foot was strapped on the footplate to ensure more stability during the motion and better contact between plantar surface of the foot and footplate. Instrumentation took place as described below then the participants rested before starting the testing for approximately 30 minutes.

5.3.2.1 Peak vascular conductance protocols

The VC_{peak} was obtained during the reactive hyperemia that followed release of an arterial occlusion cuff placed around the lower leg just distal to the popliteal fossa. After brief collection of baseline data, the cuff was inflated to 300 mmHg for 2 minutes. During the inflation period, isometric plantar flexion exercise was performed at 50% of maximal voluntary contraction force (MVC) for 1 minute. The occlusion cuff was rapidly deflated after optimal acquisition of the Doppler ultrasound signal to monitor for peak muscle blood flow velocity (MBV_{peak}). The VC_{peak} was obtained in the three different body positions administered in random order. Recovery for at least 30 min was allowed between VC_{peak} measurements, and in each case MBV had returned to baseline.

5.3.2.2 Transitions from rest to exercise protocols

Preliminary testing revealed that participants could sustain a repeated plantar flexion exercise consisting of 1s to extend the footplate, 1s to return it to resting position, and 1s of rest (3s duty cycle) when the load was 20% or 30% of the maximal voluntary contraction (MVC). The work rates generated by these protocols, defined respectively as lower and higher power output, could be sustained for 5min with some challenge but without fatigue. The initial body position in the start of testing was maintained for the entire duration (Figure 6.1) representing a different procedure than our previous study (Chapter 3, page 37).

The angle of movement was $\sim 20^{\circ}$ representing a displacement of 10 cm. The foot plate movement was set in the rotational axis of the ankle to isolate contractions to the calf muscles. Each participant performed six different protocols; three tests per day on four different days randomized by blocks and counterbalanced among the participants. Between each individual test on each day, participants were allowed to come off the tilt table and rest prior to the next test bout. The order for test block A was: higher power output exercise in head-up tilt (HPO_{HUT}), lower power output exercise in head-down tilt

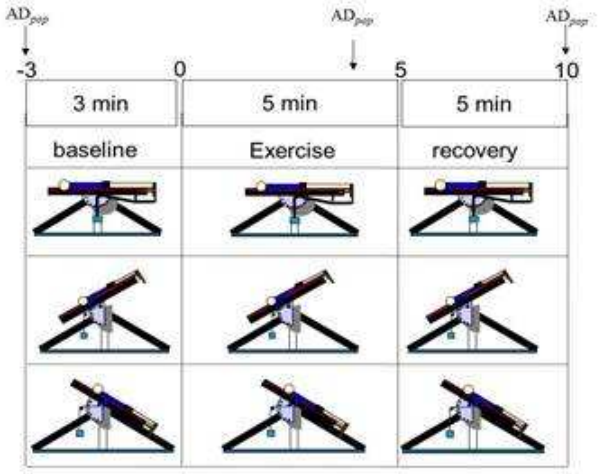

Figure 5.1: Exercising protocol used to test the impact of altered MPP on VC and MBF during transitions from rest to plantar flexion exercise in HOR, HDT and HUT positions. Arrows indicate were AD_{pop} (popliteal arterial diameter) were taken.

(LPO_{HDT}) and higher power output exercise in horizontal (HPO_{HOR}). The order for test block B was: lower power output exercise in horizontal (LPO_{HOR}), higher power output exercise in head-down tilt (HPO_{HDT}) and lower power output exercise in head-up tilt (LPO_{HUT}). These blocks were distributed based on the best physiological approach to minimize any carry over, cumulative effect of one condition to another, especially on the early stages of the subsequent protocols and to avoid muscle fatigue. The four days of testing were performed two times per week separated by at least 48 hours. This break minimized any carry over and cumulative effect of the exercise protocols, as well as muscle adaptation to the stimulus.

The angles of tilting for HDT (35°) and HUT (45°) accomplished a reduction and increase in muscle perfusion pressure (MPP) estimated to the middle of the calf muscle of approximately −44mmHg and +55mmHg, respectively. Changing the angle of the tilt table required adjusting the load to achieve the same tension on the cable attached to the foot plate. This was accomplished by raising and lowering 2.4 ± 0.4Kg (power output, = 1.6 ± 0.2 W) for LPO_{HDT}, 3.0 ± 0.4Kg (2.0 ± 0.2 W) for LPO_{HOR}, 3.8 ± 0.4Kg (2.6 ± 0.2 W) for LPO_{HUT}, 5.4 ± 0.7Kg (3.6 ± 0.5 W) for HPO_{HDT}, 6.0 ± 0.7Kg (4.0 ± 0.5 W) for HPO_{HOR} and 6.8 ± 0.7Kg (4.6 ± 0.5 W) for HPO_{HUT}. In the horizontal position, the lower power output exercises elicited an EMG response of ∼20% MVC, while at the higher power output the EMG response was ∼30% MVC measured during brief isometric contraction.

All tests were conducted in a quiet room where room temperature was kept constant at 19.4 ± 0.9 °C to ensure minimal skin blood flow at rest, with humidity at 39.9 ± 4.8%, and barometric pressure at 730.8 ± 2.4 mmHg.

5.3.3 Data Acquisition

Muscle blood flow velocity (MBV) in the popliteal artery, heart rate (HR) and blood pressure (BP) were measured on a beat-by-beat basis. For all trials in the experiments, MBV responses were determined from the intensity weighted mean of a 4 MHz pulsed Doppler ultrasound probe (Neurovision Doppler Ultrasound, Model 500, Multigon Industries, Mt. Vernon, USA). This flat ultrasound probe was secured by surgical tape to the skin surface over the popliteal artery embedded at a 45° angle of insonation relative to the skin and adjusted according to the measured angle between the skin and the popliteal artery.

Prior to each trial in the experiments, the popliteal artery was investigated to establish proper alignment, and to determine the depth and gate to assure full insonation of the artery lumen. The intensity of the Doppler spectrum was verified by audio and visual feedback. The probe location was marked on the skin to assist repositioning of the probe during the test and on subsequent tests. During the test, a researcher stood up beside the participants to follow the tilt table movement up or down. If it was necessary, the researcher manipulated the probe to preserve the best Doppler signal as determined by constant use of auditory and visual feedback. If the ultrasound signal quality was compromised the test trial was excluded from the analysis.

Diameter of the popliteal artery (AD_{pop}) was measured with an 8-12 MHz echo Doppler ultrasound probe (Diagnostic Ultrasound system, Model M5, Shenzen Mindray Bio-medical electronics CO., LTD., Shenzen, China). These popliteal artery images were recorded and stored for analysis. AD_{pop} measurements were performed in B-mode with the echo Doppler ultrasound probe positioned just proximal to the site of velocity measurements. AD_{pop} images were taken as the average of three separate measurements during diastole at baseline and at the end of recovery and during the relaxation phase between contractions in the fourth minute of exercise.

Arterial blood pressure was estimated from a photoplethysmograph cuff placed around the middle finger of the left hand (Finometer, Finapres Medical Systems, Arnhem, the Netherlands). Mean arterial pressure (MAP) was determined as the area under the curve between successive heart beats. Heart rate (HR) was continuously calculated from the RR-interval obtained from an electrocardiogram (Pilot 9200, Colin Medical Instruments Corp, San Antonio, Texas, USA). Oxygen saturation (SaO_2) was obtained by a pulse oximeter

with the probe placed in the index finger of the left hand (Ohmeda 3740 Pulse oximeter, Lousville, CO., USA).

The muscle activity was measured by electromyography (EMG) through six skin surface disposable electrodes pre-gelled (Blue Sensor, Medicost, Inc., Olstykke, Denmark) placed longitudinally on the distal half of the medial and lateral gastrocnemius (GMH and GLH, respectively) and soleus muscles in the right leg in a bipolar configuration with the inter-electrodes distance of 2 cm. Prior to the data collection, participants were shaved, abraded and cleaned with isopropyl alcohol to reduce skin impedance. Following this procedures, the electrodes were fixed on the skin where the reference electrode was placed over the head of the fibula. EMG electrodes placement was marked on the skin by a pen marker and recorded to ensure a more precise return during subsequent tests. A custom built amplifier (Waterloo, Ontario, Canada, bandwidth 20-500Hz, common mode rejection rate $>$ 90db, input impedance 2MΩ) was used to amplify the raw EMG.

MBV, MAP, HR, EMG and SaO_2 were continuously collected at 1000 Hz using a data acquisition system (Powerlab, ADInstruments, Colorado Springs, USA). This system was connected to the computer and all the variables were continuously recorded using data analysis software (Chart, version 5.5, ADInstruments, Colorado Springs, USA). EMG data were collected at 1000 Hz and processed with custom software (Matlab, version 7.0, The Mathworks, Natick, Massachusetts, USA).

5.3.4 Data Analysis

5.3.4.1 Peak vascular conductance

The MBV_{peak} was detected as the average of the three highest consecutive beats after cuff release. AD_{pop} value used was the closest to the time of the MBV_{peak}. Then, peak muscle blood flow (MBF_{peak} in $mL{\cdot}min^{-1}$) was determined as the product of MBV_{peak} and AD_{pop} cross-sectional area as $MBF_{peak} = MBV_{peak} \times \pi r^2 \times 60$; where r is the vessel radius. To calculate peak vascular conductance (VC_{peak} in $mL{\cdot}min^{-1}{\cdot}mmHg^{-1}$) MBF_{peak} was divided by MPP measured at the same time (Figure 5.2).

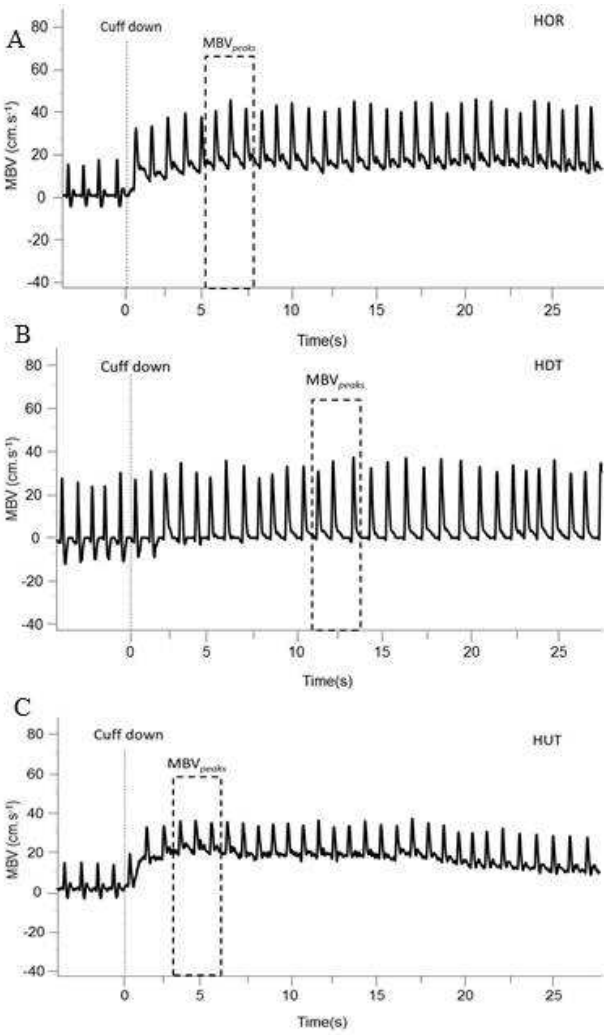

Figure 5.2: Representative patterns of beat-by-beat muscle blood flow velocity waveforms after cuff release in the peak vascular conductance (VC_{peak}) tests in (A) horizontal (HOR), (B) head-down tilt (HDT), (C) head-up tilt (HUT). Dashed lines indicate cuff release (cuff down) and rectangular dashed box depicts the three beats averaged to represent peak muscle blood flow velocity (MBV_{peak}).

The relative vasodilation during the rest to exercise transitions was estimated from the effective vascular conductance during relaxation (VC_{relax}) calculated from complete cardiac cycles during the relaxation phase of the duty cycle from the muscle blood flow (MBF_{relax}) and the corresponding muscle perfusion pressure (MPP) then expressed as a percentage of the VC_{peak} ($\%VC_{peak}$)(Figure 5.3).

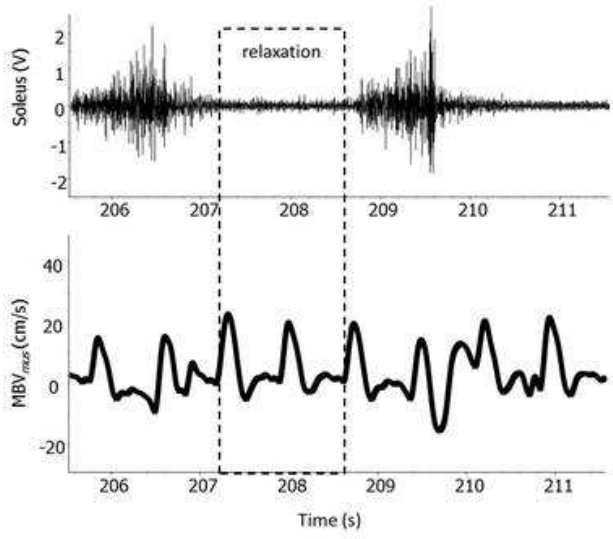

Figure 5.3: Example of isolation of complete cardiac cycles (onset of systole to end of diastole) during the relaxation phase of exercise for calculation of effective vascular conductance (VC_{relax}). In this example, the average MBV over two complete cardiac cycles was used to calculate MBF which was divided by corresponding average MPP (not shown) to yield VC_{relax}.

5.3.4.2 Transitions from rest to exercise

MBF was calculated from MBV and the corresponding AD_{pop}. MPP (mmHg) was estimated by MAP (mmHg) and the distance between subjects heart and the middle of the calf muscle for all body positions as demonstrated elsewhere (see, page 20-21).

5.3.4.3 Electromyography

The offset of the raw electromyographic signal was removed and rectified (full wave rectification). The rectified signal passed through a 2nd order, dual pass Butterworth digital

filter with a cut-off frequency of 5Hz. The area under of the rectified signal for each contraction was used to calculate the integrated EMG (iEMG) blocked into 3 seconds window for each exercise protocol. The fast Fourier transformation (FFT) analysis was used to estimate mean power frequency (MPF) on the raw signal. The EMG signal was normalized by the maximal voluntary contraction (MVC) performed prior to each day of testing to account for the inter participant and day to day variability. Then, the EMG data was averaged in the last minute of exercise expressed as the percentage of MVC.

5.3.5 Statistical Analysis

MBV_{peak}, AD_{pop}, MBF_{peak}, MPP, and VC_{peak} were analysed by one-way analysis of variance for repeated measures with respect to body position (HOR, HDT, and HUT). For EX experiments, exercise, $T_{63\%}$, G and EMG data underwent a one-way analysis of variance (ANOVA) for repeated measures of body position (HOR, HDT, and HUT) for the dependent variables in lower and higher power outputs. Time (baseline and recovery), VC_{relax} and $\%VC_{peak}$ data were analysed by a two-way ANOVA for repeated measures with main effects of body position and time. If required, prior to the analysis data were rank-transformed to address concerns of non-normal distribution. The Student-Newman-Keuls post-hoc test was performed to identify the statistically significant main effects and interactions. The level of significance for main effects and interactions was set at $p < 0.05$. Data were presented as means $\pm$ standard deviation (SD).

5.4 Results

5.4.1 Muscle blood flow velocity patterns in different body positions

The post-hoc analysis of the one-way repeated measures ANOVA indicated that AD_{pop} and VC_{peak} were not different for any body position, while MBV_{peak}, MBF_{peak} and MPP were different between all body positions ($p < 0.05$). MBV_{peak}, MBF_{peak} and MPP were higher during 45°HUT but lower during 35°HDT (Table 5.1).

Table 5.1: MBV_{peak}, AD_{pop}, MBF_{peak}, MPP and VC responses during VC_{peak} protocol during HOR, HDT and HUT conditions.

Variables	HOR	HDT	HUT
MBV_{peak} ($cm \cdot s^{-1}$)	34.5 ± 7.9	19.8 ± 7.0^{a}	52.0 ± 10.3^{ab}
AD_{pop} (cm)	0.64 ± 0.06	0.63 ± 0.06	0.63 ± 0.06
MBF_{peak} ($mL \cdot min^{-1}$)	673.6 ± 226.1	378.2 ± 134.9^{a}	997.4 ± 284.2^{ab}
MPP (mmHg)	93.2 ± 8.0	54.9 ± 11.4^{a}	142.6 ± 6.6^{ab}
VC ($mL \cdot min^{-1} \cdot min^{-1}$)	7.2 ± 2.1	7.1 ± 1.8	7.0 ± 1.9

Values are mean ± of 11 participants. Peak muscle blood flow velocity (MBV_{peak}), popliteal arterial diameter at MBV_{peak} (AD_{pop}), peak muscle blood flow (MBF_{peak}), muscle perfusion pressure at MBV_{peak} (MPP), effective vascular conductance (VC), horizontal (HOR), head-down tilt (HDT) and head-up tilt (HUT). Statistically significant differences compared to: [a]HOR and [b]HDT, $p<0.05$.

5.4.2 Transitions from rest to exercise in lower output tests

The two-way repeated measures ANOVA indicated a main effect of body position for MBF and VC ($p < 0.05$). The post-hoc analysis demonstrated that baseline values for MBF were not statistically significant differences between LPO_{HOR} and LPO_{HDT} but were lower during LPO_{HUT} ($p < 0.05$, Table 5.2, Figure 5.4A). The baseline values for VC were significantly different between all body positions being higher during LPO_{HDT} but lower during LPO_{HUT} ($p < 0.05$, Table 5.2, Figure 5.4B).

MBF and VC demonstrated a rapid increase immediately after the onset of exercise for all body positions (Figure 5.4A, 5.4B). The post-hoc analysis of the one-way repeated

measure ANOVA indicated that the rate of increase was faster in the LPO_{HUT} and slower in LPO_{HDT} as observed in the patterns of the responses and significant effect of body position ($p < 0.05$, Figure 5.4A, 5.4B) and the more rapid $T_{63\%}$ ($p < 0.05$, Table 5.2). The gain values for MBF were not statistically different between all body positions but tended to be higher in LPO_{HOR} than the other two positions ($p = 0.06$, Table 5.2). VC gain was not significantly different during LPO_{HDT} and LPO_{HOR} ($p = 0.13$) but it was lower during LPO_{HUT} ($p < 0.05$, Table 5.2). The post-hoc analysis of the one-way repeated measure ANOVA indicated that MBF values during exercise were higher during LPO_{HOR} than LPO_{HDT} and LPO_{HUT} ($p < 0.05$, Table 5.2) with no differences between and LPO_{HDT} and LPO_{HUT} (Figure 5.4A). VC values during exercise were higher in LPO_{HDT} ($p < 0.05$) but were lower during LPO_{HUT} ($p < 0.05$, Table 5.2, Figure 5.4B). The post-exercise MBF and VC responses were greater in LPO_{HDT} and lower in LPO_{HUT} ($p < 0.05$, Table 5.2). The post-hoc analysis showed that in the last minute of recovery, MBF and VC returned to the baseline levels for all body positions (Figure 5.4A, 5.3B).

Table 5.2: Dynamic responses of MBF and VC during exercise in LPO_{HOR}, LPO_{HDT} and LPO_{HUT}.

Variables	time points	LPO_{HOR}	LPO_{HDT}	LPO_{HUT}
MBF ($mL \cdot min^{-1}$)	baseline	51.5 ± 25.5	41.8 ± 15.3	28.6 ± 14.0^{ab}
	$T_{63\%}$	16.5 ± 10.9	37.6 ± 15.5^{a}	9.5 ± 6.3^{b}
	G	133.4 ± 65.0	105.1 ± 57.0	92.3 ± 48.1
	exercise	185.9 ± 70.2	146.9 ± 62.1^{a}	120.9 ± 54.8^{a}
	recovery	53.6 ± 32.8	49.0 ± 28.4	26.6 ± 9.9^{ab}
VC ($mL \cdot min^{-1} \cdot min^{-1}$)	baseline	0.7 ± 0.4	1.0 ± 0.4	0.2 ± 0.1^{ab}
	$T_{63\%}$	16.9 ± 9.8	35.6 ± 14.1^{a}	9.9 ± 7.6^{b}
	G	1.5 ± 0.7	2.0 ± 1.1	0.6 ± 0.4^{ab}
	exercise	2.2 ± 0.9	2.9 ± 1.3^{a}	0.9 ± 0.4^{ab}
	recovery	0.7 ± 0.4	1.1 ± 0.6	0.2 ± 0.1^{ab}
test block order		B1	A2	B3

Values are mean ± SD of 11 participants. Muscle blood flow (MBF), vascular conductance (VC), lower power output in horizontal (LPO_{HOR}), lower power output in head-down tilt (LPO_{HDT}), lower power output in head-up tilt (LPO_{HUT}), time to reach 63% of the response ($T_{63\%}$), gain of the response (G). Statistically significant compared to: [a]LPO_{HOR}; [b]LPO_{HDT}; [c]LPO_{HUT}; [d]baseline in the same conditions ($p < 0.05$). Test Block Order indicates for each of the Blocks (see 5.3.4.2 Transitions from rest to exercise (EX), page 76) the order of that specific condition for comparison of baseline data (see Table 2.2, page 24).

5.4.3 Transitions from rest to exercise in higher output tests

The two-way repeated measures ANOVA indicated a main effect of body position and time (baseline and recovery) and interactions between body position and time for MBF and VC ($p < 0.05$). The post-hoc analysis indicated that baseline values for MBF were not different between HPO_{HOR} and HPO_{HDT} but were lower during HPO_{HUT} ($p < 0.05$, Table 5.2, Figure 5.4C). The baseline values for VC were significantly different between all body positions with higher VC during HPO_{HDT} and lower VC during HPO_{HUT} ($p < 0.05$, Table 5.2, Figure 5.4D). MBF and VC demonstrated a rapid increase immediately after the onset of exercise for all body positions (Figure 5.4C, 5.4D). The one-way repeated measure ANOVA post-hoc analysis demonstrated that the rate of increase was faster in the

HPO_{HUT} and slower in HPO_{HDT} as observed in the patterns of the responses and significant effect of body position ($p < 0.05$, Figure 5.4C, 5.4D) and the more rapid $T_{63\%}$ ($p < 0.05$, Table 5.2). The gain values of MBF were not different between HPO_{HOR} and HPO_{HUT} but were lower in HPO_{HDT} ($p < 0.05$, Table 5.2). VC gain was not different between HPO_{HDT} than HPO_{HOR} but it was lower during HPO_{HUT} ($p < 0.05$, Table 5.2). The post-hoc analysis of the one-way repeated measure ANOVA indicated that MBF values during exercise were not different between HPO_{HOR} and HPO_{HUT} but lower in HPO_{HDT} ($p < 0.05$, Table 5.2, Figure 5.4C, 5.4D). VC values during exercise were higher in HPO_{HDT} compared to HPO_{HOR} and HPO_{HUT} ($p < 0.05$, Table 5.2), but were lower during HPO_{HUT} ($p < 0.05$, Table 5.2, Figure 5.4D). The post-exercise MBF and VC response was greater in HDT and lower in HUT ($p < 0.05$, Table 5.2). The post-hoc analysis showed that in the last minute of recovery, MBF and VC returned to the baseline levels during HPO_{HOR} and HPO_{HUT} but did not in HPO_{HDT} (Figure 5.4C, 5.4D).

Table 5.3: Dynamic responses of MBF and VC during exercise in HPO_{HOR}, HPO_{HDT} and HPO_{HUT}.

Variables	time points	HPO_{HOR}	HPO_{HDT}	HPO_{HUT}
MBF ($mL \cdot min^{-1}$)	baseline	44.2 ± 14.0	46.7 ± 17.7	32.8 ± 10.7^{ab}
	$T_{63\%}$	25.3 ± 8.9	44.5 ± 9.7^{a}	13.4 ± 6.7^{ab}
	G	197.2 ± 83.9	135.9 ± 55.6^{a}	187.0 ± 60.6^{b}
	exercise	241.4 ± 93.2	182.6 ± 54.6^{a}	219.8 ± 64.2
	recovery	47.7 ± 19.0	118.1 ± 59.4^{ab}	38.0 ± 16.2^{b}
VC ($mL \cdot min^{-1} \cdot min^{-1}$)	baseline	0.6 ± 19.0	1.2 ± 0.8^{a}	0.2 ± 0.1^{ab}
	$T_{63\%}$	23.9 ± 10.1	39.0 ± 15.1^{a}	13.3 ± 6.7^{ab}
	G	2.2 ± 1.1	2.5 ± 1.1^{a}	1.3 ± 0.5^{ab}
	exercise	2.8 ± 1.2	3.7 ± 1.2^{a}	1.5 ± 0.5^{ab}
	recovery	0.7 ± 0.3	2.9 ± 1.8^{ad}	0.3 ± 0.1^{ab}
test block order		A3	B2	A1

Values are mean ± SD of 11 participants. Muscle blood flow (MBF), vascular conductance (VC), higher power output in horizontal (HPO_{HOR}), higher power output in head-down tilt (HPO_{HDT}), higher power output in head-up tilt (HPO_{HUT}), time to reach 63% of the response ($T_{63\%}$), gain of the response (G). Statistically significant compared to: [a]HPO_{HOR}; [b]HPO_{HDT}; [c]HPO_{HUT}; [d]baseline in the same conditions ($p < 0.05$). Test Block Order indicates for each of the Blocks (see 5.3.4.2 Transitions from rest to exercise (EX), page 76) the order of that specific condition for comparison of baseline data (see Table 2.2, page 24).

