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THE EFFECT OF POSTURE ON MUSCLE ENDURANCE, FATIGUE AND BLOOD FLOW

Thesis submitted for the degree of Doctor in Philosophy at the University of Dublin, Trinity College.

Submitted December 2004

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III. DECLATION

I declare that this thesis is entirely my own work, with the exception of the curve fitting of the blood flow responses which was performed by Dr. Simon Green. This thesis has not been previously submitted as an exercise for a degree in this or any other university. Trinity College Dublin library may lend or copy this thesis on request.

Signature:.....December, 2004

Mikel Egaña Etxeberria, BSc (Physical Education and Sports); MSc (Exercise Physiology)

IV. LIST OF ABREVIATIONS

A	amplitude
ABS	absolute
ANOVA	analysis of variance
ADP	adenosine-diphosphate
ATP	adenosine-triphosphate
A-V O_2 difference	arterio-venous oxygen difference
beats.min^{-1}	beats per minute
BLac	blood lactate concentration: unit mM.l^{-1}
Ca^{2+}	calcium
cm	centimeter
CNS	central nervous system
CO	cardiac output
CO_2	carbon dioxide
ECG	electrocardiogram
EMG	eleoctromiogram
F_{max}	maximum force
g	gram
g	gravitational acceleration
GL	gastrocnemius lateralis
GM	gastrocnemius medialis
H_2	hydrogen
HR	heart rate
HR max	maximal heart rate
Hz	hertz
kg	kilogram
km.h^{-1}	kilometers per hour
l	liter
LBNP	low body negative pressure
LCD	liquid crystal display
l.min^{-1}	liter per minute
m	meter

MAP	mean arterial pressure
max	maximum
mg	milligram
min	minute
$ml.kg^{-1}.min^{-1}$	milliliter per kilogram per minute
ml	milliliter
mm	millimeter
mmHg	millimeter of mercury
MRT	mean response time
mV	minivolt
MVC	maximum voluntary contraction
n	number of subjects
N	newton
$N.s^{-1}$	newton per second
N_2	nitrogen
O_2	oxygen
PCr	phosphocreatine
PO_2	partial pressure of oxygen
Pi	inorganic phosphate
REL	relative
RER	respiratory exchange ratio
RMS	root mean square
rpm	revolution per minute: unit of cadence
s	second
SD	standard deviation
SUP	supine
SV	stroke volume
TD	time delay
TPR	total peripheral resistance
UPR	upright
V	volt
$\dot{V}CO_2$	rate of carbon dioxide production
$\dot{V}_E$	minute ventilation: unit $L.min^{-1}$

$\dot{V}O_2$	rate of oxygen uptake
$\dot{V}O_{2\ max}$	maximal oxygen uptake
vs	versus
W	watt: unit of power
wk	week
$W.kg^{-1}$	watt per kilogram
yr	year
%	percentage
°	degree

V. ABSTRACT

Tilting the body from the supine to an upright position increases the time on a maximal graded cycle test by ~15%. However, this effect during constant-load cycling is unknown, and in addition, it is also unknown if this postural effect is observed during exercise involving isolated muscle groups. Therefore, we tested the effect of upright versus supine posture on endurance during graded and high-intensity constant-load cycling exercise (Chapter 2) and on the strength, endurance, fatigue (Chapters 3 and 4) and blood flow (Chapter 5) during graded and high-intensity constant-load isometric plantar flexion exercise.

In the cycling study, subjects performed two graded tests and three high-intensity constant-load tests to the point of failure. During experiment 1 in Chapter 3, subjects performed three graded tests (0°, 47° and 90° tilt angles, intermittent contractions); and then during experiment 2, four constant-force tests (70% of maximum force, F_{max}; intermittent contractions, 0°, 32°, 47° and 67° tilt angles) to the point of failure. In Chapter 4-experiment 1, subjects performed eight constant force tests (maximum duration of 20 min) at four low to moderate intensities (30%, 40%, 50% and 60% F_{max}; intermittent contractions) and two tilt angles (0° and 67°); and then, during experiment 2, four constant force tests to the point of failure at two high intensities (80%, 90% F_{max}; intermittent contractions) and two tilt angles (0° and 67°). In Chapter 5, the kinetic response of the calf blood flow was assessed using venous occlusion plephysmography during four constant-force exercises (3 bouts of 6 minutes in each condition) at two intensities (30 and 70%F_{max}) and two tilt angles (0° and 67°).