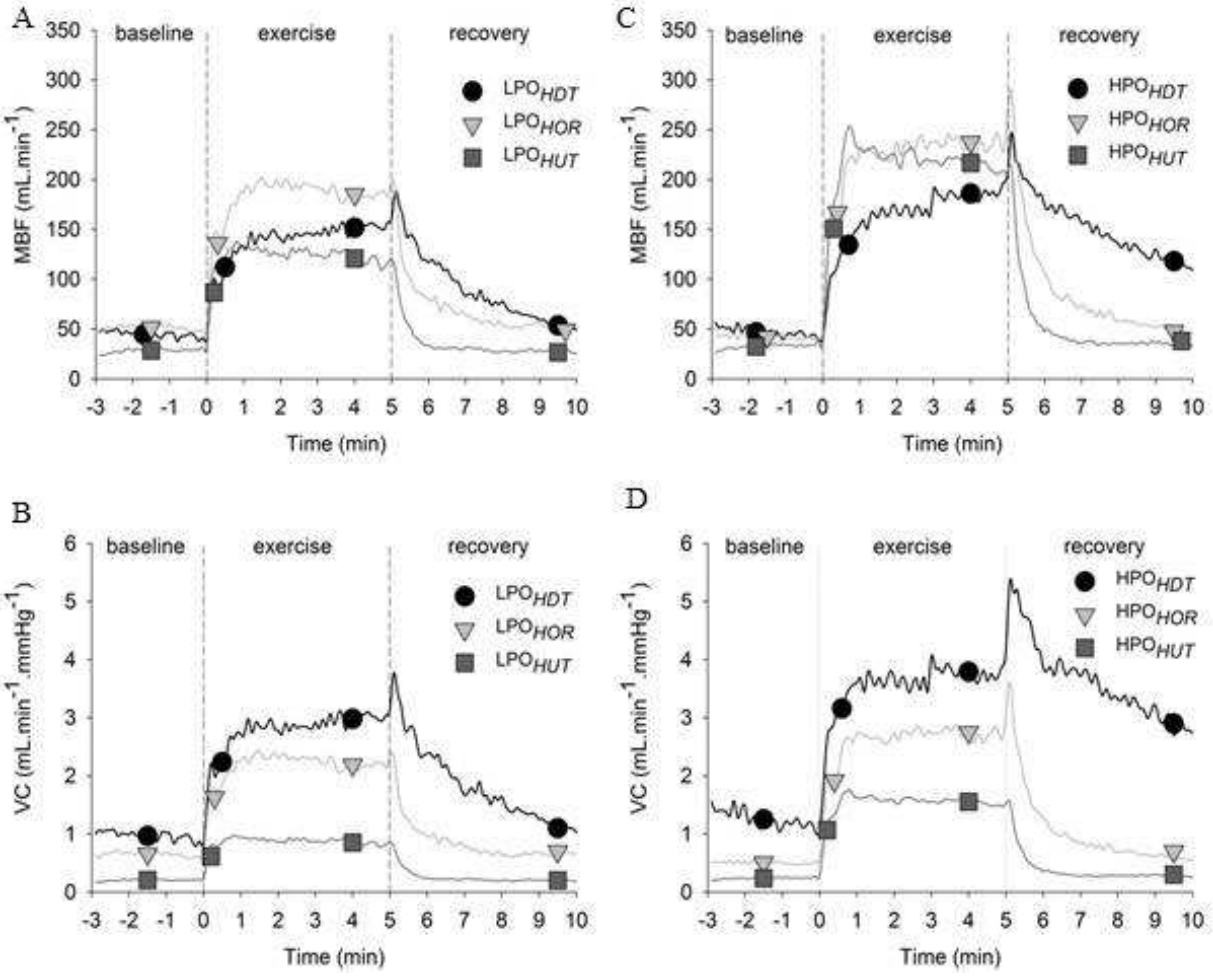

Figure 5.4: Time course of changes in MBF and VC during dynamic plantar flexion exercise performed in lower (A and B) and higher (C and D) power outputs. Lines indicate group response, symbols indicate the points at which statistical analysis were performed and dashed vertical lines indicate the start and cessation of exercise. Data are the mean analysed over 6 seconds time bins including contraction and relaxation phases of the duty cycles. SD was omitted to improve data visualization, but are presented in the Table 5.2. If necessary symbols were offset for clarity.

5.4.4 Vascular conductance expressed as percentage of VCpeak

The two-way repeated measures ANOVA indicated a main effect of body position and time and an interaction between body position and time for vascular conductance during relaxation (VC_{relax}) and VC_{relax} expressed as percentage of VC_{peak} ($\%VC_{peak}$) ($p < 0.05$). The post-hoc analysis indicated that VC_{relax} and $\%VC_{peak}$ were significantly higher during HDT and lower during HUT from baseline to recovery for both lower and higher power outputs ($p < 0.05$, Figure 5.5A, 5.5B, 5.5C, 5.5D).

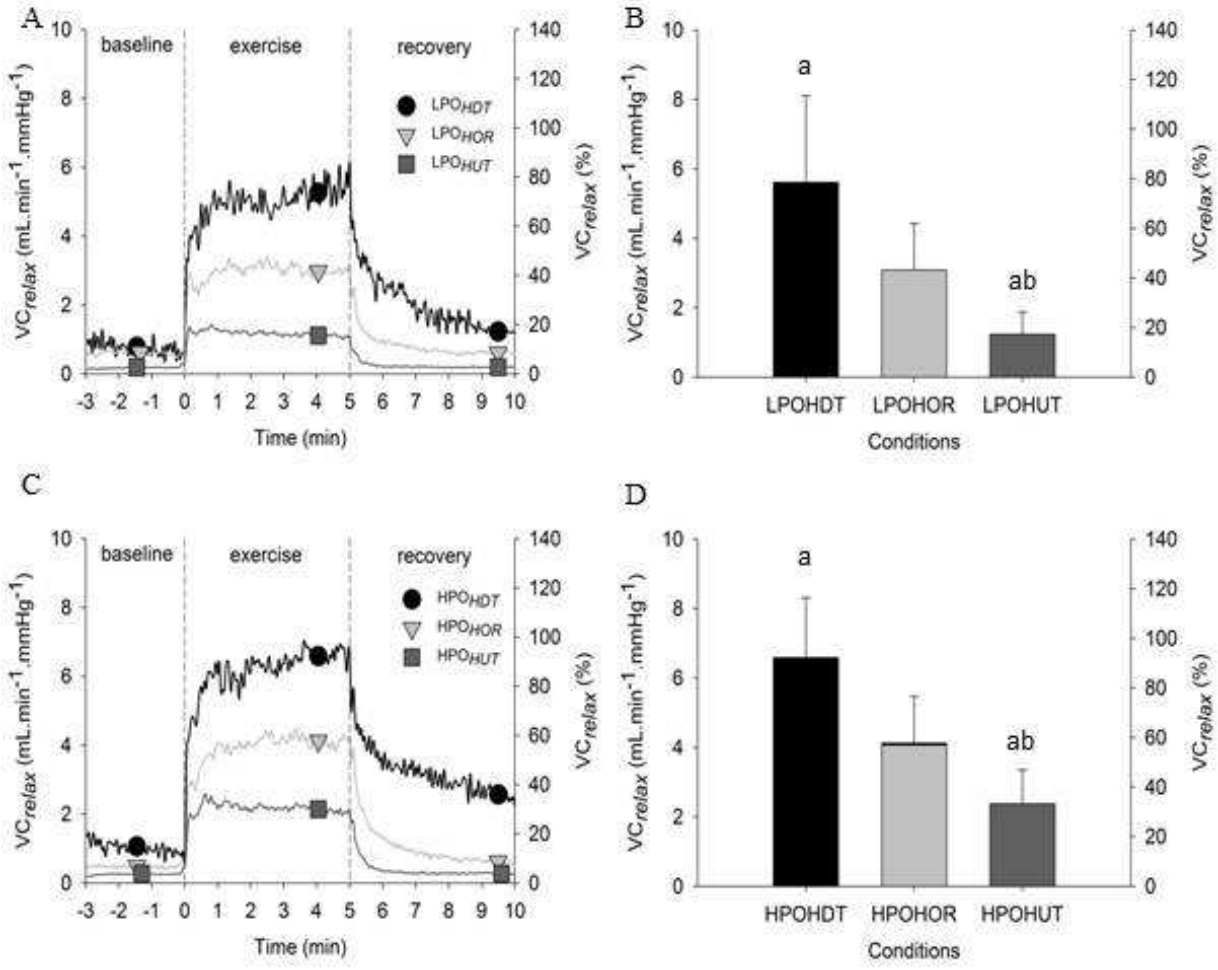

Figure 5.5: Time course of changes in VC_{relax} (mL·min^{-1}·mmHg^{-1}) and $\%VC_{relax}$ as percentage of change in vascular conductance response ($\%VC_{relax} = VC_{relax}/VC_{peak} \times 100$) during dynamic plantar flexion exercise performed in lower (A) and higher power outputs (C). Lines indicate group response, symbols indicate the points at which statistical analysis were performed and dashed vertical lines indicate the start and cessation of exercise. The bar graphs represent exercising VC_{relax} and $\%VC_{relax}$ during lower (B) and higher power outputs (D). Data are the mean ± SD analysed over the relaxation phases between muscle contraction phases of the duty cycles. Statistically significant compared to: [a]HOR and [b]HDT ($p < 0.05$). If necessary symbols were offset for clarity.

5.4.5 Muscle activity responses during exercise

The one-way repeated measures ANOVA post-hoc analysis showed that muscle activity (EMG) was not different during lower power output exercise for all body positions (Figure 5.6A) and for HPO_{HOR} and HPO_{HUT} during higher power output exercise, excepted for gastrocnemius medial head (GMH). However, muscle activity was higher during HPO_{HDT} for all muscle groups ($p < 0.05$, Figure 5.6B). Total muscle activity and EMG_{index} were not different during lower power output for all body positions and for HPO_{HOR} and HPO_{HUT}, but they were higher during HPO_{HDT} ($p < 0.05$, Figure 5.6C, 5.6D).

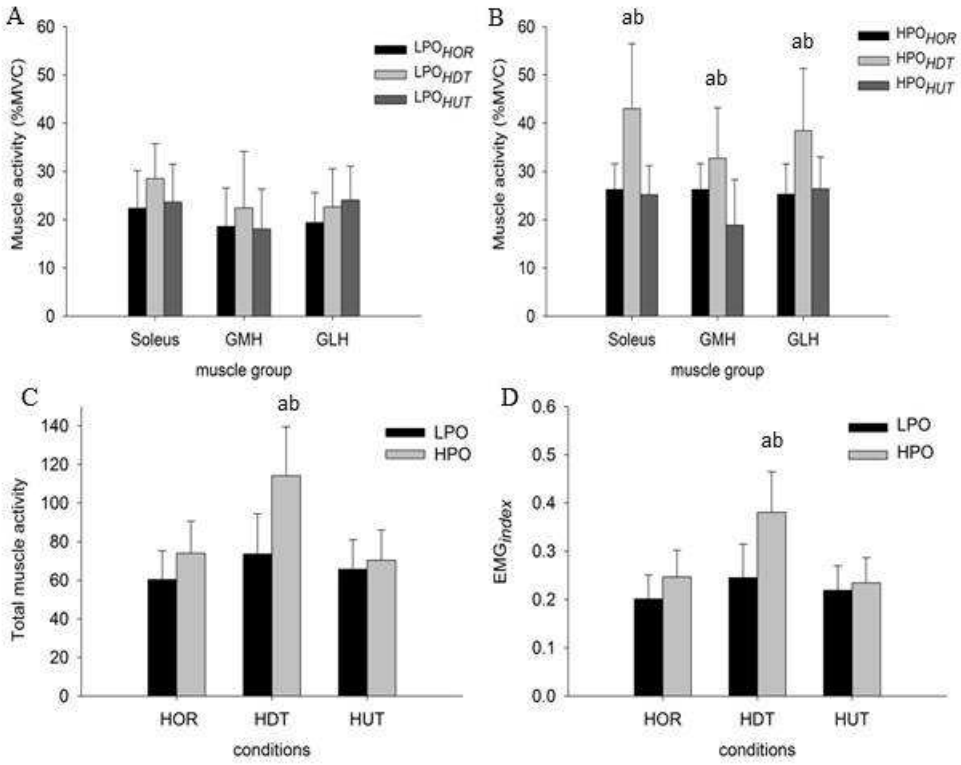

Figure 5.6: Muscle activity in soleus, gastrocnemius medial head (GMH) and gastrocnemius lateral head (GLH) during plantar flexion exercise during lower (A) and higher power outputs (B) in horizontal (LPO_{HOR}), head-down tilt (LPO_{HDT}) and head-up tilt (LPO_{HUT}) positions. Total muscle activity (C) was calculated as the sum of the mean for soleus and gastrocnemius muscle groups activity as a percentage of MVC and EMG_{index} (D) was calculated as the sum of the mean for soleus and gastrocnemius muscle groups activity divided by the sum of the MVCs during plantar flexion exercise during lower (LPO) and higher power outputs (HPO) in horizontal (HOR), head-down tilt (HDT) and head-up tilt (HUT) positions. Data are the mean ± SD averaged in the last two minutes of exercise. Statistically significant compared to: [a]HOR and [b]HUT ($p < 0.05$).

5.4.6 Normalized muscle blood flow by muscle activity

The relationship between MBF and an index of the sum of electromyographic signals from the muscles (EMG_{index}) for the corresponding time was used to account for the differences in muscle activity related to body position (MBF/EMG_{index}) and used to determine com-

pensations in MBF. The one-way repeated measures ANOVA post-hoc analysis showed that this ratio was not different between LPO_{HDT} and LPO_{HUT}, but both conditions were lower than LPO_{HOR} ($p < 0.05$, Figure 5.7A). The MBF/EMG_{index} was not different between HPO_{HOR} and HPO_{HUT} but both conditions were higher than HPO_{HDT} ($p < 0.05$, Figure 5.7B).

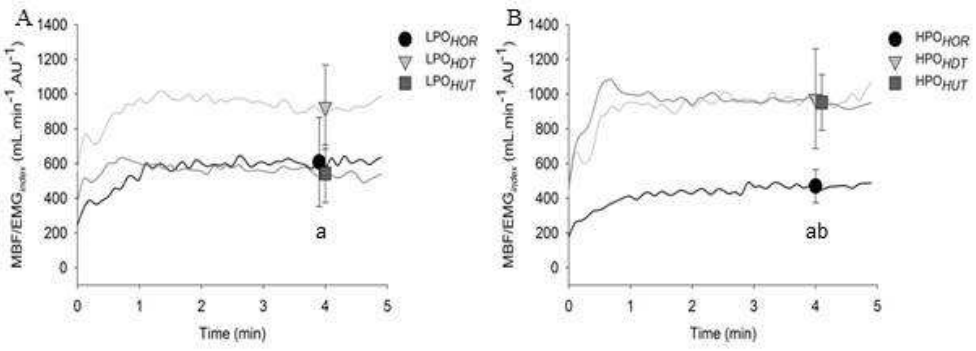

Figure 5.7: Time course of changes in MBF/EMG_{index} during dynamic plantar flexion exercise performed in lower (A) and higher (B) power outputs. Symbols indicate group response which statistical analysis was performed. Data are the mean analyzed over 6 seconds time bins including contraction and relaxation phases of the duty cycles. Statistically significant compare to: [a]HOR and [b]HUT ($p < 0.05$). Symbols were offset for clarity.

5.4.7 MAP and HR responses during lower and higher power output exercises

The two-way repeated measures ANOVA indicated a main effect of body position and time for MAP during lower power output ($p < 0.05$) and a main effect of time and interaction between body position and time for higher power output ($p < 0.05$). The post-hoc analysis demonstrated that baseline, exercise and recovery values for MAP were not different between LPO_{HOR} and LPO_{HUT}, but were higher during LPO_{HDT} ($p < 0.05$, Table 5.3). The baseline and recovery values for MAP were not different for all body positions during higher power output exercise. During exercise, MAP values were not different between HPO_{HOR} and HPO_{HUT} but were higher during HPO_{HDT} ($p < 0.05$, Table 5.3).

The two-way repeated measures ANOVA indicated a main effect of body position and time and an interaction between body position and time for HR during lower and higher power outputs ($p < 0.05$). The post-hoc analysis showed that baseline HR was not different

between LPO_{HOR} and LPO_{HUT}, but was slightly lower during LPOHDT ($p < 0.05$, Table 5.3). During exercise, HR values were not different between all body positions in LPO ($p < 0.05$, Table 5.3). At the recovery, HR was not different between LPO_{HOR} and LPO_{HDT}, but slightly higher during LPO_{HUT} ($p < 0.05$, Table 5.3). The baseline, exercise and recovery HR were not different between HPO_{HDT} and HPO_{HOR}, but was higher in HPO_{HUT} ($p < 0.05$, Table 5.3).

Table 5.4: MAP and HR at baseline, exercise (last 2min of exercise) and recovery (last minute) during LPO_{HDT}, LPO_{HOR}, LPO_{HUT}, HPO_{HDT}, HPO_{HOR} and HPO_{HUT}.

Variables	time points	LPO_{HOR}	LPO_{HDT}	LPO_{HUT}
MAP (mmHg)	baseline	80.9 ± 5.8	91.5 ± 6.7^{a}	84.1 ± 5.9^{b}
	exercise	85.3 ± 5.8	95.8 ± 8.1^{a}	87.6 ± 6.6^{b}
	recovery	83.0 ± 5.6^{f}	93.5 ± 8.7^{af}	84.1 ± 6.4^{b}
HR (bpm)	baseline	62.4 ± 8.0	55.7 ± 9.0^{a}	64.4 ± 8.8^{b}
	exercise	65.3 ± 9.4	62.5 ± 9.3	64.7 ± 8.5
	recovery	61.8 ± 7.6	59.8 ± 10.2^{f}	66.0 ± 9.8^{ab}
Variables	time points	HPO_{HOR}	HPO_{HDT}	HPO_{HUT}
MAP (mmHg)	baseline	85.2 ± 6.9	86.2 ± 7.5	83.9 ± 7.1
	exercise	89.3 ± 6.1	95.3 ± 9.3^{d}	88.5 ± 5.7^{e}
	recovery	86.6 ± 7.9	88.1 ± 7.4	84.7 ± 6.7
HR (bpm)	baseline	58.5 ± 6.7	55.5 ± 8.9	70.0 ± 11.8^{de}
	exercise	63.7 ± 7.3	65.8 ± 9.8	70.6 ± 12.3^{de}
	recovery	59.6 ± 6.0	55.6 ± 8.7	70.4 ± 13.0^{de}

Values are mean ± SD of 11 participants. Mean arterial pressure (MAP), heart rate (HR), lower power output in horizontal (LPO_{HOR}), lower power output in head-down tilt (LPO_{HDT}), lower power output in head-up tilt (LPO_{HUT}), higher power output in horizontal (HPO_{HOR}), higher power output in head-down tilt (HPO_{HDT}), higher power output in head-up tilt (HPO_{HUT}). Statistically significant compared to: [a]LPO_{HOR}; [b]LPO_{HDT}; [c]LPO_{HUT}; [d]HPO_{HOR}, [e]HPO_{HDT}, and [f]baseline in the same body position ($p < 0.05$).

5.5 Discussion

In the presented study, it was shown the functional limitations of the vascular system to compensate for altered MPP during exercise. This is the first time that HOR, HDT and HUT positions were tested to determine the maximal dilatory capacity of the vascular system of the calf muscles. The findings confirmed the hypothesis that following intense, ischemic muscle contractions, VC_{peak} response would not differ between body positions indicating that VC_{peak} would be independent of changes in MPP. The second hypothesis was also supported by the results demonstrating that the rate of increase for MBF and VC at the onset of exercise would be more rapid in HUT and slower during HDT for both the lower and higher output exercise levels. Finally, the results confirmed that recruitment of greater VC was able to fully compensate for the reduced MPP in LPO_{HDT} to maintain MBF and therefore DO_2 at the same levels as LPO_{HUT}. However, as predicted, the recruitment of VC during HPO_{HDT} restricted the increase in MBF limiting the DO_2 with consequent metabolic stress as suggested by the increased EMG only in this condition.

5.5.1 Peak Vascular Conductance

Complete occlusion of the circulation during 2 minutes associated with one minute of intense, ischemic isometric muscle contraction at 50% MVC were able to elicit VC_{peak} due to the high accumulation of vasodilators metabolites around the resistance vessels of the calf muscle. Such method was similar to the previous method applied by Snell, Martin, Buckey, and Blomqvist, 1987 and Martin et al., 1991 which reported $\sim 10-30$ fold increases in VC. To the best of our knowledge, this is the first time VC_{peak} has been assessed in conditions which MPP was reduced by -44mmHg (HDT) and increased by $+55$mmHg (HUT). The current study results demonstrated $\sim$12 fold increases in VC in HOR, $\sim$8 fold increases in HDT and $\sim$29 fold increases in HUT. The differences between studies can be attributed to the methods employed, occlusion plethysmography (Martin et al., 1991, Snell et al., 1987) versus Doppler ultrasound, body position and physical fitness. Based on the evidences provided by this study, baseline VC is considered an important component in assessing percentage changes. However, the main finding from the present study was that VC_{peak} was similar regardless of body position indicating that VC_{peak} was independent of limb position and MPP. This result was anticipated as the accumulation of metabolites during one minute of ischemic, isometric exercise (50% MVC) was expected to be sufficient to achieve maximal vasodilation and to be independent of body position. Consistent VC_{peak} in each body position allows investigation of the regulation of MBF under different exercise

challenges with altered MPP and estimation of the fractional contribution of VC during exercise.

Nevertheless, the blood flow velocity pattern in different body positions was highly dependent on MPP despite similar VC_{peak} (Figure 5.2A, 5.2B, 5.2C). The MBV pattern showed elevation of blood flow throughout the cardiac cycle (i.e. in systole and diastole) in HOR and HUT. Conversely, the MBV pattern under condition with reduced MPP (HDT) demonstrated periods of no forward flow. The velocity waveform prior to cuff release presented brief periods of reverse flow (negative) during diastole indicating a high resistance vascular bed (Arbeille, Berson, Achaibou, Bodard, and Locatelli, 1995). Therefore, it was anticipated after cuff deflation that the reverse flow would be eliminated of the velocity wave for all body positions (Figure 5.2A, 5.2B, 5.2C).

5.5.2 Baseline VC and MBF

MBF and VC baseline values were different between body positions. MBF and VC were lower in HUT and higher in HDT. The mechanisms underlying these differences relate to the interactions of metabolic demand that would affect vasodilation, shear rate with release of vasoactive compounds from the endothelium, myogenic responses of the vascular smooth muscle, and local and systemic sympathetic vasoconstriction (for more details see Chapter 3). At rest, MBF is sufficient to maintain the metabolites released from the muscles in low concentrations. Despite nitric oxide (NO) in skeletal muscle is released (Shoemaker, Halliwill, Hughson, and Joyner, 1997), flow rates and shear stress are relatively low in the basal condition. However, the potential effects of vasodilatory factors released from the endothelium contributing to the differences in VC and MBF is not known.

In the HUT position, the MPP was ∼55 mmHg greater than HOR and ∼100 mmHg greater than in HDT. This higher MPP raises distending pressure on the vessel increasing transmural pressure across the arterioles stimulating smooth muscle contraction mediated by myogenic reflex response (Toska and Walloe, 2002, Imadojemu et al., 2001). The leg veins are distended due to the hydrostatic effect on the venous blood column activating the veno-arteriolar reflex inducing local arteriolar vasoconstriction of the corresponding arteriole (Tschakovsky and Hughson, 2000, Henriksen and Sejrsen, 1977). In this position, the arterial baroreflex response is activated increasing sympathetic vasoconstriction (Cui et al., 2003, Toska and Walloe, 2002). Combined, these factors result in reduced VC due to the rise in vascular resistance in HUT (Harms et al., 2003). In the HDT, the hydrostatic effect is reduced emptying the veins as result vascular smooth muscle relax due to the myogenic response, removal of venoarteriolar reflex (Sheriff et al., 2007, Rowell, 1993) and

possibly reduced baroreflex activity (Toska, Eriksen, and Walloe, 1994) (for more details see Chapter 3).

In the present study, it is necessary to consider whether the experimental design could have contributed to the differences in baseline. The block design consisted of three separate tests on each day and the order of testing was kept constant with at least 30 minutes between any two tests. Before the next test, the participants were allowed to move from the tilt table to rest. Small differences were noted during the baseline periods for VC and MBF between lower and higher power outputs. However, it is though highly unlikely that these differences were caused by prior tests on that day. For example, the VC and MBF were slightly higher in the LPO_{HOR} and the HPO_{HUT} than the HPO_{HOR} and LPO_{HUT} respectively, but in both cases the slightly higher values were observed in the first test of the day eliminating any possibility of carryover effect (Table 5.2).

5.5.3 Adaptive responses of vascular conductance and muscle blood flow to exercise

The current study is the first to compare the effect of HOR, HDT and HUT positions on the vascular adaptations to calf muscle exercise. VC response was consistently higher in HDT compared to HOR and HUT and lower in HUT than the other two body positions (Figure 5.4B, 5.4D). As a consequence of altered MPP, MBF responses were different between body positions and work rates (Figure 5.4A, 5.4C). There were rapid increases in VC that permitted increases in MBF following the onset of muscle contractions. Such VC increases were a consequence of both changes in vasodilation and muscle pump effect that effectively enhanced the perfusion pressure gradient across the working muscle resulting in an increase in virtual conductance (Nadland et al., 2009, Shoemaker and Hughson, 1999, Shoemaker et al., 1998, Tschakovsky et al., 1996).

The adaptive VC responses were quite different between HOR, HDT and HUT. The rate of increase in VC and the amplitude of increase in VC were different between body positions for both lower and higher power output exercises. These results are supported by previous studies comparing arm below and above heart level (Walker et al., 2007, Saunders and Tschakovsky, 2004) and with the legs below compared to heart level (Nadland et al., 2009). The rate of increase in VC was fastest for the HUT position, but slowest for the HDT position (Table 5.2, Figure 5.4B, 5.4D). The greater amplitude of change for VC in face of reduced arterial perfusion pressure (HDT) in comparison with elevated arterial perfusion pressure (HUT) reflects the demand for vasodilation when MPP is diminished in the HDT position.

The mechanism accounting for the VC response was probably the greater local accumulation of vasodilator metabolites, as the muscle pump was unable to contribute to the increase in VC and MBF due to the minimized emptying effect of the veins and the lack of a hydrostatic column in the HDT position (Saunders and Tschakovsky, 2004, Tschakovsky et al., 1996). A longer time was required to achieve this greater vasodilation as indicated by the longer time to reach steady-state ($T_{63\%}$) in the HDT position. The blunted VC response with elevated arterial perfusion pressure (HUT) reflects the lower demand for vasodilation (Saunders and Tschakovsky, 2004). The mechanism accounting for the VC response was probably the less accumulation of vasodilator metabolites as the muscle pump is more effective contributing to the increase in MBF (Nadland et al., 2009, Laughlin and Joyner, 2003, Folkow et al., 1971). A shorter time was necessary to achieve the required vasodilation was shown by the shorter $T_{63\%}$ in the HUT position.

The MBF responses also had different response times ($T_{63\%}$) following the onset of exercise between conditions. MBF for the HUT position reached $T_{63\%}$ faster, while HDT was the slowest compared to the other two positions (Table 5.2, Figure 5.4A, 5.4C). These results are supported by previous studies comparing arm below and above heart level (Hughson et al., 1996) and with the legs below compared to heart level (Egana and Green, 2005, MacDonald et al., 1998, Leyk et al., 1994). However, the difference in MPP between conditions resulted in different MBF responses during exercise. MBF was higher in the LPO_{HOR} position compared to LPO_{HDT} and LPO_{HUT} (Figure 5.4A). However, MBF for HPO_{HOR} and HPO_{HUT} were similar but were both higher than HPO_{HDT} (Figure 5.4C). The confirmation of inadequate MBF was observed from the relationship between MBF and an index of the sum of electromyographic signals from the muscles (EMG_{index}) demonstrating that MBF in HPO_{HDT} was lower than HPO_{HOR} and HPO_{HUT} with no differences between HPO_{HOR} and HPO_{HUT} (Figure 5.7B).

MBF was probably sufficient in each condition to maintain a relatively low local concentration of vasodilatory metabolites during lower power output exercise, but probably more accentuated during LPO_{HDT}. For the LPO_{HDT}, the VC response was $\sim$80% VC_{peak} compared to $\sim$45% VC_{peak} in the LPO_{HOR} position and only $\sim$20% in LPO_{HUT} (Figure 5.5A). However, the VC in the HPO_{HDT} position reached $\sim$92% VC_{peak} than $\sim$57% in HPO_{HOR} and $\sim$33% in HPO_{HUT} (Figure 5.5B) as consequence MBF in HPO_{HDT} was considerably reduced compared to HPO_{HOR} and HPO_{HUT} positions (Figure 5.4C and Figure 5.7B). Despite the greater recruitment of VC in HPO_{HDT}, VC was not able to fully compensate for the reduction in MPP due to the functional limitation of VC as consequence MBF was compromised in face of reduced MPP. This suggested that the dilatory contribution to the increase in MBF was close to its maximum in the HDT position where the muscle pump contributes little to the change in MBF (Saunders and Tschakovsky, 2004, Tschakovsky

et al., 1996). Conversely, during HUT, the contribution from the muscle pump is more effective allowing further increases in MBF (Nadland et al., 2009, Laughlin and Joyner, 2003, Folkow et al., 1971 with less extent of the vasodilation (Nadland et al., 2009).

5.5.4 Consequences of muscle blood flow to Metabolic Demand

The finding that MBF was lower in the HPO_{HDT} compared to the other body positions suggested that DO_2 was insufficient to meet the demands for aerobic metabolism. The EMG and MBF/EMG_{index} data provide further support for this evidence. Muscle fatigue is associated with an increase in amplitude of EMG as there is an increased requirement for muscle fiber recruitment to maintain muscle power output (Cheng and Rice, 2005, Klass, Guissard, and Duchateau, 2004). In the current study, it was found an increase in EMG only during HPO_{HDT} (Figure 5.6B) suggesting that to accomplish the work rate it was necessary to recruit more muscle fibers as fibers that had been recruited but engaged anaerobic metabolism had ceased to generate the appropriate muscle force. Conversely, MBF must have increased to meet the metabolic requirements as there was no change in EMG relative to LPO_{HOR}, LPO_{HDT} and LPO_{HUT} (Figure 5.6A).

The implications of these data are that reduced MPP caused slower adaptation of MBF which limited O_2 availability following the onset of exercise resulting in slower adaptation of oxidative metabolism and a greater O_2 deficit. This in turn would require greater breakdown of phosphocreatine (PCr) lowering intracellular PCr concentrations with concomitant increases in Pi, ADP, H^+ and lactate. Overall, this would increase the relative stress of the exercise challenge and advance the onset of muscle fatigue. In contrast, elevated MPP caused faster adaptation of MBF which increase O_2 availability following the onset of exercise resulting in faster adaptation of oxidative metabolism and a lower O_2 deficit. This in turn would require lower breakdown of phosphocreatine (PCr) resulting in higher intracellular PCr concentrations and lower accumulation of metabolic by products (Pi, ADP, H^+ and lactate).

5.6 Conclusion

In summary, intense ischemic isometric muscle contraction was able to elicit similar VC_{peak} regardless of body position indicating that VC_{peak} was independent of limb position and MPP. The rate of increase for VC and MBF following the onset of exercise were accelerated in HUT and delayed during HDT due to changes in vasodilation and muscle pump activity altering perfusion pressure gradient across the working muscle resulting in changes in virtual conductance. Recruitment of greater VC was able to fully compensate for the reduced MPP in LPO_{HDT} to maintain MBF and thus DO_2 at the same levels as LPO_{HUT}. However, as predicted, a functional limitation to the recruitment of VC during HPO_{HDT} restricted the increase in MBF, as confirmed by the relationship between MBF and EMG_{index} analysis, limiting the DO_2 with consequent metabolic stress as suggested by the higher EMG activity in this body position. The lower MBF in the HPO_{HDT} suggested that DO_2 was insufficient to meet the demands for aerobic metabolism. This slower adaptation of MBF which limited O_2 availability following the onset of exercise would result in slower adaptation of oxidative metabolism and a greater O_2 deficit increasing the relative stress of the exercise challenge which could advance the onset of muscle fatigue.

Chapter 6

Effects of altered arterial oxygen inspired fraction and arterial perfusion pressure on vascular conductance and muscle blood flow during exercise in humans

6.1 Overview

This study tested the hypothesis during the combined challenges of altered inspired O_2 concentration (F_IO_2) and posture that vascular conductance (VC) to the exercising muscle would change to compensate for changes in muscle perfusion pressure (MPP) and arterial O_2 such that muscle blood flow (MBF) can completely compensate to maintain estimated O_2 delivery (DO_{2est}). Ten healthy volunteers exercised at lower (LPO) and higher power outputs (HPO) (repeated plantar flexion contractions at ∼20% and 30% maximal voluntary contraction, respectively) in horizontal (HOR), 35° head-down tilt (HDT) and 45° head-up tilt (HUT) under normoxia and hypoxia on separate days randomized by blocks. Muscle blood flow velocity was measured by Doppler ultrasound, popliteal arterial diameter was measured by ultrasound imaging, MBF was calculated, arterial O_2 saturation (SaO_2%) was measured by a finger pulse oximeter, DO_{2est} was estimated by MBF times SaO_2 and VC was calculated by dividing MBF by MPP determined beat-by-beat from a finger cuff. Exercise VC, MBF and DO_{2est} were higher in hypoxia than normoxia during LPO and HPO. Exercise MBF and DO_{2est} in hypoxia were higher in LPO_{HOR} than LPO_{HDT} and LPO_{HUT},v but was lower in LPO_{HUT}. Exercise VC in hypoxia was higher in LPO_{HDT} and lower in LPO_{HUT}. The higher VC in LPO_{HDT} during hypoxia compensated for the reduced MPP and SaO_2 increasing MBF to maintain DO_{2est}. During HPO in hypoxia, MBF and DO_{2est} tended to be higher in HPO_{HOR} with no differences between HPO_{HDT} and HPO_{HUT}. VC was higher during HPO_{HDT} and lower in HPO_{HUT}. The greater VC in HPO_{HDT} in hypoxia compensated the reduced MPP and O_2 availability such that MBF and DO_{2est} were not different between HPO_{HDT} and HPO_{HUT}. However, the relationship between MBF and EMG activity demonstrated that MBF was reduced during HPO_{HDT} indicating that MBF and DO_{2est} were not sufficient, a conclusion also supported by the markedly elevated VC, MBF and DO_{2est} post-exercise recovery responses. VC during the relaxation phase of the duty cycle (VC_{relax}) demonstrated to be at the maximal vasodilatory capacity during HPO_{HDT} indicating that the ability to recruit further increases in VC was limited. Thus, MBF and subsequently DO_2 were compromised during reduced MPP and SaO_2.

6.2 Introduction

Muscle blood flow (MBF) increases to match oxygen delivery (DO_2) to the metabolic demand during exercise with constant intensity (Walker et al., 2007, Laughlin and Joyner, 2003). This matching between MBF to DO_2 to metabolic demand is accomplished through a combined effect of progressive vasodilatory mechanisms and potential contribution of the muscle pump (Walker et al., 2007, Tschakovsky et al., 2006, Lutjemeier et al., 2005, Tschakovsky and Sheriff, 2004, Shoemaker and Hughson, 1999). This metabolic regulation of MBF is mainly responsible to promote the maintenance of steady state conditions (Shoemaker and Hughson, 1999). In submaximal steady-state exercise performed with small muscle mass, MBF determines DO_2 because there is no alteration in the arterial O_2 content (Walker et al., 2007). However, in conditions with altered inspired O_2 fraction (F_IO_2), arterial O_2 content (CaO_2) and arterial O_2 partial pressure (PaO_2) changes and compensatory adjustments in MBF are required to maintain the delivery of O_2 (Gonzalez-Alonso et al., 2001). The capacity of MBF to increase DO_2 is directly dependent on O_2 content on the blood (Calbet, 2000).

Alterations in F_IO_2 to manipulate CaO_2 and PaO_2, such as hypoxia, have been used to investigate MBF and DO_2 adjustments during exercise. Previous investigations reported that manipulation of CaO_2 and PaO_2 via hypoxia resulted in compensatory adjustments in MBF to maintain DO_2 during submaximal one or two-legged knee extension exercise in semi-supine position (Gonzalez-Alonso et al., 2001, Koskolou et al., 1997) or maintenance of MBF with reduction in DO_2 during submaximal alternated two-legged kicking exercise in sitting position (MacDonald et al., 2001). Manipulations of limb position relative to the heart to alter arterial and venous pressure also have been used to investigate MBF and DO_2 adaptations during exercise. In the previous investigation (Chapter 5) and Walker et al., 2007 provided evidences that MBF during exercise was reduced in the face of reduced muscle perfusion pressure (MPP) despite the greater vascular conductance (VC) during head-down tilt (HDT), indicating that VC response was not able to fully compensate for the diminished MPP. This functional limitation to the recruitment of VC during HDT restricted the increase in MBF limiting the DO_2 with consequent metabolic stress suggesting that DO_2 was insufficient to meet the demands for aerobic metabolism (Chapter 5). However, the effects of altered F_IO_2 (hypoxia) in conjunction with altered arterial perfusion pressure on MBF, DO_2 and VC responses during exercise in humans are not known.

The purpose of the current study was to investigate MBF, DO_{2est} and VC responses during submaximal calf exercise in three different body positions that affect the gravity-dependent distribution of arterial and venous pressures under altered F_IO_2 (normoxia, $F_IO_2 = 0.21$ and hypoxia, $F_IO_2 = 0.14$). It was hypothesized that under hypoxia within

the same body position VC to the exercising muscle would change to compensate for changes in perfusion pressure and O_2 availability such that MBF through the popliteal artery can completely compensate to maintain DO_{2est}. It was also hypothesized that in hypoxic gas breathing during lower power output exercise, VC response would be able to fully compensate for the changes in F_IO_2 and MPP so that MBF to the working muscle would be maintained as well as DO_{2est} regardless of body position. However, during higher power output exercise, VC response would not be able to completely compensate for the reduction in F_IO_2 and MPP due to a functional limitation to the recruitment of VC that would restrict the increase in MBF leading to a limitation in DO_{2est}.

6.3 Methods

6.3.1 Participants

Ten healthy, non-smoking male volunteers, not specifically exercise trained, with average physical characteristics of: age 27.2 ± 3.6 years, height 176.7 ± 5.5 cm, body mass 78.6 ± 6.9 Kg and body mass index 25.2 ± 2.5 Kg/m^2 participated in this study. The participants average maximal isometric voluntary contraction force (MVC) produced during plantar flexion exercise in prone horizontal position was 61.0 ± 3.2 Kg. Participants received complete written and verbal details of the experimental procedures and potential risks involved prior to signing an information consent form approved by the Office of Research Ethics of the University of Waterloo. All participants were instructed to refrain from consuming alcohol, caffeinated beverages, engaging in vigorous exercise for 24 hours prior to testing, and from consuming a large meal within two hours of testing.

6.3.2 Experimental Design

Participants, who had previously completed tests of peak vascular conductance (VC_{peak}) (see Chapter 5, page 69) reported to the laboratory four times for the manipulations of the gas breathing conditions to alter F_IO_2 (normoxia, 21% O_2 or F_IO_2 = 0.21 and hypoxia, 14% O_2 or F_IO_2 = 0.14) associated with changes in body position during submaximal plantar flexion exercise. Briefly, after arrival in the laboratory, they assumed a prone position with their heads supported by a massage table head piece, shoulder blocks were adjusted accordingly, their arms were placed with the shoulders and elbows positioned at approximately 90°. Two belts, one located on the chest and other on the hips were

used to secure the participants during testing. Straps from these belts were attached to the tilt table to maintain body position. A footplate was connected to the tilt table allowing plantar flexion exercise to be performed. The participants foot was strapped on the footplate to ensure more stability during the motion and better contact between plantar surface of the foot and footplate. Instrumentation took place as described below then the participants rested before starting the testing for approximately 30 minutes. For the experiments involving manipulations of gas concentrations, participants breathed hypoxic air through a face mask connected via a sterile air filter to a large non-pressurized bag (Non-diffusing gas collection bag 60L, Hans Rudolph Inc., Kansas City, Missouri, USA) containing medical grade gas mixtures with 14% O_2 and balance nitrogen from a cylinder tank (Praxair Canada Inc., Mississauga, Ontario, Canada).

6.3.2.1 Altered arterial perfusion pressure and inspired O_2 fraction exercise protocols

The participants were able to sustain a repeated plantar flexion exercise comprised of 1s to extend the footplate, 1s to return it to resting position, and 1s of rest representing 3s duty cycle in time with a metronome with the load set to 20% or 30% MVC. These protocols generated work rates defined as lower and higher power output that could be sustained for 7min with some challenge but without fatigue. The foot plate movement was set in the rotational axis of the ankle to isolate calf muscle contractions which the angle of movement set at $\sim 20^{\circ}$ representing a displacement of 10 cm.

Each participant performed six different protocols, three tests per day on four different days randomized by blocks and counterbalanced. Participants were permitted to come off the tilt table and rest for at least 30min between each individual test on each day to ensure that variables were back to the baseline levels. The altered F_IO_2 tests consisted of 3min baseline followed by 3min exercise in normoxia ($F_IO_2 = 0.21$), 4min exercise in hypoxia and 3min of recovery in hypoxia ($F_IO_2 = 0.14$) (Figure 6.1). The order for test block A was: higher power output exercise in head-up tilt (HPO_{HUT}), lower power output exercise in head-down tilt (LPO_{HDT}) and higher power output exercise in horizontal (HPO_{HOR}). The order for test block B was: lower power output exercise in horizontal (LPO_{HOR}), higher power output exercise in head-down tilt (HPO_{HDT}) and lower power output exercise in head-up tilt (LPO_{HUT}). These blocks were distributed based on the best physiological approach to minimize any carry over, cumulative effect of one condition to another, especially on the early stages of the subsequent protocols and to avoid muscle fatigue. The four days of testing were performed two times per week separated by at least 48 hours. This break minimized any carry over and cumulative effect of the exercise

protocols as well as muscle adaptation to the stimulus.

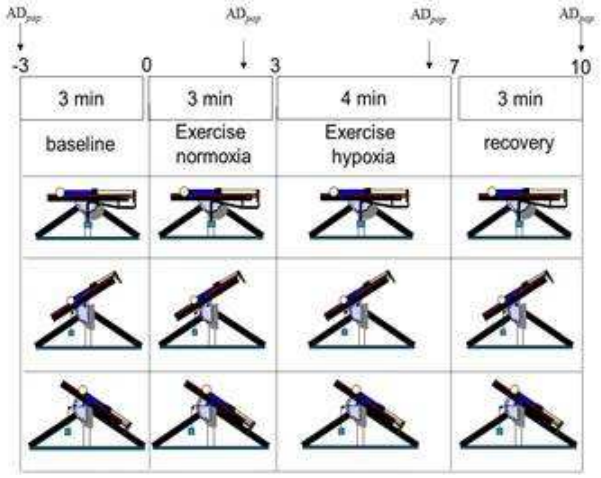

Figure 6.1: Exercising protocol used to investigate MBF, DO_{2est} and VC responses during submaximal calf exercise in HOR, HDT and HUT positions under altered F_IO_2 (normoxia, $F_IO_2 = 0.21$ and hypoxia, $F_IO_2 = 0.14$). Arrows indicate were AD_{pop} (popliteal arterial diameter) were taken.

The angle of tilting accomplished an average reduction in MPP estimated to the middle of the calf muscle of $\sim$−44 mmHg for HDT (35°) and $\sim$+55 mmHg for HUT position (45°). The gas breathing changes accomplished a reduction of $\sim$8% in SaO_2 from normoxia ($F_IO_2 = 0.21$) to hypoxia ($F_IO_2 = 0.14$). Changing the angle of the tilt table required adjusting the load to achieve the same tension on the cable attached to the foot plate as previously described elsewhere (see methods section in Chapter 5 for more details, page 70).

All tests were conducted in a quiet room where room temperature was kept constant at 19.4 ± 0.9 °C to ensure minimal skin blood flow at rest, with humidity at 39.9 ± 4.8%, and barometric pressure at 730.8 ± 2.4 mmHg.

6.3.3 Data Acquisition

Muscle blood flow velocity (MBV) in the popliteal artery, heart rate (HR) and blood pressure (BP) were measured on a beat-by-beat basis. MBV responses were determined from the intensity weighted mean of a 4 MHz pulsed Doppler ultrasound probe (Neurovision Doppler Ultrasound, Model 500, Multigon Industries, Mt. Vernon, USA) for all trials in the experiments. This flat ultrasound probe was secured by surgical tape to the skin surface

over the popliteal artery embedded at a 45° angle of insonation relative to the skin and adjusted according to the measured angle between the skin and the popliteal artery.

The popliteal artery was investigated to establish proper alignment, and to determine the depth and gate to assure full insonation of the artery lumen prior to each trial. The intensity of the Doppler spectrum was verified by audio and visual feedback. The probe location was marked on the skin to assist repositioning of the probe during the test and on subsequent tests. If it was necessary, the researcher manipulated the probe to preserve the best Doppler signal as determined by constant use of auditory and visual feedback. If the ultrasound signal quality was compromised during the last minute of exercise in normoxia and/or hypoxia the test trial was excluded from the analysis. This occurred with one participant in HDT conditions due to difficulties with the Doppler ultrasound signal resulting in loss of some data in those trials. Therefore, the data analysis for LPO_{HDT} and HPO_{HDT} were based on nine rather than ten participants.

Diameter of the popliteal artery (AD_{pop}) was measured with an 8-12 MHz echo Doppler ultrasound probe (Diagnostic Ultrasound system, Model M5, Shenzen Mindray Bio-medical electronics Co., Ltd., Shenzen, China). These popliteal artery images were recorded and stored for analysis. AD_{pop} measurements were performed in B-mode with the echo Doppler ultrasound probe positioned just proximal to the site of velocity measurements. AD_{pop} images were taken as the average of three separate measurements during diastole at baseline and at the end of recovery and during the relaxation phase between contractions in the third and sixth minutes of exercise.

Arterial blood pressure was estimated from a photoplethysmograph cuff placed around the middle finger of the left hand (Finometer, Finapres Medical Systems, Arnhem, the Netherlands). Mean arterial pressure (MAP) was determined as the area under the curve between successive heart beats. HR was continuously calculated from the RR-interval obtained from an electrocardiogram (Pilot 9200, Colin Medical Instruments Corp, San Antonio, Texas, USA). Oxygen saturation (SaO_2) was obtained by a pulse oximeter with the probe placed in the index finger of the left hand (Ohmeda 3740 Pulse oximeter, Louisville, CO., USA).

The muscle activity was measured by electromyography (EMG) as described elsewhere (for more details see methods section Chapter 5, page 72). Briefly, the EMG surface electrodes were placed longitudinally on the distal half of the medial and lateral gastrocnemius and soleus of the right leg. Participants were shaved, abraded and cleaned with alcohol and electrodes were fixed on the skin with the reference electrode on the head of the fibula. A custom built amplifier was used to amplify the raw EMG signal.

MBV, MAP, HR, EMG and SaO_2 were continuously collected at 1000 Hz using a data

acquisition system (Powerlab, ADInstruments, Colorado Springs, USA). This system was connected to the computer and all the variables were continuously recorded using data analysis software (Chart, version 5.5, ADInstruments, Colorado Springs, USA). EMG data were collected at 1000 Hz and processed with custom software (Matlab, version 7.0, The Mathworks, Natick, Massachusetts, USA).

6.3.4 Data Analysis

Beat-by-beat data were time aligned, linearly interpolated and averaged over two complete contraction/relaxation cycles (6 seconds) and over two trials to produce a single data set per person for each of the six conditions. In the current study, MBF, DO_{2est}, VC and EMG in different body positions (HOR, HDT and HUT) were all determine in the last minute of exercise in normoxia (2-3min) and compared with the last minute of exercise in hypoxia (6-7min).

MBF was calculated as the product between MBV and the corresponding AD_{pop} cross-sectional area as MBF = MBV$\times\pi r^2\times$60; where r is the vessel radius. The DO_{2est} was calculated as MBF (mL·min^{-1}) times SaO_2. MPP (mmHg) was estimated by MAP (mmHg) and the distance between subjects heart and the middle of the calf muscle for all body positions as demonstrated elsewhere (Chapter 2, page 22). VC (mL·min^{-1}·mmHg^{-1}) was determined by MBF (mL·min^{-1}) divided by MPP (mmHg) as VC = MBF/MPP.

The EMG data analyses were described elsewhere (see methods section Chapter 5 for more details, page 74-75). Briefly, the EMG data was rectified and filtered (2nd order, dual pass Butterworth, cut off frequency of 5Hz). The area under the rectified signal determined integrated EMG (iEMG) and the fast Fourier transformation (FFT) determined mean power frequency (MPF). The EMG signal was normalized by the maximal voluntary contraction (MVC) and then averaged in the last minute of exercise expressed as percentage of MVC (%MVC).

6.3.5 Statistical Analysis

Data underwent a two way analysis of variance (ANOVA) for repeated measures with main effects of body position (HOR, HDT, and HUT) and time (baseline and recovery) for the dependent variables during lower and higher power outputs at baseline and the last minute of recovery. Exercise data were analysed by a two way analysis of variance (ANOVA) for repeated measures with main effects of body position (HOR, HDT, and HUT) and altered F_IO_2 (normoxia and hypoxia) for the dependent variables during lower and

higher power outputs in the last minute of normoxia and hypoxia. If required, prior to the analysis data were rank-transformed to address concerns of non-normal distribution. The Student-Newman-Keuls post-hoc test was performed to identify the statistically significant differences. The level of significance for main effects and interactions was set at $p < 0.05$. Data were presented as means ± standard deviation (SD).

6.4 Results

6.4.1 Lower output tests responses under altered FIO_2

The two-way repeated measures ANOVA indicated a main effect of body position and time (baseline and recovery) and interactions between body position and time for MBF and DO_{2est} ($p < 0.05$) and a main effect of body position and time for VC ($p < 0.05$). The post-hoc analysis demonstrated that the baseline values for MBF and DO_{2est} were not different between LPO_{HDT} and LPO_{HOR} but lower in LPO_{HUT} ($p < 0.05$, Table 6.1, Figure 6.2A, 6.2B). The baseline values for VC were higher in LPO_{HDT} and lower during LPO_{HUT} ($p < 0.05$, Table 6.1, Figure 6.2C).

During exercise, the two-way repeated measures ANOVA indicated a main effect of body position and interactions between body position and F_IO_2 for MBF and DO_{2est} ($p < 0.05$) and main effect of body position and F_IO_2 and interactions between body position and F_IO_2 for VC ($p < 0.05$). Mean values of MBF during exercise were not different between normoxia and hypoxia in the same condition for LPO_{HOR} and LPO_{HUT}, but during LPO_{HUT}, DO_{2est} was lower in hypoxia than normoxia ($p < 0.05$). During LPO_{HDT}, MBF and DO_{2est} were higher in hypoxia than normoxia ($p < 0.05$, Table 6.1, Figure 6.2D, 6.2E). Mean VC values during exercise were not different between normoxia and hypoxia in the same condition for LPO_{HOR} and LPO_{HUT}, but were higher in hypoxia than normoxia for LPO_{HDT} ($p < 0.05$, Table 6.1, Figure 6.2F).

Differences existed between body positions for both the normoxic and hypoxic phases of the tests. During exercise in normoxia, MBF and DO_{2est} were higher in LPO_{HOR} than LPO_{HDT} and LPO_{HUT} ($p < 0.05$) with no differences between LPO_{HUT} and LPO_{HDT} (Table 6.1, Figure 6.2D, 6.2E). During exercise in hypoxia, MBF and DO_{2est} were higher in LPO_{HOR} than ($p < 0.05$), but lower in LPO_{HUT} compared to LPO_{HDT} (Table 6.1, Figure 6.2D, 6.2E). VC values during exercise in both normoxia and hypoxia were higher in LPO_{HDT} but were lower during LPO_{HUT} ($p < 0.05$, Table 6.1, Figure 6.2F).

The post-hoc analysis indicated that MBF values in the same conditions were higher during recovery than baseline for all body positions ($p < 0.05$, Table 2.1, Figure 6.2A).

However, DO_{2est} values were higher during recovery compared to baseline only for LPO_{HDT} ($p < 0.05$) with no differences for LPO_{HOR} and LPO_{HUT} (Table 6.1, Figure 6.2B). The post-exercise recovery for MBF and DO_{2est} between body positions were lower in LPO_{HUT} compared to LPO_{HDT} and LPO_{HOR} ($p < 0.05$) with no differences between LPO_{HOR} and LPO_{HDT} (Table 6.1, Figure 6.2A, 6.2B). Within the same condition, VC was higher during recovery than baseline for LPO_{HDT} ($p < 0.05$) with no differences between LPO_{HOR} and LPO_{HUT} (Table 6.1, Figure 6.2C). VC values between body positions were higher during LPO_{HDT} but lower in LPO_{HUT} ($p < 0.05$, Table 6.1, Figure 6.2C).

Table 6.1: MBF, DO_{2est} and VC at baseline, exercise (normoxia and hypoxia) and recovery during LPO_{HOR}, LPO_{HDT} and LPO_{HUT} conditions.