During graded and constant-load cycling exercise, performance time was greater ($p<0.05$) in the upright compared with supine posture, (17.9±3.5 vs. 16.1±3.1 min for graded; 13.2±8.7 vs. 5.2±1.9 min for constant-load) but this improvement in the upright posture was 10-fold larger for the constant-load exercise (n=10). In addition, the postural effect on endurance during the constant-load exercise was larger for men than women and was related to

height, the estimated distance of the hydrostatic column and the early exercise response of oxygen uptake. During calf exercise, strength was not affected ($P > 0.05$) by tilt angle in any of the experiments. In Chapter 3, consistent with the observation of the cycling study, time to failure during the graded test was significantly higher at 47° (25.9 ± 2.0 min) and 90° (25.1 ± 3.0 min) compared to 0° (22.2 ± 2.6 min); and also, during the constant-force test time to failure was longer at 32° (7.1 ± 3.6 min), 47° (8.0 ± 5.2 min) and 67° (8.6 ± 5.6 min) compared with 0° (4.0 ± 2.6 min). When the graded or constant-force exercises were performed with the arterial flow to the leg eliminated (i.e. ischaemia), there were no differences in exercise time between the horizontal and inclined positions. In Chapter 4 muscle fatigue was affected by posture at moderate to high intensities (> 40 % F_{max}), but not at low intensities (< 40 % F_{max}). During Chapter 5, the fast phase (A_1) of the kinetic response was significantly higher in the inclined than the supine position at both intensities, demonstrating that leg blood flow was increased over the first few minutes of calf exercise in the inclined position.

These results demonstrate that head-up tilt improves endurance during cycling and plantar flexion exercise in both graded and constand-load exercise, that this improvement in the upright posture is much larger for the constant-load exercise, and that this effect during plantar flexion exercise occurs in the absence of an effect on strength and depends on an intact peripheral circulation. Moreover, the postural effect on muscle endurance appears to be due to a greater blood flow into the leg, but this is only evident at high intensities (i.e. 70 % F_{max}), because at low intensities (i.e. 30 % F_{max}) a similar postural response in blood flow does not increase fatigue-resistance.

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Chapter 1*:* **General introduction**

Chapter 1: GENERAL INTRODUCTION

A change in posture elicits alterations in various cardiovascular and metabolic parameters. Significant differences have been observed after a change in whole-body and/or single muscle group position on

a) the performance time to failure (Eiken, 1988, Leyk *et al.*, 1994a, Terkelsen *et al.*, 1999),

b) the rate of muscle fatigue (Fitzpatrick *et al.*, 1996, Wright *et al.*, 1999), and

c) the rate of increase in muscle blood flow (MacDonald *et al.*, 1998).

The present work tries to further explore the postural effects on human muscle endurance, muscle fatigue and muscle blood flow. This introduction is divided in four main sections:

1. To better understand the postural effects on performance the first section describes the cardiovascular and metabolic adaptations to acute exercise.
2. The literature suggests that oxygen availability plays an important role in mediating the postural effect on fatigue (Haseler *et al.*, 1998, Hogan *et al.*, 1999a), so, in order to better assimilate the postural effects on muscle fatigue the second section describes the role of oxygen supply in muscle fatigue
3. The third section reviews the literature regarding postural effects on performance, fatigue and oxygen uptake and blood flow dynamics.
4. It has been suggested that the rate at which blood flow increases at the onset of exercise is linked to the postural effect on performance and fatigue, so the last section will review specifically the physiological and mechanical factors that affect the rapid (from 0 to 10 s) onset of muscle blood flow.

1.1 Cardiovascular adaptations to acute exercise

With the onset of dynamic exercise, O_2 requirements increase in response to, and in proportion to, the metabolic needs of exercising muscles. The cardiovascular system responds by increasing blood flow and O_2 delivery to exercising muscles. However, at the same time, adequate blood supply to essential organs such as the brain and the heart is maintained by a redistribution of cardiac output. These responses require changes in both the central and peripheral circulations. Changes in the central circulation include increases in heart rate and stroke volume to bring about an increase in cardiac output. The peripheral circulation response is also designed to maximize O_2 delivery. Resistance vessels in the exercising muscle dilate in response to local metabolites, whereas vessels in non-exercise tissue, such as the splanchnic bed, vasoconstrict. The effect is to shunt blood to exercising tissues. The following section will consider individually the changes of these components of the central and peripheral circulation during acute exercise.

1.1.1 Maximal Oxygen Consumption ($\dot{V}O_2$ max)

Maximal functional capacity during dynamic exercise is limited by the body's ability to deliver and utilise oxygen (O_2) to meet the metabolic needs of the exercising muscles. Physiologists define this maximal exercise capacity as the maximal oxygen uptake ($\dot{V}O_2$ max, (Mitchell & Blomqvist, 1971)). Maximal $\dot{V}O_2$ is the greatest amount of O_2 that can be extracted and utilised from the inspired air during incremental exercise. $\dot{V}O_2$ max is a highly reproducible measurement of an individual, similar to height and weight, showing little day-to-day variation, and an objective benchmark for assessing exercise capacity and the cardiovascular response to dynamic exercise. $\dot{V}O_2$ max is physiologically limited by the ability of the cardiopulmonary system to deliver and the ability of the exercising muscles to use O_2. Rearrangement of the Fick equation yields the following equation:

$\dot{V}O_2$ max = Max cardiac output × Max. arterial-venous (A-V) O_2 difference

or

$\dot{V}O_2$ max = Heart rate × Stroke volume × A-V O_2 difference.