Variables	time points	LPO_{HOR} (n=10)	LPO_{HDT} (n=9)	LPO_{HUT} (n=10)
MBF ($mL \cdot min^{-1}$)	baseline	48.4 ± 17.2	44.3 ± 13.5	27.3 ± 10.0^{ab}
	normoxia	188.7 ± 67.1	146.3 ± 34.2^{a}	127.2 ± 58.8^{a}
	hypoxia	195.8 ± 72.4	167.3 ± 38.1^{bd}	119.5 ± 53.2^{de}
	recovery	61.7 ± 26.7^{f}	82.8 ± 34.4^{f}	31.1 ± 11.2^{fgh}
DO_{2est} ($mLO_2 \cdot min^{-1}$)	baseline	46.9 ± 16.7	43.2 ± 13.2	26.6 ± 9.7^{ab}
	normoxia	183.4 ± 65.6	142.2 ± 33.2^{a}	123.9 ± 57.2^{a}
	hypoxia	176.9 ± 65.4	152.6 ± 34.3^{d}	109.6 ± 48.5^{cde}
	recovery	54.6 ± 23.4	74.2 ± 34.7^{f}	29.6 ± 9.7^{gh}
VC ($mL \cdot min^{-1} \cdot min^{-1}$)	baseline	0.6 ± 0.2	0.9 ± 0.4^{a}	0.2 ± 0.1^{ab}
	normoxia	2.2 ± 0.7	2.9 ± 0.8^{a}	0.9 ± 0.4^{ab}
	hypoxia	2.1 ± 0.8	3.3 ± 0.8^{bd}	0.8 ± 0.4^{de}
	recovery	0.7 ± 0.3	1.8 ± 1.0^{fg}	0.2 ± 0.1^{gh}
test block order		B1	A2	B3

Values are mean ± SD. Number of participants (n), muscle blood flow (MBF), estimated O_2 delivery (DO_{2est}), vascular conductance (VC), lower power output in horizontal (LPO_{HOR}), lower power output in head-down tilt (LPO_{HDT}) and lower power output in head-up tilt (LPO_{HUT}). Statistically significant compared to: [a]LPO_{HOR}, [b]LPO_{HDT} and [c]LPO_{HUT} in normoxia, [d]LPO_{HOR} and [e]LPO_{HDT} in hypoxia, [f]baseline in the same condition, [g]recovery in LPO_{HOR} and [h]recovery in LPO_{HDT} ($p < 0.05$). Test Block Order indicates for each of the Blocks (see page 98).

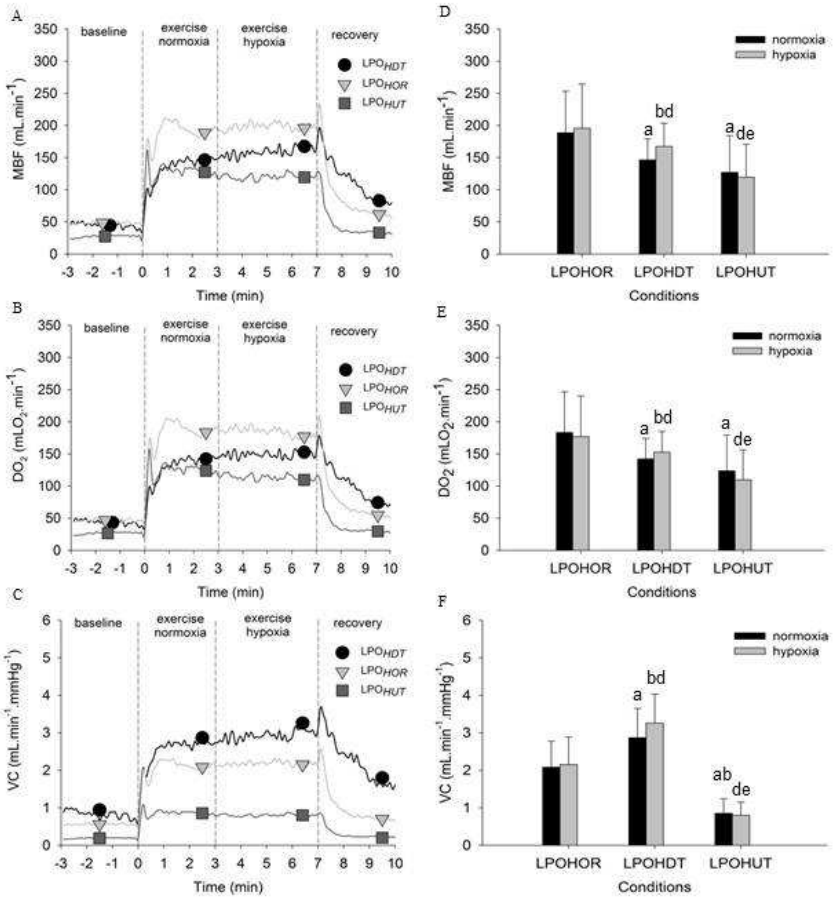

Figure 6.2: Time course of changes in MBF (A), DO_{2est} (B) and VC (C) during dynamic plantar flexion exercise performed in lower power output under normoxia ($F_IO_2 = 0.21$) and hypoxia ($F_IO_2 = 0.14$). Lines indicate group response, symbols indicate the points at which statistical analysis were performed and dashed vertical lines indicate the start, gas concentration shift and cessation of exercise. Data are the mean analysed over 6 second time bins including contraction and relaxation phases of the duty cycles. MBF (D), DO_{2est} (E) and VC (F) during dynamic plantar flexion exercise performed in lower power output under normoxia and hypoxia. Data are the mean ± SD averaged during the last minute of exercise in normoxia and hypoxia. Statistically significant compared to: $^{a}LPO_{HOR}$, $^{b}LPO_{HDT}$ and $^{c}LPO_{HUT}$ in normoxia and $^{d}LPO_{HOR}$ and $^{e}LPO_{HDT}$ in hypoxia ($p < 0.05$). If necessary the symbols were offset for clarity.

6.4.2 Higher output tests responses under altered FIO_2

The two-way repeated measures ANOVA indicated a main effect of body position and time (baseline and recovery) for MBF, DO_{2est} and VC ($p < 0.05$). The post-hoc analysis demonstrated that the baseline values for MBF, DO_{2est} and VC were higher in HPO_{HDT}, but lower in HPO_{HUT} ($p < 0.05$, Figure 6.3A, 6.3B, 6.3C).

During exercise, the two-way repeated measures ANOVA indicated a main effect of body position and F_IO_2 for MBF, DO_{2est} and VC ($p < 0.05$). The post-hoc analysis indicated that mean values of MBF and DO_{2est} during exercise were not different between normoxia and hypoxia in the same condition for HPO_{HUT}, but were higher during hypoxia than normoxia in HPO_{HOR} and HPO_{HDT} (Table 6.2, Figure 6.3D, 6.3E). Mean VC values during exercise were not different between normoxia and hypoxia in the same condition for HPO_{HOR} and HPO_{HUT} but were higher in hypoxia than normoxia for HPO_{HDT} ($p < 0.05$, Table 6.2, Figure 6.3).

Normoxic and hypoxic phases of the tests presented differences between body positions. MBF and DO_{2est} during normoxia were higher in HPO_{HOR} compared to HPO_{HDT} ($p < 0.05$) and tended to be higher compared to HPO_{HUT} ($p = 0.064$) with no differences between HPO_{HDT} and HPO_{HUT} ($p = 0.236$). During hypoxia, MBF and DO_{2est} tended to be higher in HPO_{HOR} compared to HPO_{HDT} ($p = 0.052$) and HPO_{HUT} ($p = 0.077$) with no differences between HPO_{HDT} and HPO_{HUT} ($p = 0.808$, Figure 6.3D, 6.3E). VC values during exercise between body positions during both normoxia and hypoxia were higher in HPO_{HDT} ($p < 0.05$), but were lower during HPO_{HUT} ($p < 0.05$, Table 6.2, Figure 6.3F).

The post-hoc analysis indicated that MBF and DO_{2est} values were higher during recovery compared to baseline for all body positions in the same conditions ($p < 0.05$, Table 6.2, Figure 6.3A, 6.3B). MBF and DO_{2est} values between body positions were higher during HPO_{HDT} than HPO_{HOR} and HPO_{HUT} ($p < 0.05$), but lower in HPO_{HUT} ($p < 0.05$, Table 6.2, Figure 6.3C). Within the same conditions, VC values were higher during recovery compared to baseline for HPO_{HDT} and HPO_{HUT} ($p < 0.05$) and tended to be for HPO_{HOR} ($p = 0.063$, Figure 6.3C). VC responses between body positions were greater in HPO_{HDT} than HPO_{HOR} and HPO_{HUT} ($p < 0.05$), but lower in HPO_{HUT} ($p < 0.05$, Table 6.2, Figure 6.3C).

Table 6.2: MBF, DO_{2est} and VC at baseline, exercise (normoxia and hypoxia) and recovery during HPO_{HOR}, HPO_{HDT} and HPO_{HUT} conditions.

Variables	time points	LPO_{HOR} (n=10)	LPO_{HDT} (n=9)	LPO_{HUT} (n=10)
MBF ($mL \cdot min^{-1}$)	baseline	36.8 ± 11.4	47.8 ± 15.3	30.8 ± 11.1^{b}
	normoxia	227.5 ± 79.7	186.8 ± 55.4^{a}	203.3 ± 74.3
	hypoxia	249.5 ± 95.7^{a}	221.6 ± 68.7^{bd}	210.3 ± 84.2
	recovery	55.1 ± 23.8^{f}	155.2 ± 72.3^{fg}	43.5 ± 18.9^{fh}
DO_{2est} ($mLO_2 \cdot min^{-1}$)	baseline	35.9 ± 11.1	46.4 ± 14.8	29.9 ± 10.7^{b}
	normoxia	221.0 ± 77.3	180.9 ± 53.3^{a}	197.0 ± 72.4
	hypoxia	228.4 ± 86.7	201.0 ± 60.8^{bd}	190.8 ± 75.0
	recovery	49.6 ± 21.3^{f}	138.2 ± 63.6^{fg}	38.7 ± 16.0^{fgh}
VC ($mL \cdot min^{-1} \cdot min^{-1}$)	baseline	0.4 ± 0.1	1.0 ± 0.4^{a}	0.2 ± 0.1^{ab}
	normoxia	2.4 ± 0.8	3.6 ± 1.0^{a}	1.4 ± 0.5^{ab}
	hypoxia	2.5 ± 0.9	4.1 ± 1.2^{bd}	1.4 ± 0.6^{de}
	recovery	0.5 ± 0.2	3.3 ± 1.7^{fg}	0.3 ± 0.1^{fgh}
test block order		A3	B2	A1

Values are mean ± SD. Number of participants (n), muscle blood flow (MBF), estimated O_2 delivery (DO_{2est}), vascular conductance (VC), higher power output in horizontal (HPO_{HOR}), higher power output in head-down tilt (HPO_{HDT}) and higher power output in head-up tilt (HPO_{HUT}). Statistically significant compared to: [a]HPO_{HOR}, [b]HPO_{HDT} and [c]HPO_{HUT} in normoxia, [d]HPO_{HOR} and [e]HPO_{HDT} in hypoxia, [f]baseline in the same condition, [g]recovery in HPO_{HOR} and [h]recovery in HPO_{HDT} ($p < 0.05$). Test Block Order indicates for each of the Blocks (see page 98).

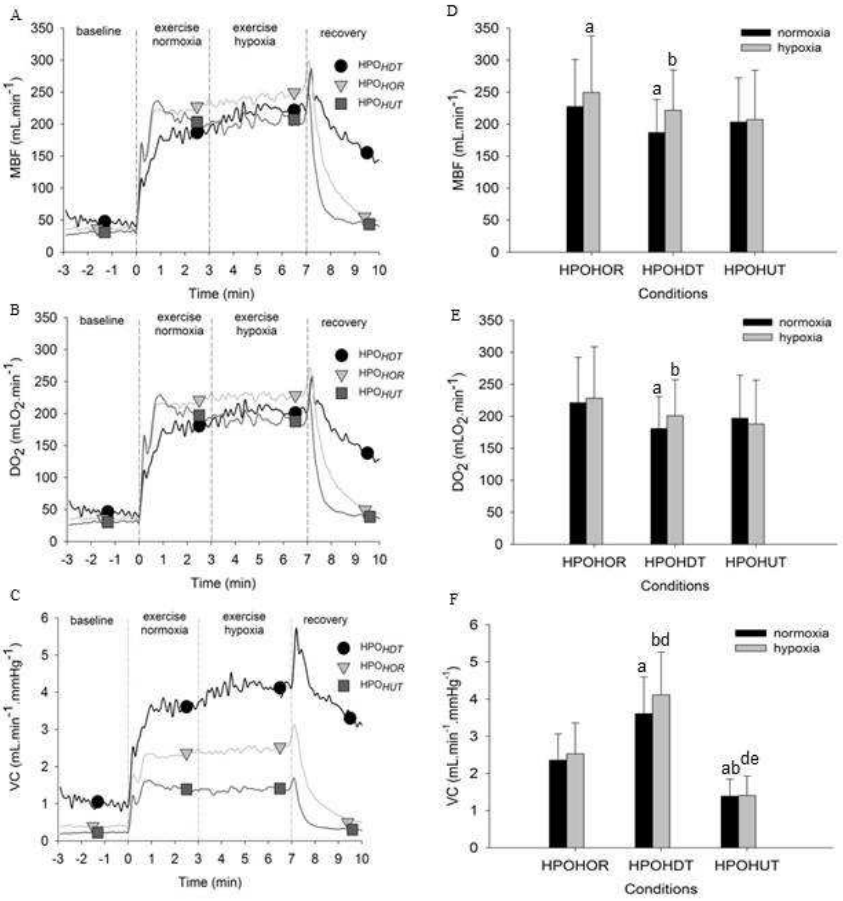

Figure 6.3: Time course of changes in MBF (A), DO_{2est} (B) and VC (C) during dynamic plantar flexion exercise performed in highber power output under normoxia ($F_IO_2 = 0.21$) and hypoxia ($F_IO_2 = 0.14$). Lines indicate group response, symbols indicate the points at which statistical analysis were performed and dashed vertical lines indicate the start, gas concentration shift and cessation of exercise. Data are the mean analysed over 6 second time bins including contraction and relaxation phases of the duty cycles. MBF (D), DO_{2est} (E) and VC (F) during dynamic plantar flexion exercise performed in higher power output under normoxia and hypoxia. Data are the mean ± SD averaged during the last minute of exercise in normoxia and hypoxia. Statistically significant compared to: $^{a}HPO_{HOR}$, $^{b}HPO_{HDT}$ and $^{c}HPO_{HUT}$ in normoxia and $^{d}HPO_{HOR}$ and $^{e}HPO_{HDT}$ in hypoxia ($p < 0.05$). If necessary the symbols were offset for clarity.

6.4.3 Vascular conductance expressed as percentage of the peak vascular conductance under altered FIO_2

The two-way repeated measures ANOVA indicated a main effect of body position and interaction between body position and F_IO_2 for vascular conductance during relaxation (VC_{relax}) and VC_{relax} expressed as percentage (% VC_{relax}) for lower power output ($p < 0.05$). The post-hoc analysis demonstrated that the baseline VC_{relax} and $\%VC_{relax}$ were significantly higher during LPO_{HDT} and lower during LPO_{HUT} ($p < 0.05$, Figure 6.4A). VC_{relax} and $\%VC_{relax}$ responses during exercise were different between normoxia and hypoxia for LPO_{HDT} ($p < 0.05$) with no differences within LPO_{HOR} and LPO_{HUT} (Figure 6.4C). In both normoxia and hypoxia during lower power output, VC_{relax} and $\%VC_{relax}$ during exercise were higher in LPO_{HDT} and lower in LPO_{HUT} position ($p < 0.05$, Figure 6.4C).

In higher power output there were main effects of body position and F_IO_2 and interaction between body position and F_IO_2 for VC_{relax} and $\%VC_{relax}$ ($p < 0.05$). The post-hoc analysis indicated that the baseline VC_{relax} and $\%VC_{relax}$ were significantly higher during HPO_{HDT} and lower during HPO_{HUT} ($p < 0.05$, Figure 6.4B). In exercise, VC_{relax} and $\%VC_{relax}$ responses were different between normoxia and hypoxia for HPO_{HDT} and HPO_{HOR} ($p < 0.05$), but not in HPO_{HUT} (Figure 6.4D). During higher power output, VC_{relax} and $\%VC_{relax}$ exercise were higher in HPO_{HDT} and lower in HPO_{HUT} in both normoxic and hypoxic conditions ($p < 0.05$, Figure 6.4D).

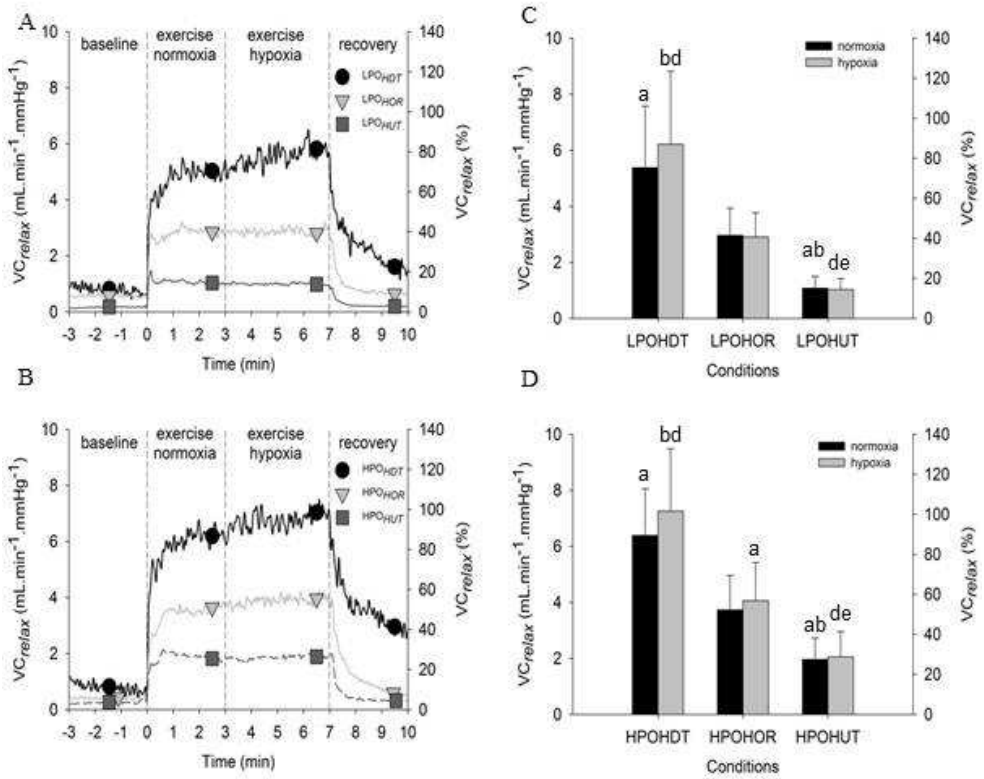

Figure 6.4: Time course of changes in VC_{relax} ($mL \cdot min^{-1} \cdot mmHg^{-1}$) and $\%VC_{relax}$ as percentage of change in vascular conductance response ($\%VC_{relax} = VC_{relax}/VC_{peak} \times 100$) during dynamic plantar flexion exercise performed in lower (A) and higher power outputs (B) under normoxia ($F_IO_2 = 0.21$) and hypoxia ($F_IO_2 = 0.14$). Lines indicate group response, symbols indicate the points at which statistical analysis were performed and dashed vertical lines indicate the start and cessation of exercise. The bar graphs represent exercising VC_{relax} and $\%VC_{relax}$ during lower (C) and higher power outputs (D) under normoxia and hypoxia. Data are the mean ± SD analysed over the relaxation phases between muscle contraction phases of the duty cycles in normoxia and hypoxia. Statistically significant compared to: [a]HOR and [b]HDT and [c]HUT in normoxia and [d]HOR and [e]HDT in hypoxia ($p < 0.05$). If necessary symbols were offset for clarity.

6.4.4 Muscle activity responses during exercise

In lower power output, the two-way repeated measures ANOVA indicated a main effect of body position and interaction between body position and F_IO_2 for soleus, total muscle activity (TMA) and EMG_{index} and interaction between body position and F_IO_2 for gastrocnemius medial head (GMH) and gastrocnemius lateral head (GLH) ($p < 0.05$). The post-hoc analysis showed that during lower power output, EMG activity was not different between normoxia and hypoxia in the same condition for all muscles and body positions (Figure 6.5A, 6.5B, 6.5C). However, TMA and EMG_{index} were different between normoxia and hypoxia in the same conditions for all body positions (Figure 6.5D, 6.5E). In lower power output in normoxia, EMG activity was not different between body positions for all muscles, TMA and EMG_{index} (Figure 6.5A-E). However, in hypoxia, soleus, GLH, TMA and EMG_{index} responses during exercise were higher in LPO_{HDT} than LPO_{HOR} and LPO_{HUT} ($p < 0.05$) with no differences between LPO_{HOR} and LPO_{HUT} positions (Figure 6.5A, 6.5C, 6.5D, 6.5D, respectively).

In higher power output, the two-way repeated measures ANOVA indicated a main effect of body position and interaction between body position and F_IO_2 for all muscle groups, TMA and EMG_{index} ($p <0.05$). The post-hoc analysis demonstrated that in higher power output, EMG activity was different between normoxia and hypoxia during HPO_{HDT} with no differences during HPO_{HOR} and HPO_{HUT} for all muscle groups, TMA and EMG_{index} ($p < 0.05$, Figure 6.5A-E). In normoxia, GMH, TMA and EMG_{index} demonstrated lower values during HPO_{HUT} compared to HPO_{HDT} and HPO_{HOR} ($p < 0.05$) with no differences between HPO_{HOR} and HPO_{HDT} (Figure 6.5B, 6.5D, 6.5E). Soleus showed lower values during HPO_{HUT} compared to HPO_{HDT} ($p < 0.05$), but not different than HPO_{HOR} (Figure 6.5A). GLH was not different between body positions in normoxia (Figure 6.5C). In hypoxia during higher power output, EMG activity for all muscle groups, TMA and EMG_{index} were higher in HPO_{HDT}, but lower in HPO_{HUT} ($p < 0.05$, Figure 6.5A-E).

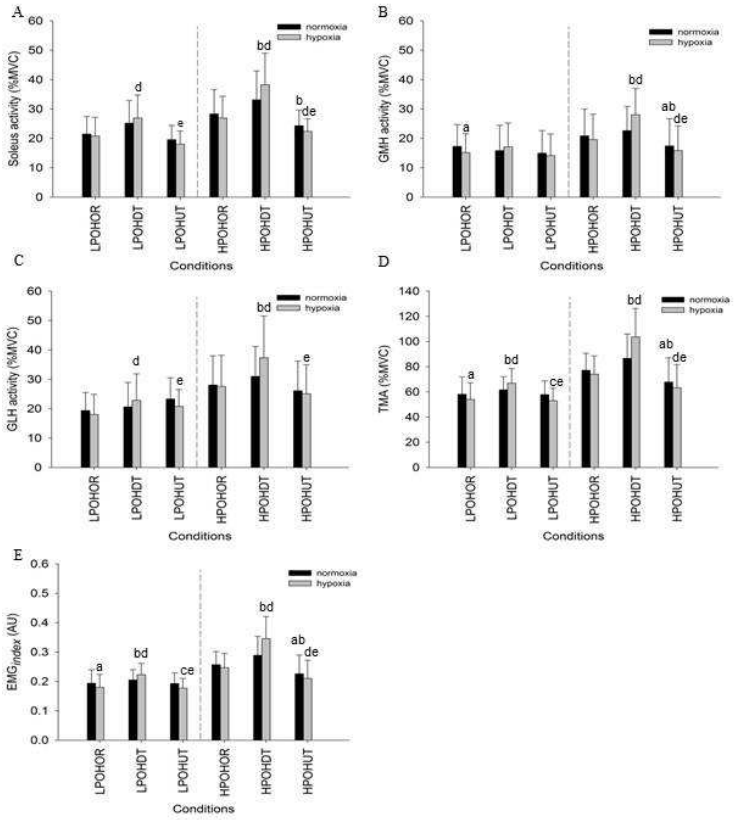

Figure 6.5: (A) Muscle activity in soleus, (B) gastrocnemius medial head (GMH), (C) gastrocnemius lateral head (GLH), (D) total muscle activity (TMA) and (E) EMG_{index} during plantar flexion exercise during lower and higher power outputs under horizontal (HOR), head-down tilt (HDT) and head-up tilt (HUT) positions under normoxia (F_IO_2 = 0.21) and hypoxia (F_IO_2 = 0.14). Total muscle activity was calculated as the sum of the mean for soleus and gastrocnemius muscle groups activity as a percentage of MVC and EMG_{index} was calculated as the sum of the mean for soleus and gastrocnemius muscle groups activity divided by the sum of the MVC for both workloads and for all body positions. Data are the mean ± SD averaged during the last minute of exercise in normoxia and hypoxia. Statistically significant compared to: [a]HOR, [b]HDT and [c]HUT in normoxia and [d]HOR and [e]HDT in hypoxia ($p < 0.05$).

6.4.5 Normalized muscle blood flow by muscle activity under altered FIO_2

The two-way repeated measures ANOVA indicated a main effect of body position and F_IO_2 for MBF/EMG_{index} during lower power output ($p < 0.05$). The post-hoc analysis indicated that MBF/EMG_{index} was different between normoxia and hypoxia during LPO_{HOR} ($p < 0.05$) with no differences between LPO_{HDT} and LPO_{HUT} (Figure 6.6C). During lower power output in normoxia and hypoxia, MBF/EMG_{index} was higher in LPO_{HOR} compared to LPO_{HDT} and LPO_{HUT} ($p < 0.05$) with no differences between LPO_{HDT} and LPO_{HUT} (Figure 6.6C).

The two-way repeated measures ANOVA indicated a main effect of body position for MBF/EMG_{index} during higher power output ($p < 0.05$). The post-hoc analysis indicated that MBF/EMG_{index} was not different between normoxia and hypoxia for all body positions (Figure 6.6D). In higher power output during normoxia and hypoxia MBF/EMG_{index} was lower in HPO_{HDT} than HPO_{HOR} and HPO_{HUT} ($p < 0.05$) with no differences between HPO_{HOR} and HPO_{HUT} (Figure 6.6D).

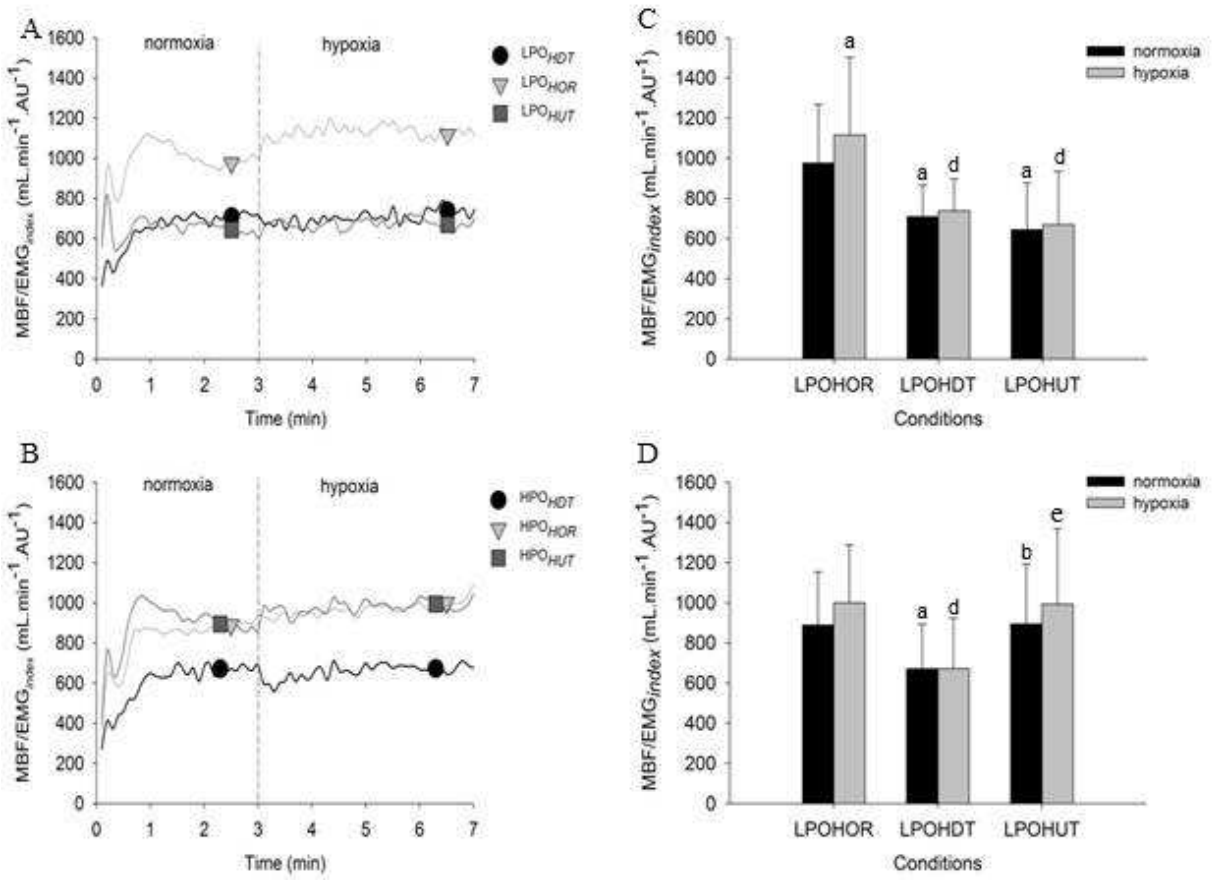

Figure 6.6: Time course of changes in MBF/EMG_{index} during dynamic plantar flexion exercise performed in lower (A) and higher (B) power outputs under normoxia (F_IO_2 = 0.21) and hypoxia (F_IO_2 = 0.14). Lines indicate group response, symbols indicate the points at which statistical analysis were performed and dashed vertical lines indicate the start, gas concentration shift and cessation of exercise. Data are the mean analysed over 6 second time bins including contraction and relaxation phases of the duty cycles. MBF/EMG_{index} during dynamic plantar flexion exercise performed in lower (C) and higher power output (D) under normoxia and hypoxia. Data are the mean ± SD averaged during the last minute of exercise in normoxia and hypoxia. Statistically significant compared to: [a]HOR, [b]HDT and [c]HUT in normoxia and [d]HOR and [e]HDT in hypoxia ($p < 0.05$). If needed symbols were offset for clarity.

6.4.6 Q, MAP and HR responses during lower and higher power output exercises during normoxia and hypoxia

The two-way repeated measures ANOVA indicated a main effect of body position and F_IO_2 for MAP during lower and higher power outputs ($p < 0.05$). The post-hoc analysis indicated that in the lower power output exercise, MAP was not different between normoxia and hypoxia in the same conditions for all body positions (Table 6.3). In both normoxia and hypoxia during lower power output, MAP was higher in LPO_{HDT} and LPO_{HUT} than LPO_{HOR} ($p < 0.05$) with no differences between LPO_{HDT} and LPO_{HUT} (Table 6.3). In higher power output exercise, MAP was lower in normoxia than hypoxia for HPO_{HOR} and HPO_{HDT} ($p < 0.05$), but not different in HPO_{HUT} (Table 6.3). In both normoxia and hypoxia, MAP was lower in HPO_{HUT} than HPO_{HOR} and HPO_{HDT} ($p < 0.05$) with no differences between HPO_{HOR} and HPO_{HDT} (Table 6.3).

The two-way repeated measures ANOVA indicated a main effect of body position and F_IO_2 for $\dot{Q}$ and HR during lower power output ($p < 0.05$). The post-hoc analysis demonstrated that $\dot{Q}$ was lower in normoxia compared to hypoxia for all body positions during lower power output (Table 6.3). In normoxia and hypoxia, $\dot{Q}$ was higher in LPO_{HOR} compared to LPO_{HDT} and LPO_{HUT} ($p < 0.05$) with no differences between LPO_{HDT} and LPO_{HUT} (Table 6.3). During higher power output, $\dot{Q}$ was lower in normoxia than hypoxia for HPO_{HOR} and HPO_{HDT} ($p < 0.05$), but not during HPO_{HUT} as indicated by the main effect of F_IO_2 (Table 6.3). $\dot{Q}$ during normoxia and hypoxia was not different for all body positions (Table 6.3).

The post-hoc analysis indicated that HR during lower power output was lower in normoxia compared to hypoxia in the same conditions for all body positions ($p < 0.05$). During lower power output, HR values during exercise in normoxia and hypoxia were lower in LPO_{HDT} than LPO_{HOR} and LPO_{HUT} ($p < 0.05$) with no differences between LPO_{HOR} and LPO_{HUT} (Table 6.3). In higher power output, HR was lower in normoxia than hypoxia for all body positions ($p < 0.05$). Normoxic and hypoxic conditions showed higher HR values during HPO_{HUT} than HPO_{HOR} and HPO_{HDT} ($p < 0.05$) with no differences between HPO_{HOR} and HPO_{HDT} (Table 6.3).

Table 6.3: $\dot{Q}$, MAP and HR in normoxia and hypoxia averaged during the last minute of exercise during LPO_{HDT}, LPO_{HOR}, LPO_{HUT}, HPO_{HDT}, HPO_{HOR} and HPO_{HUT}.

Variables	time points	LPO_{HOR}	LPO_{HDT}	LPO_{HUT}
MAP (mmHg)	normoxia	89.9 ± 5.2	95.3 ± 7.9^{a}	95.1 ± 7.2^{a}
	hypoxia	90.4 ± 5.7	95.6 ± 7.9^{d}	96.0 ± 8.3^{d}
$\dot{Q}$ ($L \cdot min^{-1}$)	normoxia	6.4 ± 1.0	5.6 ± 1.0^{a}	5.4 ± 0.7^{a}
	hypoxia	7.1 ± 1.3^{a}	5.9 ± 1.1^{bd}	5.9 ± 0.9^{acd}
HR (bpm)	normoxia	63.6 ± 9.4	60.7 ± 8.7^{a}	65.3 ± 9.3^{b}
	hypoxia	70.1 ± 11.2^{a}	65.8 ± 10.1^{bd}	72.1 ± 10.7^{de}
Variables	time points	HPO_{HOR}	HPO_{HDT}	HPO_{HUT}
MAP (mmHg)	normoxia	96.0 ± 7.0	95.1 ± 7.6	91.8 ± 8.2^{ab}
	hypoxia	98.2 ± 7.6^{a}	97.7 ± 7.4^{b}	92.7 ± 7.6^{de}
$\dot{Q}$ ($L \cdot min^{-1}$)	normoxia	5.9 ± 0.7	6.0 ± 1.0	6.2 ± 0.9
	hypoxia	6.3 ± 0.7^{a}	6.6 ± 1.4^{d}	6.4 ± 0.8
HR (bpm)	normoxia	61.3 ± 8.8	64.6 ± 9.6	70.7 ± 10.5^{ab}
	hypoxia	66.3 ± 9.6^{a}	72.2 ± 11.6^{b}	75.4 ± 12.4^{cde}

Values are mean ± SD of 10 participants. Mean arterial pressure (MAP), cardiac output($\dot{Q}$) heart rate (HR), lower power output in horizontal (LPO_{HOR}), lower power output in head-down tilt (LPO_{HDT}), lower power output in head-up tilt (LPO_{HUT}), higher power output in horizontal (HPO_{HOR}), higher power output in head-down tilt (HPO_{HDT}) and higher power output in head-up tilt (HPO_{HUT}). Statistically significant compared to: $^{a}LPO_{HOR}$; $^{b}LPO_{HDT}$; $^{c}LPO_{HUT}$; $^{d}HPO_{HOR}$ and $^{e}HPO_{HDT}$ ($p < 0.05$).

6.5 Discussion

In the present study, it was demonstrated the capability of the vascular system to regulate MBF to maintain DO_2 in conditions with altered F_IO_2 (hypoxia) and MPP during submaximal exercise. This is the first time that HDT, HOR and HUT positions were tested in conjunction with hypoxia to determine exercise MBF, DO_{2est} and VC responses during lower and higher power output exercises. The findings confirmed the hypothesis that within the same body position VC to the exercising muscle would increase to compensate for the alterations in perfusion pressure and O_2 availability such that MBF through the popliteal artery would increase sufficiently to maintain DO_{2est}. The greater recruitment of VC in HDT compensated for the reduction in MPP and SaO_2 increasing MBF sufficiently to maintain DO_{2est} at the same level as HUT. However, the analysis of the relationship between MBF and EMG activity (EMG_{index}) demonstrated that this ratio was reduced during HPO_{HDT} in both normoxia and hypoxia. The lower MBF/EMG_{index} ratio indicated inadequate MBF limiting DO_{2est} despite the greater VC response. During HPO_{HDT} in hypoxia, the maximal vasodilatory capacity was reached indicating that the ability to compensate was exceeded. This functional limitation in the recruitment of VC might increase the metabolic stress in exercise as suggested by the higher EMG activity in this condition. The higher VC, MBF and DO_{2est} responses during post-exercise recovery in HDT is also evidence that the MBF and DO_{2est} were inadequate during the exercise phase in this position.

6.5.1 VC, MBF and DO_2est responses during exercise: comparisons between normoxia and hypoxia in the same body position

6.5.1.1 Lower power output exercise

During LPO_{HDT}, the ∼14% increase in VC on average in hypoxia resulted in similar ∼14% in MBF that maintained DO_{2est} at the same level as normoxia compensating for the reduced MPP and SaO_2 probably due to the greater accumulation of metabolites promoting vasodilation of the resistance arterioles. Despite this, there was ∼9% increase in EMG activity in this position during hypoxia compared to normoxia suggesting that oxidative energy supply might have been impaired (Figure 6.2A-F, Figure 6.5A-E). The higher VC values during post-exercise recovery in LPO_{HDT} suggested greater vasodilation probably due to the higher concentration of muscle metabolites produced during this exercise condition resulting in higher post-exercise MBF. The higher post-exercise VC, MBF and DO_{2est}

are evidences that both flow and DO_2 were probably not sufficient to meet the demand for aerobic metabolism in this position during exercise.

During LPO_{HOR}, the non-significant changes in VC (∼4%) were sufficient to alter MBF (∼4%) to maintain DO_{2est} at the same level as normoxia (Figure 6.5A-F) which was consistent with no significant changes in EMG values in this position in hypoxia compared to normoxia (Figure 6.5A-E). In the current study, the compensatory adjustments in VC and MBF to maintain DO_{2est} between normoxia and hypoxia are in line with studies that demonstrated increase in VC and MBF to maintain DO_2 during hypoxia during one or two-legged knee extension submaximal exercise in semi-supine position (Gonzalez-Alonso et al., 2001, Roach, Koskolou, Calbet, and Saltin, 1999, Koskolou et al., 1997, Rowell, Saltin, Kiens, and Christensen, 1986) or submaximal handgrip exercise (Wilkins, Schrage, Liu, Hancock, and Joyner, 2006). Such compensatory adjustments in VC and MBF during hypoxia probably minimized the risk of mismatch between DO_2 and metabolic demand during exercise (Calbet, Radegran, Boushel, and Saltin, 2009, Calbet, 2000). During LPO_{HOR}, the VC, MBF and DO_{2est} post-exercise responses suggested that both MBF and DO_2 were adequate to match the aerobic metabolism requirements in this condition during exercise.

During LPO_{HUT}, VC and MBF responses did not change significantly as a consequence DO_{2est} decreased ∼12% in hypoxia in comparison with normoxia (Table 6.1, Figure 6.2A-F) with no significant changes in EMG activity between normoxia and hypoxia (Figure 6.5A-E). The result in the current study is in agreement with the no significant compensation in leg blood flow during hypoxia resulting in reduced DO_2 during submaximal alternated two-legged kicking exercise (MacDonald et al., 2000). The lower VC response during exercise and recovery in LPO_{HUT} suggested less vasodilation probably due to the lower concentrations of muscle metabolites produced during exercise condition. The VC, MBF and DO_{2est} post-exercise responses are evidences that both flow and DO_2 were probably sufficient to meet the demand for aerobic metabolism during exercise in this position.

6.5.1.2 Higher power output exercise

During higher power output despite the ∼8% reduction in SaO_2 during hypoxia, VC was 14% higher on average during HPO_{HDT} which was accompanied by ∼19% increase in MBF that maintained DO_{2est} at the same level as normoxia, but in spite of this the average EMG increased ∼20% compared to normoxia (Figure 6.3A-F, Figure 6.5A-E). The higher EMG activity in hypoxia indicated a relative increased metabolic stress that would advance the onset of muscle fatigue. The increased VC during exercise and recovery in HPO_{HDT} suggested greater vasodilation probably due to the higher production and greater accumulation of muscle metabolites resulting in greater post-exercise hyperemia and DO_{2est}. The

increased VC, MBF and DO_{2est} post-exercise responses in HPO_{HDT} are evidences that both flow and DO_2 were probably not sufficient to meet the demand for aerobic metabolism. Together, these responses suggested impairment of oxidative energy supply to meet the demands for aerobic metabolism increasing metabolic stress during exercise as indicated by the increased EMG activity in this position that would in turn advance the onset of muscle fatigue.

In HPO_{HOR} in hypoxia, the ∼7% non-significant increase in VC was sufficient to increase MBF by ∼10% maintaining DO_{2est} at the same level as normoxia (Figure 6.3A-F) and there were no changes in EMG activity between normoxia and hypoxia in this position (Figure 6.5A-E). The results of the present study are supported by the increase in VC and MBF to maintain DO_2 during hypoxia during one or two-legged knee extension submaximal exercise in semi-supine position (Gonzalez-Alonso et al., 2001, Roach et al., 1999, Koskolou et al., 1997, Rowell et al., 1986) or submaximal handgrip exercise (Wilkins et al., 2006). The VC, MBF and DO_{2est} post-exercise responses suggested that both MBF and DO_{2est} were adequate to match the aerobic metabolism requirements during exercise in this condition.

During HPO_{HUT} in hypoxia, the slightly increase in VC and MBF responses were sufficient to maintain DO_{2est} at the same level as normoxia (Figure 6.3A-F) and there were no significant changes in EMG activity between normoxic and hypoxic gas breathing conditions (Figure 6.5A-E). The lower VC response during exercise suggested that less vasodilation was needed to achieve the required MBF response as a consequence of the elevated MPP (Nadland et al., 2009) probably due to the lower accumulation of muscle metabolites produced during this exercise condition. The VC, MBF and DO_{2est} post-exercise responses also provided additional evidences that both MBF and DO_2 were probably sufficient to meet the demand for aerobic metabolism during HPO_{HUT}.

The mechanisms accounting for the match between MBF to DO_2 to the metabolic demand during exercise with constant work rate was accomplished through the increase in VC due to vasodilatory mechanisms in response to the reduction in SaO_2 caused by the hypoxia. The hypoxic conditions reduced SaO_2, but also may result in decrease in CaO_2 and PaO_2 affecting O_2 availability with consequent decrease in the cellular PO_2 stimulating vasodilatory response (Calbet, 2000). The reduced O_2 is probably sensed by the red blood cells stimulating the release of ATP from the erythrocytes mediating vasodilatory responses (Ellsworth et al., 1995) via nitric oxide (NO) and/or endothelium-derived-hyperpolarization-factor (EDHF) and/or release of NO from S-nitrosohemoglobin due to hemoglobin deoxygenation relaxing the vascular smooth muscle (Calbet, 2000, Stamler et al., 1997, Jia et al., 1996). The accumulation of adenosine in hypoxia could activate the adenosine-sensitive K^+ channels enhancing the release of K^+ from the active muscle fiber and elevating the interstitial K^+ concentrations (Allen et al., 2008). The adenosine monophosphate-activated

protein kinase (AMPK) is probably activated in hypoxic conditions stimulating the release of NO and PG relaxing the vascular smooth muscle (Fisslthaler and Fleming, 2009, Towler and Hardie, 2007). Other vasoactive factors released during hypoxic exercise, such as CO_2, H^+, lactate probably also contribute to mediate the vasodilatory response to increase VC (Calbet, 2000).

6.5.2 VC, MBF and DO_2 responses during exercise: comparisons between body positions under normoxia and hypoxia

6.5.2.1 Lower power output exercise

During LPO_{HDT} in normoxia, the higher VC response compensated for the reduction in MPP increasing MBF sufficiently to maintain DO_{2est} at the same level as LPO_{HUT}, but both were lower than LPO_{HOR} with non-significant differences in EMG activity between body positions. These results were confirmed by the relationship between MBF and an index of the sum of electromyographic signals from the muscles (EMG_{index}) used to account for the differences in EMG activity as well as to confirm the adequacy of MBF and DO_{2est}. MBF/EMG_{index} ratio was not different between LPO_{HUT} and LPO_{HDT} but both were lower than LPO_{HOR} (Figure 6.6A, 6.6C). The greater recruitment of VC during LPO_{HDT} reached $\sim$75% VC_{peak} in normoxia reflecting the greater demand for vasodilation in this position. The current results are supported by the previous study during lower power output plantar flexion exercise in normoxia (Chapter 5).

During LPO_{HDT} in hypoxia, the increase in VC caused further increase in MBF to maintain DO_{2est} in face of reduced MPP and SaO_2. The MBF response in LPO_{HDT} during hypoxia was higher than LPO_{HUT} due to the $\sim$26% increase in EMG activity. However, MBF in hypoxia was lower during LPO_{HDT} compared to LPO_{HOR} despite the $\sim$24% increase in EMG activity. When the relationship between MBF and EMG_{index} were considered in hypoxia, this ratio did not differ between LPO_{HUT} and LPO_{HDT} but both were lower than LPO_{HOR} (Figure 6.6A, 6.6C). The greater recruitment of VC during LPO_{HDT} compensated for the reduction in MPP and SaO_2 inducing compensatory changes in MBF that maintained DO_{2est} at the same level as LPO_{HUT}, but not high enough to match MBF in LPO_{HOR}. The greater recruitment of VC during hypoxia in LPO_{HDT} reached $\sim$85%VC_{peak} reflecting the greater demand for vasodilation caused probably by the greater accumulation of muscle metabolites produced during this exercise condition inducing vasodilation of the resistance arterioles (Chapter 5) as the muscle pump does not contribute to the change in MBF in this position (Saunders and Tschakovsky, 2004, Tschakovsky et al., 1996). The post-exercise recovery in hypoxia demonstrated higher VC values in LPO_{HDT} after cessation

of exercise indicating enhanced vasodilation which is consistent with greater accumulation of muscle metabolites during exercise in this position. As a consequence of the augmented VC both MBF and DO_{2est} responses were higher during recovery suggesting inadequate MBF and DO_{2est} during exercise to meet the demands for the aerobic metabolism.

During LPO_{HUT} in normoxia and hypoxia, VC was blunted reaching only ∼15-20% VC_{peak} reflecting the lower demand for vasodilation with elevated MPP due to probably lower accumulation of muscle metabolites during exercise in this position and more effective contribution of the muscle pump to the increase in MBF (Nadland et al., 2009, Laughlin and Joyner, 2003, Folkow et al., 1971). The constrained VC response in normoxia resulted in reduced MBF compared to LPO_{HOR}. However, during hypoxia, the constrained VC resulted in decreased MBF and DO_{2est} compared to both LPO_{HOR} and LPO_{HDT}. Despite this, the post-exercise recovery suggested adequate flow and DO_{2est} in this position. These results are supported by the previous study during normoxic conditions (Chapter 5).

6.5.2.2 Higher power output exercise

During HPO_{HDT} in normoxia and hypoxia, the increase in VC values compensated for the reduction in MPP increasing MBF sufficiently to maintain DO_{2est} at the same level as HPO_{HUT}, but lower than HPO_{HOR}. In normoxia, despite ∼28% higher EMG activity and ∼62% greater VC response during HPO_{HDT} compared to HPO_{HUT}, MBF and DO_{2est} responses were not different between these body positions. Although in hypoxia, EMG activity was ∼60% higher and VC was ∼65% higher in HPO_{HDT} in comparison with HPO_{HUT}, there were no differences in MBF and DO_{2est} between these body positions. However, the ∼12% higher EMG activity and ∼53% greater VC response in HPO_{HDT} compared to HPO_{HOR} in normoxia and the ∼36% higher EMG activity and ∼53% greater VC response in HPO_{HDT} compared to HPO_{HOR} in hypoxia were not sufficient to maintain MBF and DO_{2est} at the same level as in horizontal. However, the relationship between MBF and EMG activity (MBF/EMG_{index}) in both normoxia and hypoxia demonstrated that this ratio was lower in HPO_{HDT} with no differences between HPO_{HOR} and HPO_{HUT}.

The lower MBF/EMG_{index} indicated that the increase in MBF in HPO_{HDT} was restricted despite the greater VC response indicating that VC could not fully compensate for the reduction in MPP alone or the combined effects of reduced MPP and SaO_2. The greater recruitment of VC reached ∼90%VC_{peak} with only 2-3min of normoxic exercise and ∼100%VC_{peak} in hypoxia indicating that the maximal vasodilatory capacity was achieved, thus, the ability to compensate was exceeded. This functional limitation on the VC response did not allow further increases in MBF limiting DO_{2est} in this position. The results of the present study are in line with the previous study that demonstrated limitation in

the recruitment of VC restricting MBF during plantar flexion exercise in normoxia in face of reduced MPP (Chapter 5), reduced brachial blood flow with the arm above heart level despite the increase in VC in normoxia (Walker et al., 2007) and VC limiting the increase in leg blood flow in hypoxia during one-legged exercise (Gleser, 1973).

The VC, MBF and DO_{2est} post-exercise responses also supported the inadequacy of MBF and DO_{2est} during exercise in HPO_{HDT}. The higher post-exercise VC indicated that greater amount of vasodilator muscle metabolites still present after cessation of exercise probably as a consequence of the greater accumulation of those metabolites during exercise in HPO_{HDT} inducing vasodilation of the resistance arterioles. This higher VC resulted in higher MBF and DO_{2est} during recovery suggesting that both MBF and DO_{2est} were not adequate during the exercise phase in both normoxia and hypoxia. The greater MBF observed during recovery probably represented the repayment of the O_2 deficit accumulated at the onset of exercise as consequence of the slow rate of adaptation and the higher metabolic demand during HPO_{HDT} as suggested by the higher EMG activity which was consistent with challenged aerobic metabolism (Chapter 5).

In HPO_{HUT} in normoxia and hypoxia, the lower VC ($\sim$41% in normoxia and $\sim$43% in hypoxia) and EMG activity ($\sim$12% in normoxia and $\sim$14% in hypoxia) compared to HPO_{HOR} resulted in non-significant changes in MBF and DO_{2est} responses, but there were trends for higher responses to both normoxia and hypoxia during HPO_{HOR}. However, the relationship between MBF and EMG activity (MBF/EMG_{index}) in both normoxia and hypoxia demonstrated that this ratio was not different between HPO_{HOR} and HPO_{HUT}. During HPO_{HUT}, the VC response was blunted reaching only $\sim$30% VC_{peak} in normoxia and hypoxia reflecting the lower demand for vasodilation with elevated MPP even with reduced SaO_2. The blunted VC response in normoxia and hypoxia was probably caused by less accumulation of vasodilator metabolites in the resistance arterioles due to more effective contribution of the muscle pump to the increase in MBF (Nadland et al., 2009, Laughlin and Joyner, 2003, Folkow et al., 1971) (Figure 7.4). The increase in MBF was achieved by the combined effects of vasodilation and the enhanced perfusion pressure gradient across the working muscle resulting in an increase in virtual conductance (Nadland et al., 2009, Shoemaker and Hughson, 1999, Shoemaker et al., 1998, Tschakovsky et al., 1996) with less extent of vasodilation (Nadland et al., 2009) as indicated by the use of only $\sim$30% VC_{peak} in HPO_{HUT}. The post-exercise recovery indicated that despite the constrained VC during HPO_{HUT}, MBF and DO_{2est} were adequate and probably sufficient to maintain relatively low accumulation of vasodilatory metabolites during the exercise phase. The lower MBF during recovery probably represented a lower O_2 deficit accumulated at the onset of exercise as consequence of the faster rate of adaptation and the lower metabolic demand during HPO_{HUT} as suggested by the lower EMG activity (Chapter 5).

6.6 Conclusion

In conclusion, the hypoxic challenge added in exercise was met during LPO_{HDT} by increased VC to compensate reduced MPP and O_2 availability such that MBF maintained DO_{2est}. However, during HPO_{HDT} in hypoxia, VC reached maximal vasodilatory capacity, compromising MBF and DO_{2est}. Together, these findings indicate that LPO_{HDT} in normoxia or hypoxia VC increased to maintain MBF and DO_{2est}, but during HPO functional limitation for recruitment of VC constrained MBF and DO_{2est} in normoxia and hypoxia. Elevated muscle electromyograpic signals in HPO_{HDT} were consistent with challenged aerobic metabolism associated with reduced MPP and O_2 availability that could contribute to a greater increase in the relative stress of the exercise challenge and advance the onset of muscle fatigue.

Chapter 7

General Discussion, Conclusions and Future Considerations

7.1 Summary of studies

Muscle blood flow (MBF) is determined by the interaction between perfusion pressure gradient and vascular conductance (VC) (Tschakovsky and Sheriff, 2004, Laughlin and Korzick, 2001, Hughson et al., 1996, Rowell, 1993). Based on this concept, the present work investigated the adaptations of VC, MBF and DO_2 during rest and exercise. The studies in this thesis used the changes in body position as an experimental model to induce hydrostatic pressure changes and shifts in blood volume to alter perfusion pressure by the effects of gravity. Overall, the findings from these studies provided insights about the control mechanisms regulating VC and MBF and their consequences on DO_2.

Reliable and reproducible quantitative measurements of muscle blood flow and arterial diameter at rest and exercise were essential for the experiments involved in this thesis. Previous investigations have determined that non-invasive Doppler ultrasound method can be used to obtain continuous and reliable measurements at rest, during the onset and steady-state phase of exercise with small muscle mass (Shoemaker et al., 1996, Hughson et al., 1996). In the Chapter 2, data repeatability for popliteal muscle blood flow velocity (MBV), popliteal arterial diameter (AD_{pop}), MBF and VC at rest and exercise in different body positions and work rates were tested. All variables were repeatable regardless of body position for all conditions proving that ultrasound methods are suitable to measure such dynamic responses as supported by previous investigations (Walker et al., 2007, Amundsen et al., 2002, Radegran, 1997, Shoemaker et al., 1996).

The interactions between perfusion pressure gradient and VC determine resting MBF (Rowell, 1993). In the Chapter 3, the adaptations of the lower limb resting VC and MBF during HUT and HDT postural challenges were investigated. The novel finding of this study showed that, in HDT, the increase in VC completely compensated for the reduction in MPP in the lower leg such that resting MBF was maintained. This result was in contrast with Sheriff et al., 2007 who demonstrated a transient fall in femoral vascular conductance during 20s of 15°HDT, but was similar to that observed with the arm above heart level (Tschakovsky and Hughson, 2000). As anticipated during HUT, VC was reduced in conditions under higher MPP consequently resting MBF was decreased. The results of the current study are in line with the decreased leg blood flow with elevated arterial perfusion pressure during HUT or seated position (Groothuis et al., 2005, Delis et al., 2000) as well as with the forearm below heart level (Hughson et al., 1996).

The results of the previous study (Chapter 3) were analysed as average over a beat and then averaged over the last two minutes of testing representing the net arterial response in each body position. However, this procedure did not consider important information related to MBV pattern and waveform characteristics (Thijssen et al., 2009). It was interesting to note that while the absolute MBF was not different between HDT and HOR, the patterns of MBV differed between these positions as well as in HUT. The Chapter 4 examined the changes in MBV_{ant} and MBV_{ret} patterns in the three different body positions (HOR, HDT and HUT). The primary novel finding was that the same average MBV_{net} in the popliteal artery was attained in the HOR and HDT positions even though absolute MBV_{ant} and MBV_{ret} values were greater in HDT than HOR probably due to the increase in arterial compliance and VC that compensated for the lower MPP in HDT. In contrast, lower MBV_{net} in the popliteal artery was detected in the HUT than HOR with reductions in both MBV_{ant} and MBV_{ret} in this position probably due to the lower arterial compliance and VC changing the ratio between MBV_{ant} and MBV_{ret}. The MBV patterns and waveforms characteristics during HOR and HUT in this study were consistent with previous descriptions of brachial (Baccelli et al., 1985), femoral (Blackshear et al., 1979, Risoe and Wille, 1978), popliteal (Risoe and Wille, 1978) and posterior tibial arteries (Risoe and Wille, 1978).

Exercising muscles are sensitive to changes in limb or body position due to the effects of gravity (Egana and Green, 2005) determining the dynamic responses of blood flow following the onset and steady-state exercise (Nadland et al., 2009, Hughson et al., 1996). The match between MBF to DO_2 to metabolic demand are dependent on work rates, magnitude of the hydrostatic column changes and muscle mass engaged in the exercise (Nadland et al., 2009). Therefore, the exercise model with manipulation of the body position involving a large hydrostatic column associated with different work rates involving a small muscle mass

was selected to challenge the adaptive response of VC and MBF during transitions from rest to exercise. The main findings were that the maximal dilatory capacity of the vascular system of the calf muscles was achieved following intense, ischemic muscle contractions with no differences between body positions indicating that VC_{peak} was independent of MPP. Following the onset of exercise, MBF and VC responses were delayed in HDT and accelerated in HUT in both lower and higher power outputs. These results are in agreement with faster increases in brachial blood flow observed in the arm below compared to above heart level (Hughson et al., 1996) and femoral blood flow below compared to heart level (Egana and Green, 2005, MacDonald et al., 1998, Leyk et al., 1994). In LPO_{HDT}, the increase in VC was able to counteract the reduced MPP to maintain MBF. In contrast during HPO_{HDT}, the increase in VC response could not compensate for the reduction in MPP resulting in lower MBF due to a limitation of VC to promote further increases in MBF with consequences for the delivery of O_2.

In the previous study (Chapter 5, MBF determined DO_2 responses because arterial O_2 content (CaO_2) was not altered during exercise. To further challenge the adaptive responses of VC, MBF and DO_2 during exercise in different body positions, the manipulation of the F_IO_2 was added to investigate whether VC responses would lead to compensatory changes in MBF to maintain DO_{2est}. The main findings were that within the same body position VC to the exercising muscle increased to compensate for the alterations in perfusion pressure and O_2 availability such that MBF increased sufficiently to maintain DO_{2est}. The greater recruitment of VC in HDT compensated for the reduction in MPP and SaO_2 increasing MBF sufficiently to maintain DO_{2est} at the same level as HUT. However, the analysis of the relationship between MBF and EMG activity (EMG_{index}) demonstrated that this ratio was reduced during HPO_{HDT} in both normoxia and hypoxia. The lower MBF/EMG_{index} ratio indicated inadequate MBF limiting DO_{2est} despite the greater VC response. During HPO_{HDT} in hypoxia, the maximal vasodilatory capacity was reached indicating that the ability to compensate was exceeded. This functional limitation in the recruitment of VC might increase the metabolic stress in exercise as suggested by the higher EMG activity in this condition. The higher VC, MBF and DO_{2est} responses during post-exercise recovery in HDT is also evidence that the MBF and DO_{2est} were inadequate during exercise in this position.

7.2 Resting vascular conductance and resting muscle blood flow responses following tilt

The central circulatory effects of changes in body position were relatively minor compared to the local peripheral responses of the lower legs. In HDT, the sympathetic vasoconstrictor tone in the leg arteries was probably reduced by arterial and cardiopulmonary baroreceptor activity (Nagaya et al., 1995, Rowell, 1993). The transmural pressure and wall tension were decreased inducing relaxation of the resistance arteries in the legs through myogenic response (Sheriff et al., 2007, Tschakovsky and Hughson, 2000, Rowell, 1993). The gravity-assisted shift in venous blood volume probably inhibited the veno-arteriolar reflex (Sheriff et al., 2007, Tschakovsky and Hughson, 2000). The combination of these effects altered vascular tone promoting vasodilation with consequent increase in VC that fully compensate for the reduced MPP to maintain MBF and consequently constant DO_2 (Figure 7.1).

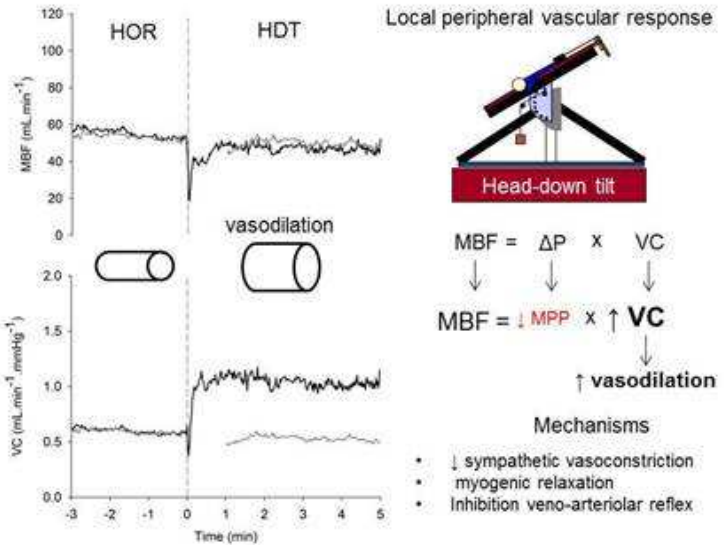

Figure 7.1: Schematic illustration of the local peripheral mechanisms probably involved in VC and MBF responses in HDT at rest. During HDT, VC increased to fully compensate for the reduction in MPP to maintain MBF at the same level as horizontal (control condition). The local peripheral (myogenic and venoarteriolar reflexes) and central cardiovascular reflexes (arterial and cardiopulmonary baroreceptors activity) mechanisms are involved in the VC response to promote vasodilation.

In HUT, the sympathetic vasoconstrictor response was augmented by the increase in the arterial and cardiopulmonary baroreceptors activity (Cui et al., 2003, Toska and Walloe, 2002, Imadojemu et al., 2001). The elevated MPP with consequent increased transmural pressure and wall tension probably induce myogenic vasoconstriction (Sheriff et al., 2007, Groothuis et al., 2005, Harms et al., 2003, Toska and Walloe, 2002, Imadojemu et al., 2001). The increase in venous pressure probably induced arteriolar vasoconstriction of the corresponding arteriole through the veno-arteriolar reflex (Tschakovsky and Hughson, 2000, Henriksen and Sejrsen, 1977). The combination of these effects resulted in vasoconstriction reducing VC and consequently resting MBF and DO_2 (Figure 7.2).

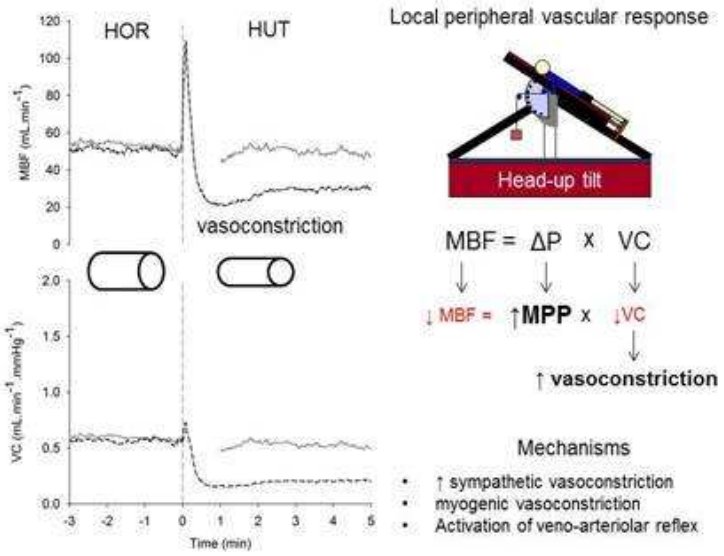

Figure 7.2: Schematic illustration of the local peripheral mechanisms probably involved in VC and MBF responses in HUT at rest. During HUT, VC decreased in face of elevated MPP resulting in a reduction in MBF. The local peripheral (myogenic and venoarteriolar reflexes) and central cardiovascular reflexes (arterial and cardiopulmonary baroreceptors activity) mechanisms are involved in the VC response to promote vasoconstriction.

The MBV patterns in the popliteal artery demonstrated marked differences between body positions within a cardiac cycle. MBV_{ant} and MBV_{ret} waveforms and patterns are not only dependent on the interactions between perfusion pressure and local vascular resistance or conductance (Baccelli et al., 1985), but also on downstream compliance (Zamir et al., 2007). In HDT, the greater increase in both MBV_{ant} and MBV_{ret} was probably due to the increase in arterial compliance and VC that compensated for the lower MPP to maintain

MBV_{net} at the same level as HOR position. In HUT, the lower MBV_{net} in the popliteal artery and the reductions of MBV_{ant} and MBV_{ret} was probably due to the lower arterial compliance and VC changing the ratio between MBV_{ant} and MBV_{ret}.

In HDT, the lower MPP decreased distending and transmural pressures would reduce stretching of the arterioles probably leading to an increase in arterial compliance (Zamir et al., 2007, Grassi et al., 1995) due to the relaxation of the vascular smooth muscle inducing vasodilation (Zamir et al., 2007). During HDT, the effects of induced vasodilation via local peripheral reflexes and central cardiovascular mechanisms increase both arterial compliance and VC resulting in higher MBV_{ant} and MBV_{ret} that maintained MBV_{net} at the same level as horizontal. Likewise, in HUT, the elevated MPP increasing distending and transmural pressures would enhance the stretching of the arterioles resulting in lower arterial compliance (Zamir et al., 2007). The combined effects of induced vasoconstriction through local peripheral reflexes and central cardiovascular mechanisms decrease both arterial compliance and VC resulting in lower MBV_{ant} and MBV_{ret} consequently lower MBV_{net} than HOR and HDT positions.

7.3 Vascular conductance and muscle blood flow adaptive responses in exercise

7.3.1 7.2.1 At the onset of exercise

VC and MBF demonstrated a biphasic response as previously reported by others (Nadland et al., 2009, Walker et al., 2007, Saunders and Tschakovsky, 2004, Hughson et al., 1996). The rapid increase in VC allowed the increases in MBF at the onset of exercise as a consequence of vasodilation and muscle pump effect. The increased VC suggested a reduction in downstream resistance to permit the increase in MBF through smaller resistance vessels (Shoemaker et al., 1996). This vasodilatory response associated with muscle pump action effectively enhanced the perfusion pressure gradient across the working muscle resulting in an increase in virtual conductance (Nadland et al., 2009, Shoemaker and Hughson, 1999, Shoemaker et al., 1998, Tschakovsky et al., 1996). At the onset of exercise in HDT, both VC and MBF adaptive responses were delayed but in HUT such adaptive responses were accelerated in both lower and higher power output exercises. It is important to note that the amplitude of the increase in VC was inversely dependent upon baseline perfusion pressure and vessel tone supported by Shoemaker et al., 1996.

The VC response at the onset of exercise in HDT was dependent almost or exclusively

on vasodilation because muscle pump could not contribute to the increase in VC due to the minimized emptying effect of the veins during muscle contractions and the lack of a hydrostatic column in this position (Saunders and Tschakovsky, 2004, Tschakovsky et al., 1996). A greater local accumulation of vasodilator metabolites probably occurred taking longer time to achieve the required vasodilation as indicated by the slower rate of increase of VC to reach steady-state (Figure 7.3).

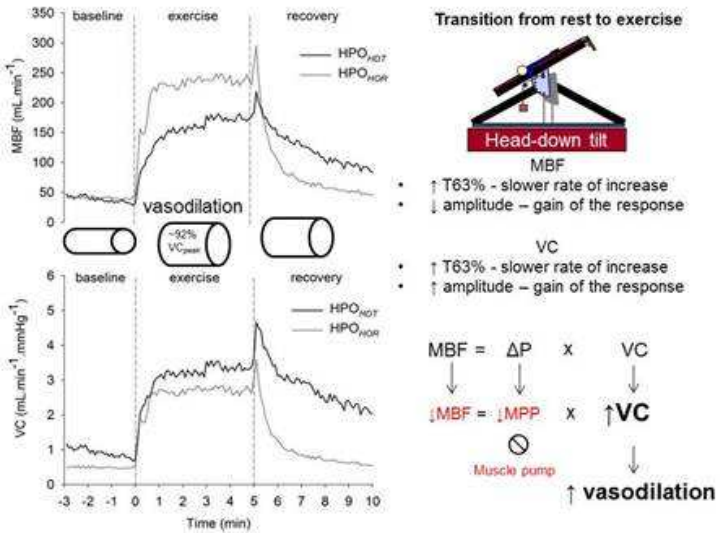

Figure 7.3: Schematic illustration of the VC and MBF responses in HDT during exercise. In HDT, VC and MBF responses at the onset of exercise were delayed due to the lack of contribution of the muscle pump and the greater vasodilation required in this position due to the greater accumulation of vasodilator metabolites.

In HUT, the faster VC response at the onset of exercise reflected the combined effects of vasodilation and muscle pump action. In this position, the more effective muscle pump contributed to the increase in MBF (Nadland et al., 2009, Laughlin and Joyner, 2003, Folkow et al., 1971) by the enhanced perfusion pressure gradient across the working muscle with less extent of vasodilation (Nadland et al., 2009) increasing virtual conductance (Nadland et al., 2009, Shoemaker and Hughson, 1999, Shoemaker et al., 1998, Tschakovsky et al., 1996). Lower VC values in HUT suggested that less accumulation of vasodilator metabolites were required to achieve the desired increase in VC resulting in shorter time to reach steady-state (Figure 7.4).

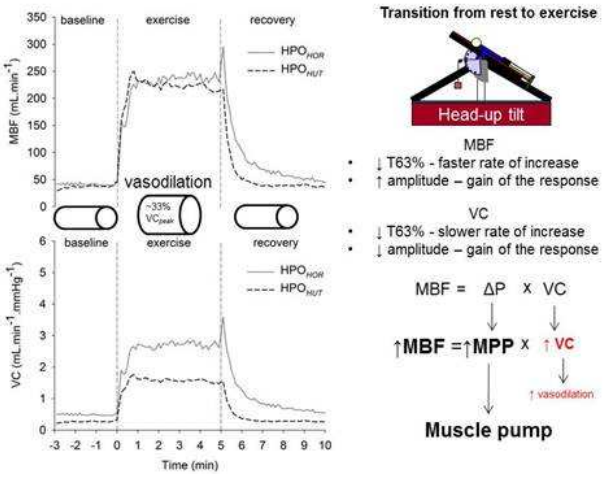

Figure 7.4: Schematic illustration of the VC and MBF responses in HUT during exercise. In HUT, VC and MBF responses at the onset of exercise were accelerated due to the combined effects of more efficient muscle pump and less requirement of vasodilation due to lower accumulation of vasodilator metabolites.

7.3.2 Exercise

The majority of the effects of increased MPP probably occurred in the first phase of blood flow adaptation described previously, thus, during the second phase of exercise the additional increase in MBF was the result of further increases in VC. VC and MBF responses were different between body positions and work rates as a consequence of altered MPP alone or with the combination between altered MPP and F_IO_2. During LPO_{HDT}, the higher VC response reached ∼80% VC_{peak} in normoxia and 85%VC_{peak} in hypoxia reflecting a greater demand for vasodilation. The recruitment of greater VC in normoxia and hypoxia fully compensated for the reduced MPP during LPO_{HDT} to maintain MBF and therefore DO_2. During HPO_{HDT} in normoxia, VC increased even further than during LPO_{HDT}, but MBF was reduced compared with HPO_{HOR} and HPO_{HUT} indicating that the increase in VC could not counteract the reduction in MPP. The recruitment of VC during HPO_{HDT} reached ∼92% VC_{peak} in normoxia indicating that the maximal vasodilatory capacity was almost reached. During HPO_{HDT} in hypoxia, the combined effects of reduced MPP and F_IO_2 were counteracted by the greater recruitment of VC to maintain MBF and DO_{2est}. However, the analysis of the relationship between MBF and EMG activity (EMG_{index}) demonstrated that MBF was reduced during HPO_{HDT} in hypoxia restricting the increase in MBF limiting DO_{2est} despite the greater VC response indicating that the ability to

compensate was exceeded as indicated by the achievement of the maximal vasodilatory capacity ($\sim$100%VC_{peak}).

The increase in VC during LPO_{HDT} and HPO_{HDT} in normoxia and hypoxia was probably due to the greater accumulation of metabolites promoting vasodilation of the resistance arterioles as the muscle pump does not contribute to the change in MBF in this position (Saunders and Tschakovsky, 2004, Tschakovsky et al., 1996) (Figure 7.3). The lower MBF in both gas breathing conditions indicated that DO_2 was insufficient to meet the demands for aerobic metabolism increasing the metabolic stress of exercise as suggested by the higher EMG activity during HPO_{HDT} in both normoxia and hypoxia.

The higher post-exercise VC in HDT indicated that higher concentration of vasodilator metabolites still present after cessation of exercise resulting in higher MBF suggesting inadequacy of flow and DO_2 in the exercise phase during normoxia and hypoxia. The greater MBF observed during recovery probably represented the repayment of the O_2 deficit accumulated at the onset of exercise as consequence of the slow rate of adaptation and the higher metabolic demand during HPO_{HDT} as suggested by the higher EMG activity which was consistent with challenged aerobic metabolism. The higher post-exercise VC in HDT also indicated that local peripheral reflexes are operating to maintain the vasodilation requirements after the cessation of exercise.

The slower adaptation of MBF during conditions with reduced MPP indicated inadequacy of flow limiting O_2 availability following the onset of exercise. As a consequence the adaptation of oxidative metabolism would be slow resulting in greater O_2 deficit requiring a greater breakdown of phosphocreatine (PCr) increasing intracellular inorganic phosphate concentrations (Pi) and also accumulation of metabolic by products such as ADP, H^+ and lactate. This would in turn indicate a relative increased metabolic stress of the exercise and advance the onset of muscle fatigue.

During LPO_{HUT}, the VC response was blunted reaching only $\sim$20% VC_{peak} in normoxia and hypoxia, while in HPO_{HUT} VC response reached $\sim$30%VC_{peak} in normoxia and hypoxia, reflecting the lower demand for vasodilation with elevated MPP even with reduced SaO_2. The blunted VC response in normoxia and hypoxia was probably caused by less accumulation of vasodilator metabolites in the resistance arterioles due to more effective contribution of the muscle pump to the increase in MBF (Nadland et al., 2009, Laughlin and Joyner, 2003, Folkow et al., 1971) (Figure 7.4). The increase in MBF was achieved by vasodilation and the enhanced perfusion pressure gradient across the working muscle resulting in an increase in virtual conductance (Nadland et al., 2009, Shoemaker and Hughson, 1999, Shoemaker et al., 1998, Tschakovsky et al., 1996).

During LPO_{HUT} in normoxia and hypoxia, VC was constrained reducing MBF, but the

post-exercise recovery suggested that both MBF and DO_2 were adequate. During HPO_{HUT} in normoxia and hypoxia, the post-exercise recovery suggested that both MBF and DO_2 were adequate and probably sufficient to maintain relatively low local concentrations of vasodilatory metabolites. The lower VC values in HUT during the post-exercise recovery also indicated that local peripheral reflexes are operating again after the cessation of exercise. In HUT, with elevated MPP, the accelerated adaptation of VC and MBF would increase O_2 availability following the onset of exercise resulting in faster adaptation of MBF and oxidative metabolism with consequent lower O_2 deficit and less accumulation of the metabolic by products during exercise in this body position.

7.3.3 Hypoxic Exercise

During hypoxic exercise in all body positions, the changes in VC caused proportional changes in MBF sufficient to maintain DO_2 at the same level as normoxia for both lower and higher power outputs. The mechanisms accounting for the match between MBF to DO_2 to the metabolic demand during exercise with constant work rate was accomplished through the increase in VC due to vasodilatory mechanisms in response to the reduction in SaO_2 caused by hypoxia.

The reduction of F_IO_2 involved decrease in SaO_2 and probably CaO_2 and PaO_2 affecting O_2 availability decreasing cellular PO_2 stimulating vasodilatory responses (Calbet, 2000). The reduced O_2 is probably sensed by the red blood cells stimulating the release of ATP from the erythrocytes mediating vasodilatory responses (Ellsworth et al., 1995) via nitric oxide (NO) and/or endothelium-derived-hyperpolarization-factor (EDHF) and/or release of NO from S-nitrosohemoglobin due to hemoglobin deoxygenation relaxing the vascular smooth muscle (Calbet, 2000, Stamler et al., 1997, Jia et al., 1996).

The accumulation of adenosine in hypoxia could activate the adenosine-sensitive K^+ channels enhancing the release of K^+ from the active muscle fiber and elevating the interstitial K^+ concentrations (Allen et al., 2008). The adenosine monophosphate-activated protein kinase (AMPK) is probably activated in hypoxic conditions stimulating the release of NO and PG relaxing the vascular smooth muscle (Fisslthaler and Fleming, 2009, Towler and Hardie, 2007). Other vasoactive factors released during hypoxic exercise, such as CO_2, H^+, lactate probably also contribute to mediate the vasodilatory response to increase VC (Calbet, 2000) (Figure 7.5). The compensatory adjustments in VC and MBF during hypoxia probably minimized the risk of mismatch between DO_2 and metabolic demand during exercise (Calbet et al., 2009, Calbet, 2000).

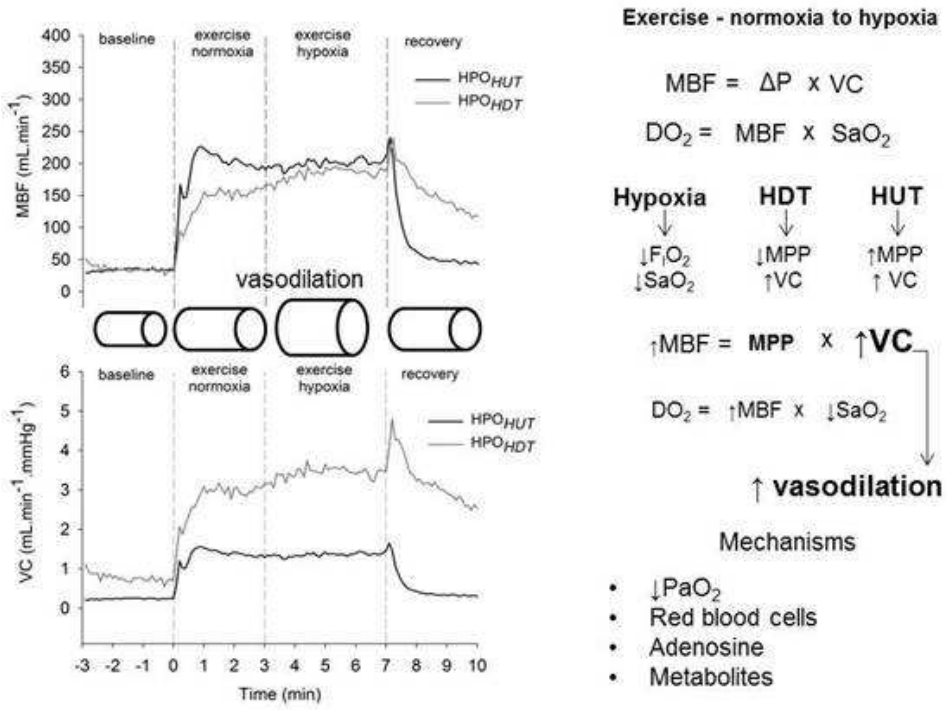

Figure 7.5: Schematic illustration of the mechanisms probably involved in VC and MBF responses in HDT and HUT during exercise in normoxia and hypoxia.

7.4 Conclusion

In conclusion, this thesis provided evidence that at rest in HDT, the increase in VC fully compensated for the reduction of MPP to maintain MBF. However, during HUT, the decrease in VC reduced resting MBF. The mechanisms accounting for these responses involved local peripheral and central cardiovascular reflexes. Following the onset of lower and higher power output exercises, MBF and VC responses were delayed during HDT but accelerated during HUT. In the steady-state during LPO_{HDT}, the adaptations in VC completely compensated for the reduction in MPP maintaining MBF to the exercising muscles. However, during HPO_{HDT}, the ability to recruit increases in VC was limited, compromising MBF. In HUT, during lower and higher power output exercises, VC was blunted but sufficiently high to maintain MBF. In LPO_{HDT}, the combined effects of reduced MPP and hypoxia were counteracted by the increase in VC to sufficiently increase MBF to maintain DO_{2est}. However, in HPO_{HDT} in hypoxia, VC was not able to fully compensate for the reduction of MPP and SaO_2 due to a functional limitation in the recruitment of VC (reached maximal vasodilatory capacity) resulting in restricted MBF and DO_{2est}. During lower and higher power output exercise in HUT under hypoxia, VC was blunted but sufficient great to increase MBF to maintain the DO_{2est}. Together, these findings indicated that during LPO_{HDT} in normoxia or hypoxia VC increased to maintain MBF and DO_{2est}, but during HPO_{HDT}, a functional limitation for recruitment of VC constrained MBF and DO_{2est} in normoxia and hypoxia as demonstrated by the relationship between MBF/EMG_{index}. The elevated VC, MBF and DO_{2est} post-exercise responses in HDT also support the inadequacy of MBF and DO_2 during the exercise phase. Elevated muscle electromyograpic signals in normoxia and hypoxia during HPO_{HDT} were consistent with challenged aerobic metabolism indicating relative increased metabolic stress of the exercise that would advance the onset of muscle fatigue.

7.5 Future Directions

The studies in this thesis were designed to investigate the adaptations of VC and MBF and their consequences for DO_2 under conditions with altered MPP alone at rest and exercise or associated with F_IO_2 during exercise through non-invasive measurements. Following are some considerations for future studies.

The results of Chapter 3 demonstrated that VC increased to fully compensate the reduction in MPP (HDT) to maintain MBF, but with elevated MPP, VC response was reduced as well as MBF. The HOR and HDT positions used as an experimental model in this thesis could be combined with lower body negative pressure (LBNP). LBNP would increase arterial-venous pressure gradient affecting resting VC and MBF responses. The comparison between HOR and HDT positions with and without LBNP will provide additional details about the regulation of resting VC and MBF under altered perfusion pressure gradient. Furthermore, LBNP could be applied with the same exercise experimental design of this thesis to investigate the adaptations of VC, MBF and DO2 at the onset and steady-state exercise. LBNP would increase perfusion pressure gradient during exercise in different body positions affecting the changes in VC, MBF and DO_2 responses. This approach would provide additional information about the adaptive responses and regulation of VC, MBF and DO_2 at the onset and steady-state exercise.

Beat-by-beat analysis on HR, BP, MBF, local and total vascular resistances typically present oscillatory patterns in distinct frequencies (Ichinose et al., 2004, O'Leary, Shoemaker, Edwards, and Hughson, 2004). The cardiovascular fluctuations may result from neural autonomic modulatory activity over the heart and blood vessels or from autoregulatory responses arising from different regions in the arterial and arteriolar trees (Ichinose et al., 2004, O'Leary et al., 2004, Akselrod et al., 1985). The transfer function analysis could be used in the present data to explore the dynamic interactions between blood pressure and vascular resistance and static and dynamic cardiac baroreflex responses to changes in body position. This would provide more information about systemic and regional circulatory adjustments to postural challenges.

This thesis was not designed to deal with the relationship between MBF and at the onset and steady-state exercise. Previous investigation reported that VO_{2mus} at the onset of forearm exercise was dependent on the MBF dynamic response (Hughson et al., 1996). Positioning of the arm above heart level (Hughson et al., 1996), alterations in O_2 concentration in the gas breathing by hypoxia (MacDonald et al., 1998) slowed the rate of MBF and adaptation. In contrast, with the arm below heart level (Hughson et al., 1996), MBF and kinetics were accelerated at the onset of exercise. Further investigations applying the same

experimental models performed in this thesis during exercise combined with simultaneous measurements of VO_{2mus} are necessary to determine the relationship between changes in VC, MBF and dynamic responses at the onset and steady-state exercise providing more information about DO_2 and O_2 utilization. Such challenge could be accomplished by direct measurements of blood samples and pressure via transducers. These techniques allow measurements of PO_2, , hematocrit, plasma concentration of lactate, SaO_2, CaO_2, venous O_2 content (CvO_2), arterio-venous O_2 content difference (a-vO_2diff) as well as estimation of O_2 extraction, DO_2 and calculation of VO_{2mus}.

Also, near infrared spectroscopy (NIRS), a non-invasive technique, can be used to measure muscle oxygenation during exercise. However, MacDonald, Tarnopolsky, Green, and Hughson, 1999 reported that NIRS did not provide the same information as femoral venous blood samples at the onset of exercise. Therefore, the validity and reproducibility of NIRS measurements of muscle oxygenation at the onset of small muscle mass need to be determined prior its application. Alternatively, nuclear magnetic resonance spectroscopy (NMRS) is also non-invasive technique that has been used to measure PCr, Pi, ADP, ATP, pH responses during exercise. Previous investigations using NMRS had shown similar time course of change in intramuscular metabolism during dynamic calf exercise and during cycling exercise (Barstow, Buchthal, Zanconato, and Cooper, 1994). Together, the exercise models present in this thesis associated with the techniques previously mentioned would permit determination of the relationship between the adaptive responses of VC, MBF and VO_{2mus} to different work rates, the control mechanisms regulating such responses and their impact on muscle metabolism.

APPENDICES

Appendix A

Repeatability of the muscle blood flow response at rest and exercise during body tilt using Doppler ultrasound

A.1 Resting

Table A.1: MBV, AD_{pop}, MBF and VC during baseline, HDT, HOR, and HUT with respect to coefficient of variation during the rest protocol.

Variables	days	Baseline	HDT	HOR	HUT
	1	2.71 ± 0.20	2.36 ± 0.18	2.64 ± 0.21	1.56 ± 0.09
MBV ($cm \cdot s^{-1}$)	2	2.79 ± 0.25	2.45 ± 0.17	2.66 ± 0.22	1.54 ± 0.13
CV (%)		9.46 ± 1.53	6.62 ± 1.06	7.48 ± 1.39	9.65 ± 1.41
	1	0.64 ± 0.06	0.64 ± 0.06	0.64 ± 0.06	0.64 ± 0.06
AD_{pop} (cm)	2	0.64 ± 0.06	0.64 ± 0.06	0.64 ± 0.06	0.64 ± 0.06
CV (%)		1.07 ± 0.24	1.07 ± 0.24	1.09 ± 0.24	1.13 ± 0.23
	1	52.11 ± 3.98	45.78 ± 3.71	51.92 ± 4.52	29.93 ± 1.55
MBF ($mL \cdot min^{-1}$)	2	52.52 ± 5.33	46.90 ± 3.20	51.18 ± 4.65	30.06 ± 2.95
CV (%)		8.01 ± 1.40	6.02 ± 0.92	7.32 ± 1.31	10.11 ± 1.53
	1	0.60 ± 0.05	1.01 ± 0.10	0.54 ± 0.03	0.21 ± 0.01
VC ($mL \cdot min^{-1} \cdot min^{-1}$)	2	0.59 ± 0.06	0.99 ± 0.07	0.56 ± 0.05	0.21 ± 0.02
CV (%)		7.60 ± 1.39	6.12 ± 1.15	6.59 ± 1.19	10.41 ± 1.74

Values are mean ± SD of 15 participants in two days of testing. Muscle blood flow velocity (MBV), popliteal arterial diameter (AD_{pop}), muscle blood flow (MBF), vascular conductance (VC), horizontal (HOR), head-down tilt (HDT), head-up tilt (HUT) and coefficient of variation (CV).

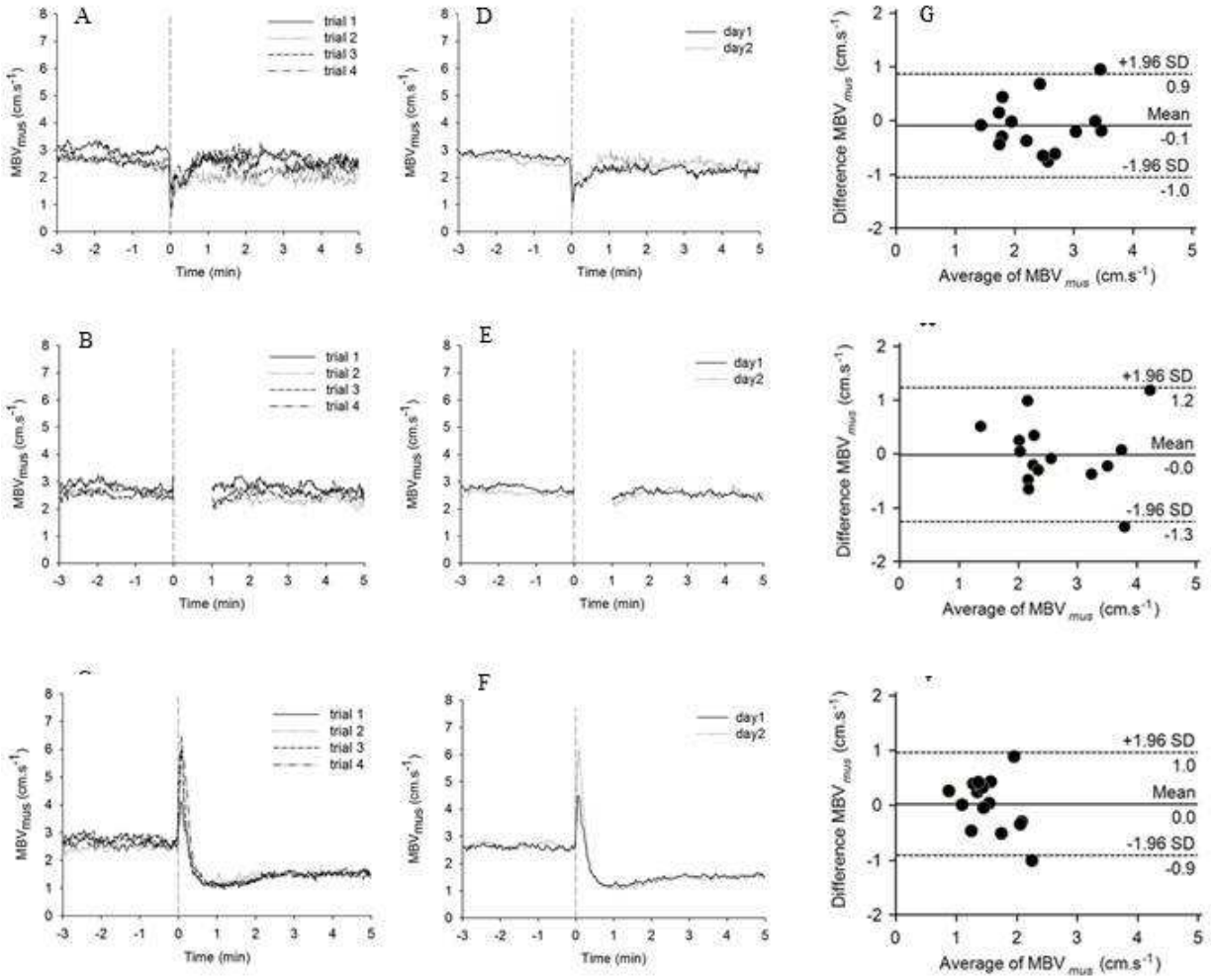

Figure A.1: The mean muscle blood flow velocity (MBV) responses to changes in body position across four different trials for HDT (A), HOR (B) and HUT (C), two days of testing for HDT (D), HOR (E) and HUT (F), and Bland-Altman Plots during HDT (G), HOR (H), and HUT (I) comparing two days of testing at rest. Lines indicate group response (mean ± SE, n =15). Dashed vertical line indicates transition from baseline to HDT and baseline to HUT. Solid horizontal lines show mean values and dashed horizontal lines represent 95% confidence limits.

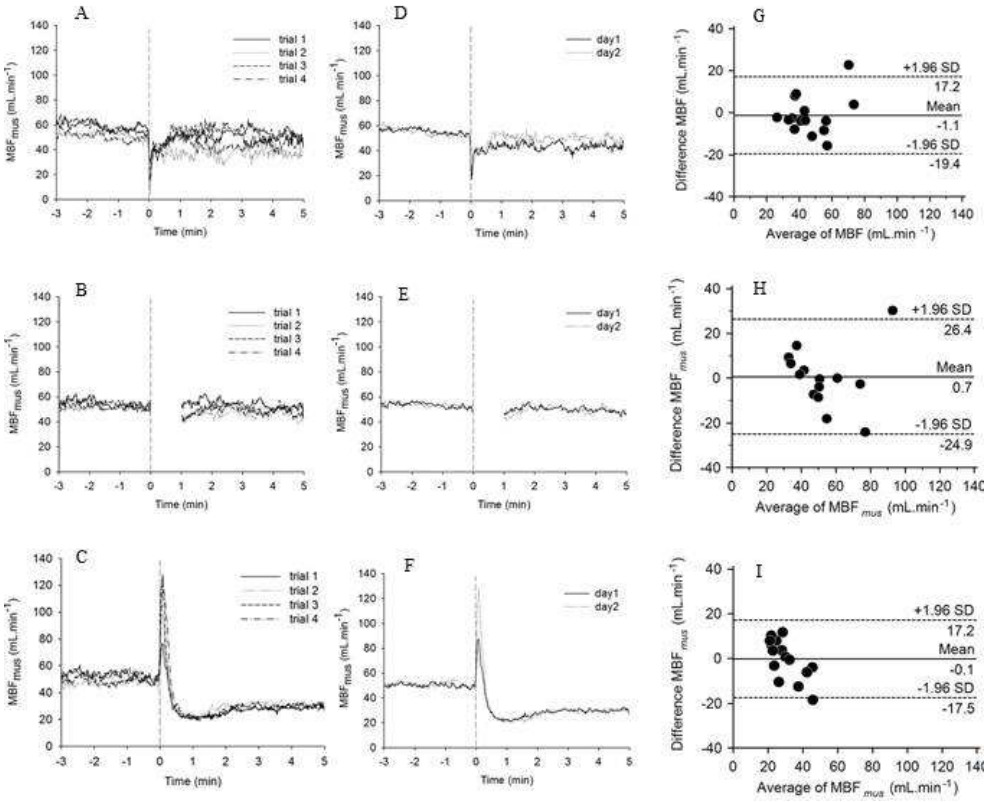

Figure A.2: The mean muscle blood flow (MBF) responses to changes in body position across four different trials for HDT (A), HOR (B) and HUT (C), two days of testing for HDT (D), HOR (E) and HUT (F), and Bland-Altman Plots during HDT (G), HOR (H), and HUT (I) comparing two days of testing at rest. Lines indicate group response (mean $\pm$ SE, n =15). Dashed vertical line indicates transition from baseline to HDT and baseline to HUT. Solid horizontal lines show mean values and dashed horizontal lines represent 95% confidence limits.

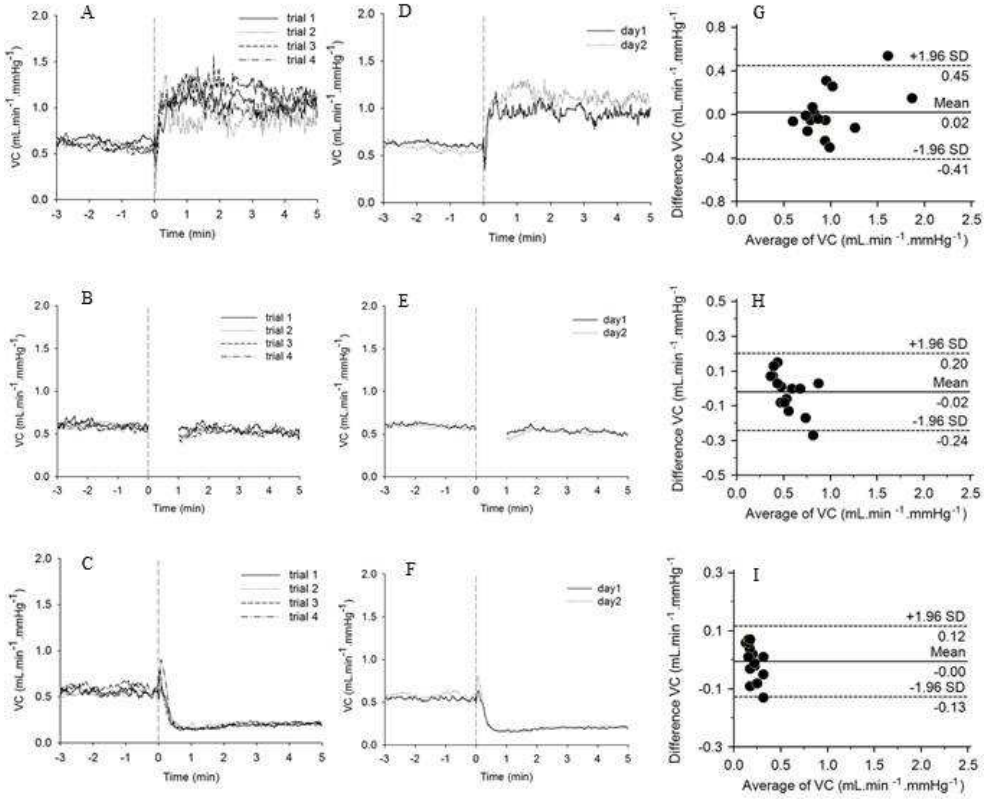

Figure A.3: The mean vascular conductance (VC) responses to changes in body position across four different trials for HDT (A), HOR (B) and HUT (C), two days of testing for HDT (D), HOR (E) and HUT (F), and Bland-Altman Plots during HDT (G), HOR (H), and HUT (I) comparing two days of testing at rest. Lines indicate group response (mean $\pm$ SE, n =15). Dashed vertical line indicates transition from baseline to HDT and baseline to HUT. Solid horizontal lines show mean values and dashed horizontal lines represent 95% confidence limits.

The Bland-Altman analyses showed that the 95% limits of agreement between the two days of testing ranged from -0.060 to 0.062 for HDT, -0.045to 0.047 for HOR and -0.047 to 0.043 for HUT. These results indicated no deviation of the mean value from 0, and relatively small variability about the mean.

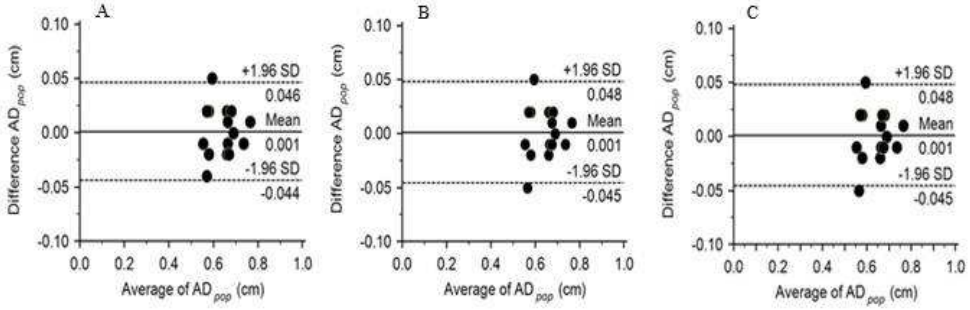

Figure A.4: Bland-Altman Plots for popliteal artery diameter (AD_{pop}) during HDT (A), HOR (B), and HUT (C) comparing two day of testing at rest. Solid horizontal lines show mean values and dashed horizontal lines represent 95% confidence limits.

A.2 Transition from rest to exercise (EX)

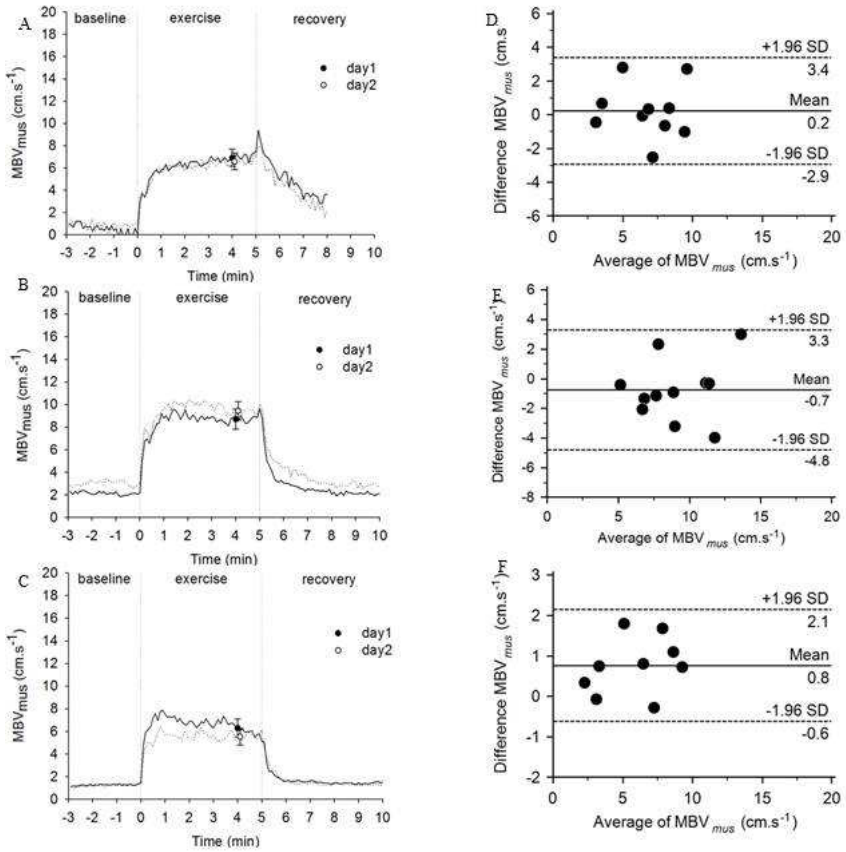

Figure A.5: The mean muscle blood flow velocity (MBV) responses to dynamic plantar flexion exercise across two days of testing during LPO_{HDT} (A), LPO_{HOR} (B) and LPO_{HUT} (C). Lines indicate group response and symbols indicate the points at which repeatability were performed. Dashed vertical lines indicate the start and cessation of exercise. Data are the mean ± SE analysed over 6 seconds time bins including contraction and relaxation phases of the duty cycles. Bland-Altman plots during LPO_{HDT} (D), LPO_{HOR} (E), and LPO_{HUT} (F) over the last 2min of exercise. Solid horizontal lines show mean values and dashed horizontal lines represent 95% confidence limits.

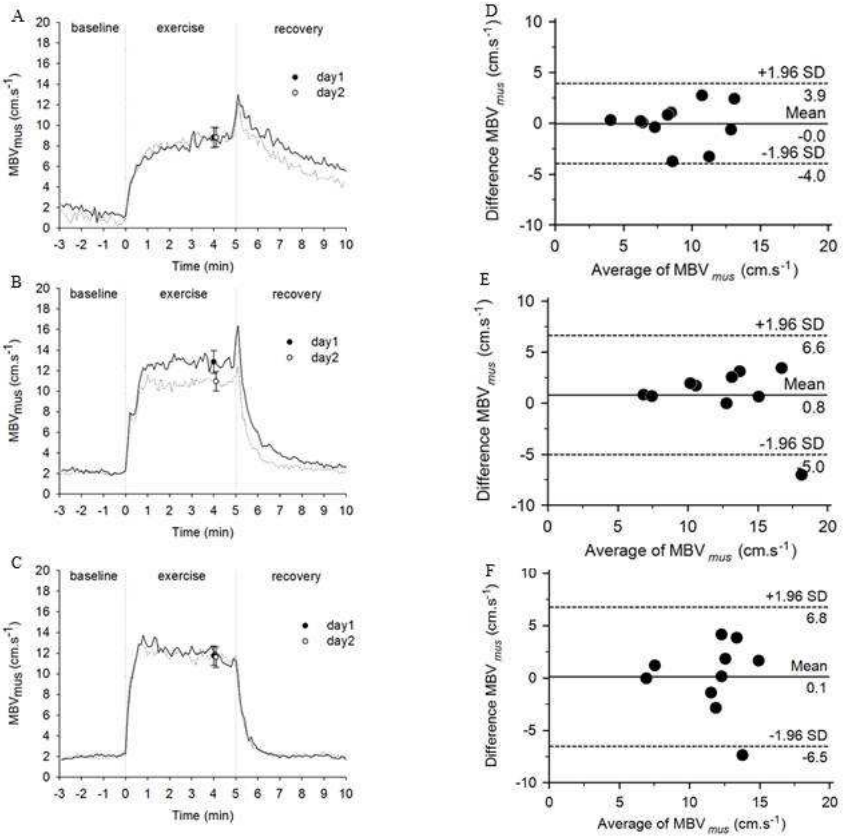

Figure A.6: The mean muscle blood flow velocity (MBV) responses to dynamic plantar flexion exercise across two days of testing during HPO_{HDT} (A), HPO_{HOR} (B) and HPO_{HUT} (C). Lines indicate group response and symbols indicate the points at which repeatability were performed. Dashed vertical lines indicate the start and cessation of exercise. Data are the mean ± SE analysed over 6 seconds time bins including contraction and relaxation phases of the duty cycles. Bland-Altman plots during HPO_{HDT} (D), HPO_{HOR} (E), and HPO_{HUT} (F) over the last 2min of exercise. Solid horizontal lines show mean values and dashed horizontal lines represent 95% confidence limits.

Table A.2: CV for MBV during lower power output exercise in (LPO_{HDT}), (LPO_{HOR}) and (LPO_{HUT}) and higher power output exercise in (HPO_{HDT}), (HPO_{HOR}) and (HPO_{HUT}) conditions during transitions from rest to exercise.

	days	1min	2min	3min	4min	5min
LPO_{HDT} (n=8)	Day1	5.3 ± 0.6	7.0 ± 0.9	7.1 ± 0.9	8.0 ± 1.1	8.2 ± 0.9
	Day2	5.3 ± 0.7	7.4 ± 0.9	8.4 ± 1.1	8.0 ± 1.0	8.2 ± 1.0
	CV (%)	6.0 ± 1.3	7.2 ± 1.8	7.1 ± 2.7	8.5 ± 0.9	3.1 ± 0.8
LPO_{HOR} (n=11)	Day1	7.6 ± 0.6	9.1 ± 0.8	8.8 ± 0.9	8.6 ± 0.9	8.8 ± 0.8
	Day2	8.4 ± 0.6	10.1 ± 0.8	10.0 ± 0.9	9.4 ± 0.7	9.5 ± 0.7
	CV (%)	7.2 ± 1.7	7.7 ± 1.5	9.4 ± 1.8	8.5 ± 1.5	6.9 ± 1.5
LPO_{HUT} (n=9)	Day1	6.8 ± 0.7	7.0 ± 0.9	6.6 ± 0.9	6.7 ± 1.0	5.8 ± 0.7
	Day2	5.3 ± 0.6	5.6 ± 0.8	5.7 ± 0.9	5.4 ± 0.7	5.6 ± 0.8
	CV (%)	11.3 ± 2.0	11.9 ± 1.4	9.3 ± 1.7	8.3 ± 1.4	5.9 ± 1.1
HPO_{HDT} (n=11)	Day1	5.7 ± 0.6	7.6 ± 0.9	8.1 ± 0.9	8.5 ± 0.9	9.5 ± 1.0
	Day2	5.9 ± 0.7	7.9 ± 0.9	8.4 ± 0.8	8.8 ± 0.9	9.3 ± 0.8
	CV (%)	7.8 ± 1.6	7.1 ± 1.3	5.2 ± 1.4	6.4 ± 1.8	6.8 ± 1.2
HPO_{HOR} (n=10)	Day1	9.9 ± 0.8	12.6 ± 1.2	13.0 ± 1.1	12.8 ± 1.1	13.2 ± 1.0
	Day2	9.5 ± 0.9	11.2 ± 1.1	11.6 ± 1.2	12.2 ± 1.4	12.2 ± 1.4
	CV (%)	4.6 ± 0.9	5.4 ± 0.8	7.4 ± 0.8	6.8 ± 1.2	8.5 ± 1.5
HPO_{HUT} (n=10)	Day1	11.3 ± 0.8	12.5 ± 1.0	12.2 ± 1.0	12.2 ± 1.1	11.0 ± 0.8
	Day2	11.2 ± 1.0	12.0 ± 1.1	11.9 ± 1.1	11.7 ± 1.0	11.5 ± 1.0
	CV (%)	8.8 ± 2.1	9.3 ± 2.4	8.8 ± 2.3	8.7 ± 2.1	7.7 ± 1.7

Values are mean ± SE of two days of testing and mean coefficient of variation (CV). Muscle blood flow velocity (MBV), lower power output exercise in head-down tilt (LPO_{HDT}), lower power output exercise in horizontal (LPO_{HOR}), lower power output exercise in head-up tilt (LPO_{HUT}), higher power output exercise in head-down tilt (HPO_{HDT}), higher power output exercise in horizontal (HPO_{HOR}) and higher power output exercise in head-up tilt (HPO_{HUT}).

A.3 Exercise experiments under altered O2 inspired fraction (hypoxia)

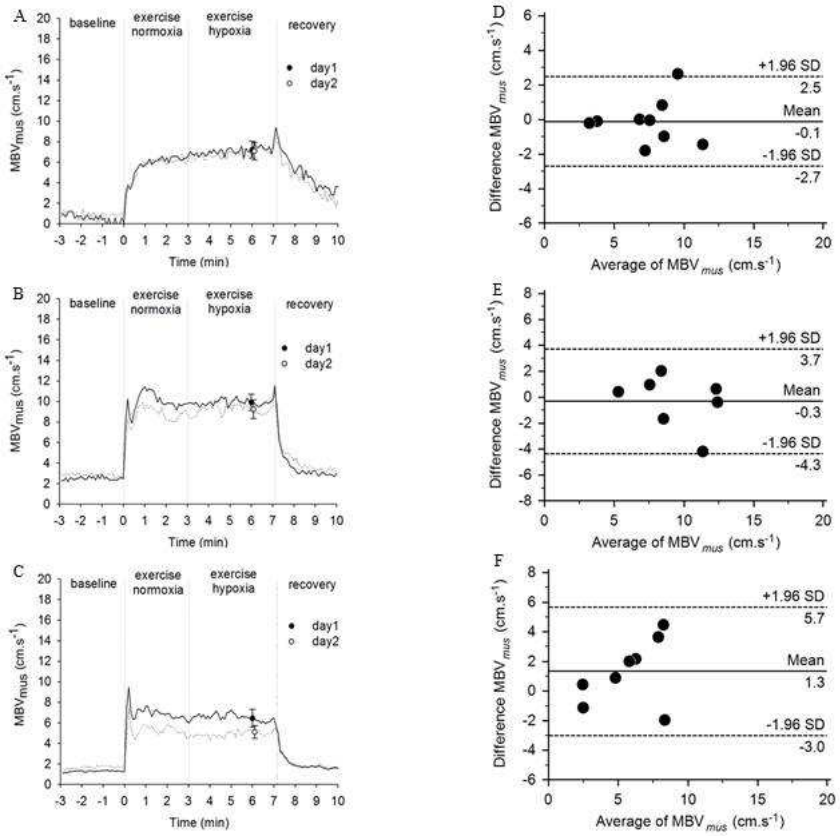

Figure A.7: The mean muscle blood velocity (MBV) responses to dynamic plantar flexion exercise across two days of testing during LPO_{HDT} (A), LPO_{HOR} (B) and LPO_{HUT} (C) under altered F_IO_2 (hypoxia). Lines indicate group response and symbols indicate the points at which Bland-Altman plots were performed. Lines indicate group response and symbols indicate the points at which repeatability were performed. Dashed vertical lines indicate the start and cessation of exercise. Data are the mean ± SE analysed over 6 seconds time bins including contraction and relaxation phases of the duty cycles. Bland-Altman plots during LPO_{HDT} (D), LPO_{HOR} (E), and LPO_{HUT} (F) over the last 2min of exercise. Solid horizontal lines show mean values and dashed horizontal lines represent 95% confidence limits.

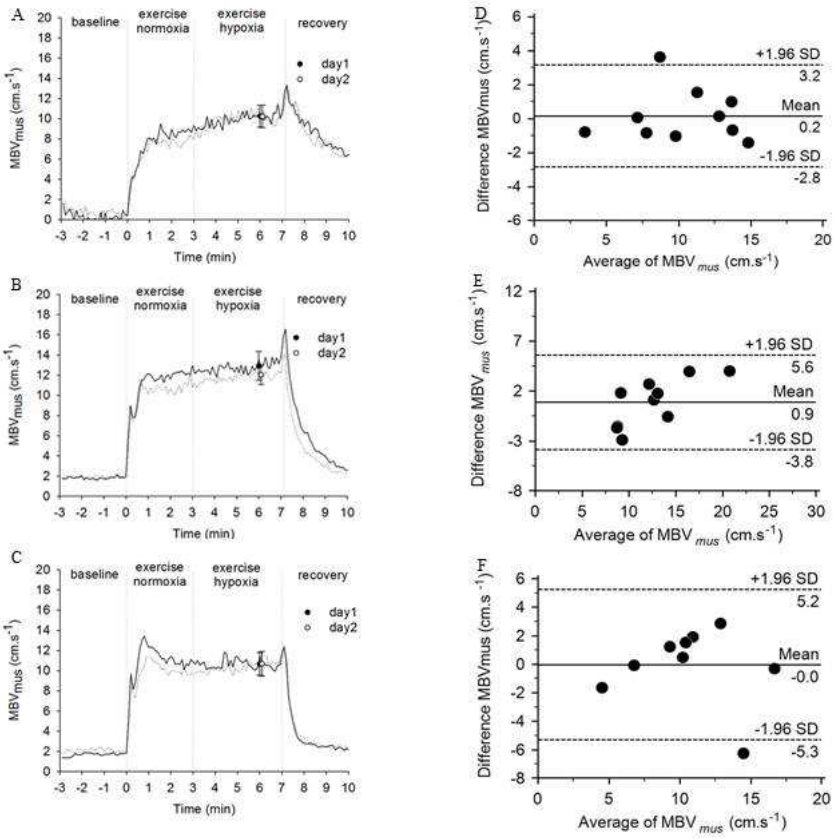

Figure A.8: The mean muscle blood velocity (MBV) responses to dynamic plantar flexion exercise across two days of testing during HPO_{HDT} (A), HPO_{HOR} (B) and HPO_{HUT} (C) under altered F_IO_2 (hypoxia). Lines indicate group response and symbols indicate the points at which Bland-Altman plots were performed. Lines indicate group response and symbols indicate the points at which repeatability were performed. Dashed vertical lines indicate the start and cessation of exercise. Data are the mean ± SE analysed over 6 seconds time bins including contraction and relaxation phases of the duty cycles. Bland-Altman plots during HPO_{HDT} (D), HPO_{HOR} (E), and HPO_{HUT} (F) over the last 2min of exercise. Solid horizontal lines show mean values and dashed horizontal lines represent 95% confidence limits.

Table A.3: CV for MBV during lower power output exercise in (LPO_{HDT}), (LPO_{HOR}) and (LPO_{HUT}) and higher power output exercise in (HPO_{HDT}), (HPO_{HOR}) and (HPO_{HUT}) conditions during hypoxic exercise.

	days	4min	5min	6min	7min
LPO_{HDT} (n=8)	Day1	6.8 ± 0.7	7.1 ± 0.8	7.0 ± 0.7	7.5 ± 0.0
	Day2	6.6 ± 0.7	6.7 ± 0.8	6.9 ± 0.8	7.3 ± 0.9
	CV (%)	6.5 ± 2.0	7.8 ± 2.2	5.5 ± 1.9	7.3 ± 2.8
LPO_{HOR} (n=11)	Day1	9.7 ± 1.0	10.0 ± 1.0	10.1 ± 0.9	9.7 ± 0.9
	Day2	8.9 ± 0.8	9.3 ± 0.8	9.5 ± 1.1	9.0 ± 1.0
	CV (%)	12.4 ± 2.1	11.1 ± 2.1	8.6 ± 2.1	7.3 ± 2.8
LPO_{HUT} (n=9)	Day1	6.6 ± 1.1	6.8 ± 1.0	6.7 ± 1.1	6.1 ± 0.9
	Day2	4.8 ± 0.7	4.8 ± 0.7	5.1 ± 0.7	5.1 ± 0.7
	CV (%)	17.6 ± 2.0	16.4 ± 1.4	15.3 ± 2.3	12.3 ± 1.7
HPO_{HDT} (n=11)	Day1	9.2 ± 0.9	9.8 ± 1.0	10.2 ± 1.1	10.3 ± 1.1
	Day2	9.1 ± 1.0	10.2 ± 1.1	10.4 ± 1.2	10.0 ± 1.0
	CV (%)	5.8 ± 1.5	3.4 ± 0.7	4.1 ± 0.9	5.5 ± 1.5
HPO_{HOR} (n=10)	Day1	12.4 ± 1.3	12.7 ± 1.4	12.6 ± 1.4	13.2 ± 1.6
	Day2	11.3 ± 0.8	11.6 ± 0.9	12.2 ± 1.0	11.9 ± 0.9
	CV (%)	6.8 ± 1.0	7.4 ± 1.2	6.5 ± 1.2	8.4 ± 1.1
HPO_{HUT} (n=10)	Day1	10.5 ± 1.1	10.9 ± 1.2	10.7 ± 1.2	10.6 ± 1.2
	Day2	9.9 ± 1.1	10.1 ± 1.1	10.4 ± 1.3	11.0 ± 1.3
	CV (%)	8.6 ± 1.4	7.3 ± 1.3	8.0 ± 1.8	7.6 ± 1.9

Values are mean ± SE of two days of testing and mean coefficient of variation (CV). Muscle blood flow velocity (MBV), lower power output exercise in head-down tilt (LPO_{HDT}), lower power output exercise in horizontal (LPO_{HOR}), lower power output exercise in head-up tilt (LPO_{HUT}), higher power output exercise in head-down tilt (HPO_{HDT}), higher power output exercise in horizontal (HPO_{HOR}) and higher power output exercise in head-up tilt (HPO_{HUT}).

Appendix B

Equipment Design

B.1 Introduction

The equipment used in the current thesis was specifically designed and built to study the effect of altered arterial blood perfusion pressure on vascular conductance (VC) and muscle blood flow (MBF) at rest and exercise by placing the heart above or below the level of the muscle.

B.1.1 Tilt table and resting protocol

This custom built tilt table device placed participants horizontally in a prone (face down) position and permitted changes in body position to different angles during resting conditions while studying the VC and MBF responses of the popliteal artery. The participants rested on the table supported by a cushion and memory foam. A massage table head piece was connected to the table for the participants to place their faces permitting unobstructed vision and breathing. Adjustable shoulder blocks were placed at the top of the table. An arm resting apparatus was placed on each side of the table with shoulders and elbows in approximately 90°. An adjustable strap system was connected to the table and to two belts by metal clips. These adjustable belts were placed on the participants chest and hips to restrict sliding, minimize muscle activity, and provide comfort and security to the participants. A total of ten straps were used, with six straps connected to the metal clips in the chest belt and the other four straps connected to the metal clips in the hip belt. The table could be tilted up or down. The Figure B.1 represents the schematic illustration with numbers indicating the parts of the equipment.

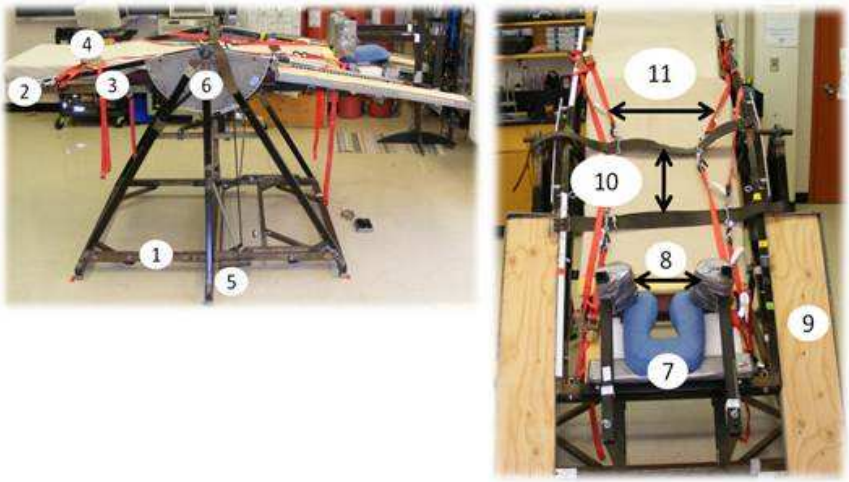

Figure B.1: Schematic illustration of the tilt table: (1) metal support; (2) metal frame; (3) wood support; (4) cushion and memory foam; (5) pedal system; (6) pivot point; (7) massage table head piece; (8) adjustable shoulder blocks; (9) arm resting apparatus; (10) belt system (chest and hips); and (11) strap system.

The angle of tilting was selected to promote changes in muscle perfusion pressure (MPP) by placing the heart below or above the level of the muscle. At 35° head-down tilt (HDT) MPP was reduced while in 45° head-up tilt (HUT) MPP was enhanced due to the effects of gravity. The Figure B.2 represents the angle of tilting used to change MPP.

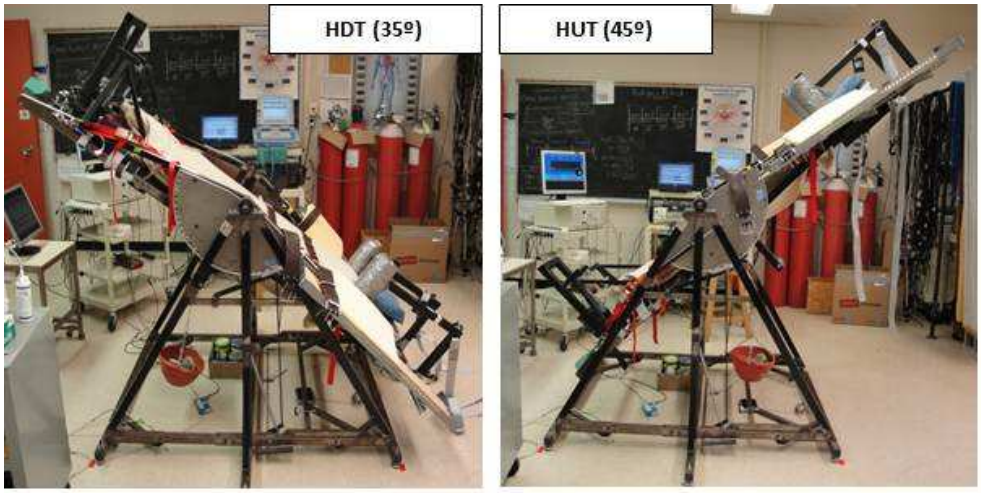

Figure B.2: Illustration of the tilt table during HDT at -35° angle (left side) and HUT at 45° angle (right side).

Participants assumed a prone position with their face in the massage table head piece. Additionally, shoulder blocks were adjusted according to participants height and their arms were placed on the resting apparatus located on the side of the table with the shoulders and elbows positioned at approximately 90°. Two belts, one located on the chest and the other on the hips, were used to secure the participants during testing. Straps were connected to the metal clips in the chest belt and hip belt and attached in the tilt table (Figure B.3).

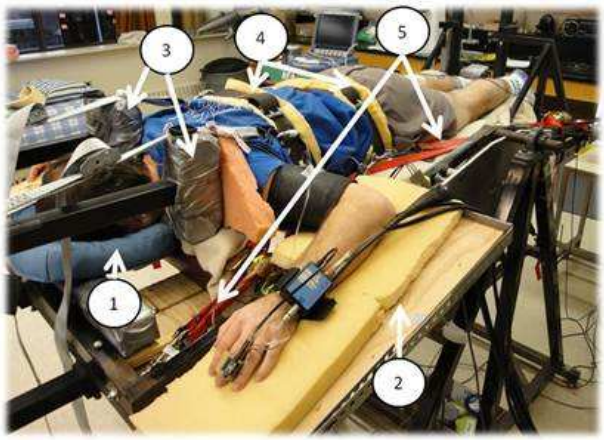

Figure B.3: Illustration of the custom tilt table device with the participant. Participants face on a (1) massage table head piece; (2) arm resting apparatus placed on the side of the table with the shoulders and elbows positioned at approximately 90°; (3) shoulder blocks adjusted according to participants height; (4) chest and hip belts worn by the participants, and (5) strap system attached in the tilt table to help to avoid, as much as possible, participant sliding and provide optimal comfort, security and stability.

B.1.2 Tilt table and exercise protocol

The tilt table custom device also allowed participants to perform isometric and dynamic plantar flexion exercises. The isometric plantar flexion exercise was used to measure the maximal voluntary contraction (MVC). For this purpose, a footplate was attached to the table via a footplate support. At the end of the footplate, there was a hook that connected the stainless steel cable with the footplate. This cable passed through three pulleys and it was connected to the load cell attached in the bottom of the metal frame of the table allowing MVC test to be performed (Figure B.4).

For dynamic plantar flexion exercise, the cable was connected to the weight system to promote the lifting of the load. The point of rotation was situated at the participants ankle to facilitate the plantar flexion exercise with range of motion between 0° and 45°. This range of motion was based on neutral position with foot at 90° to ankle, which represents 10 cm of distance moved between the beginning and the end of the plantar flexion. To ensure the correct range of motion a stopper was placed at the end of the table. In the stopper, a light system was built to promote visual feedback about the correct movement to the participants. When the back of the footplate contacted the stopper, the light was activated (turned on) indicating correct range of motion (Figure B.4).

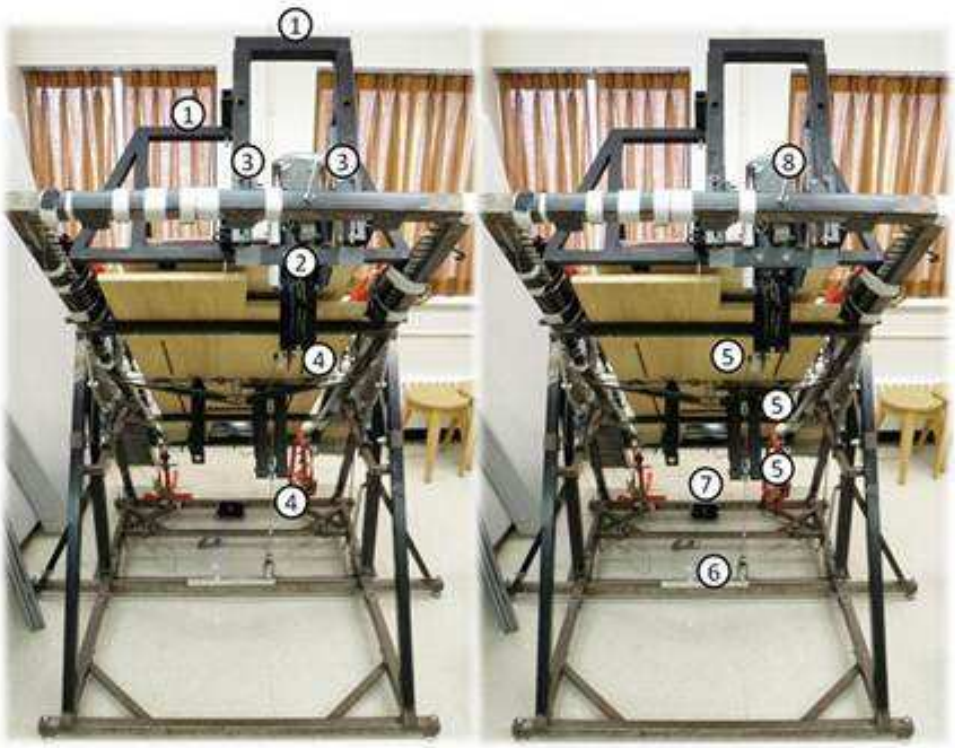

Figure B.4: Illustration of the tilt table connected to a footplate that allows participants to perform plantar flexion exercise and MVC tests. (1) footplate support; (2) footplate; (3) point of rotation (participants ankle); (4) hook used to attach the stainless steel cable with the footplate; (5) pulley system (3 pulleys); (6) load cell (MVC tests) or weight system (dynamic exercise tests); (7) light system (visual feedback about the correct range of motion); and (8) stopper placed at the end of the table.

Participants lay down on a tilt table in a prone position (face down). The footplate was connected to the tilt table allowing plantar flexion exercise to be performed. Participants foot was strapped on the footplate to ensure more stability during the motion and better contact between plantar surface of the foot and footplate (Figure B.5).

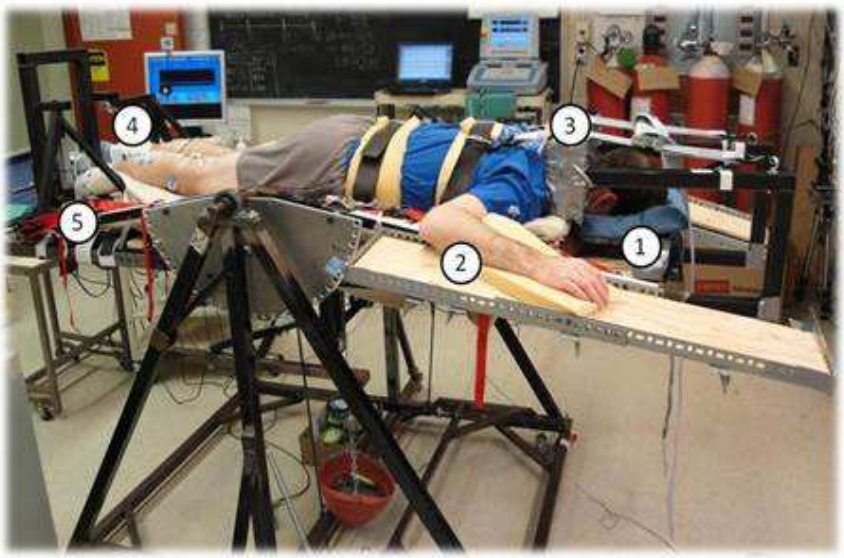

Figure B.5: Tilt table with the participants face on a (1) massage table head piece; (2) arm resting apparatus (shoulders and elbows positioned in approximately 90°); (3) shoulder blocks (adjusted according to participants height); (4) footplate connection on a tilt table (plantar flexion exercise); (5) footplate strap system (stability during motion and better contact between plantar surface of the foot and footplate).

However, it was noticed during a previous pilot study that the work output performed during HDT and HUT was similar at the neutral position (starting position at 90°), but was different during plantar flexion especially at the end of the range of motion due to the effects of gravity on work output. Therefore, more pilot tests were performed to detect how much gravity was affecting work output. Based on these previous pilot data, a correction factor for the workloads was used to account for the effects of gravity in HDT and HUT positions. As a result, the workload obtained in HOR was subtracted by 600g in HDT, then, if the load obtained in HOR was 3.0Kg, then, the load in HDT was 3.0Kg − 0.6Kg (600g) = 2.4Kg.Likewise, in HUT, 800g was added to the HOR value, then, the load in HUT was 3.0Kg + 0.8Kg (800g) = 3.8Kg. The Figure B.6 provided an example of the EMG activity comparison between HOR with HUTwithout correction factor (HUT-NCF) and HUT with correction factor.

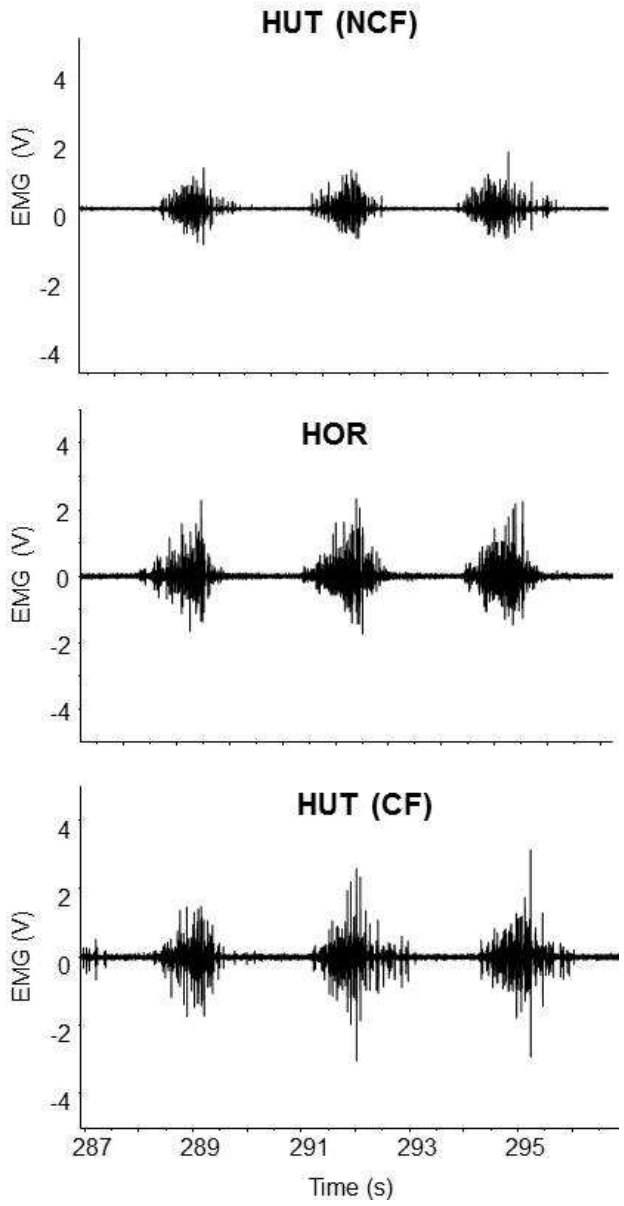

Figure B.6: Comparison of the EMG activity between horizontal (HOR, middle) compared to HUT without correction factor (HUT-NCF, top) and HUT with correction factor (HUT-CF, bottom).It was noticed that the EMG activity was similar between HOR and HUT when the correction factor was applied, but EMG activity was lower without the correction factor.

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