Maximal cardiac output describes the capacity of the heart to function as a pump and Maximal A-V O_2 difference relates to the capacity of the lungs to oxygenate blood as well as the capacity of the muscles to extract O_2.

1.1.2 Cardiac Output (CO)

Cardiac output refers to the quantity of blood that is pumped each minute by the heart and circulated throughout the body. Output from the heart, as with any pump, depends on its rate of pumping (i.e. heart rate) and the quantity of blood ejected with each stroke (i.e. stroke volume). Cardiac output is computed according to the following equation:

Cardiac output = Heart rate x Stroke volume.

The relationship between increases in cardiac output and increases in $\dot{V}O_2$ is remarkably constant. In general, cardiac output increases by roughly 6 $l.min^{-1}$ for every 1 $l.min^{-1}$ increase in oxygen consumption ($\dot{V}O_2$), and over a wide range of dynamic exercises there is a close linkage between cardiac output and oxygen uptake (Lewis *et al.*, 1983).

1.1.3 Heart rate (HR)

The heart rate increases in proportion to the demands on the cardiovascular system placed by exercise (i.e., it rises linearly with the increase in $\dot{V}O_2$, (Saltin, 1969). By definition, for a particular individual, the maximum increase in heart rate should occur at the $\dot{V}O_2$ max for that individual.

Heart rate is regulated by the sympathetic and parasympathetic nervous systems. The anticipation of physical activity inhibits the vagal nerve impulses to the heart and increases sympathetic discharge. The concerted inhibition of parasympathetic areas and activation of sympathetic areas of the medulla on the heart result in an increase in heart rate and myocardial contractility. As a person moves from rest to low to moderate levels of exercise, the increase in heart rate is predominately caused by a suppression of parasympathetic

stimulation, i.e. the parasympathetic inhibition of heart rate is removed, whereas during more strenuous exercise, heart rate increases primarily by stimulation from sympathetic nerves and the magnitude of acceleration increases in direct proportion to the intensity and duration of effort (Nobrega & Araujo, 1993, Seals *et al.*, 1994).

1.1.4 Stroke volume (SV)

Stroke volume refers to the amount of blood ejected by the left ventricle during each heartbeat and is equal to the difference in end-diastolic and end-systolic volumes. Stroke volume is determined by the interplay of 3 variables: ventricular preload or filling pressure (also called end-diastolic volume), myocardial contractility, and ventricular afterload which refers to the aortic pressure during the period when the aortic valve is open. In normal subjects under resting conditions, the stroke volume is largely dependent on the ventricular filling pressure, which in turn is dependent on the venous return.

With the onset of exercise in the upright position, the venous return increases, which in turn increases the stroke volume. The stroke volume increases up to around 60% to 70% $\dot{V}O_2$ max but then plateaus up to $\dot{V}O_2$ max (Marshall & Shepherd, 1968). Thereafter, the increase in cardiac output seen in an individual during exercise is directly related to the increase in heart rate. The second factor that regulates stroke volume is myocardial contractility. The stronger the force of myocardial contraction, the larger the amount of blood ejected from the ventricle and the larger the stroke volume. Factors that influence myocardial contractility are heart rate and sympathetic activity, both of which increase during exercise. Increased myocardial contractility also accounts for the progressive fall in end-systolic volume as exercise progresses. This rise in myocardial contractility accompanied by the fall in end-systolic volume helps to maintain stroke volume at a constant level in the face of the fall in end-diastolic volume and the rise in systolic arterial blood pressure, or afterload, at peak exercise (Poliner *et al.*, 1980, Schairer *et al.*, 1992, Tomai *et al.*, 1993, Tomai *et al.*, 1992).

1.1.5 A-V O_2 difference

The difference in O_2 concentration of the systemic arterial and venous systems reflects the extraction of O_2 by the tissues. At rest, the A-V O_2 difference is usually 4 to 5 ml.100 ml^{-1}, indicating approximately 23% extraction. During exercise, the A-V O_2 difference increases, primarily due to a fall in venous O_2 content as the O_2 extraction by muscle increases. At $\dot{V}O_2$ max, this difference may be as high as 16 to 18 ml O_2.100 ml^{-1}, corresponding to a venous O_2 content of 2 to 3 ml O_2.100 ml^{-1} (85% extraction, (Saltin, 1969). However, this is a physiological limit. The venous O_2 content does not fall further, as there is always some O_2 returning to the heart from blood flow through organs that do not fully extract O_2, such as the kidney.

1.1.6 Blood flow and Peripheral resistance

At the same time that cardiac stimulation occurs, the sympathetic nervous system also elicits changes in peripheral vascular resistance. In skin, kidneys, splachnic regions and inactive and active muscle, sympathetic-mediated vasoconstriction increases vascular resistance, which diverts blood away from these areas.

As cardiac output and blood to active muscles increase with progressive increments during exercise, blood flow to the splanchnic and renal vasculatures decreases (Musch *et al.*, 1987). In contrast, blood flow to the myocardium increases four to five-fold and that to the brain on average by 25-30 % (Thomas *et al.*, 1989). With an increasing body heat load, the temperature control centres of the hypothalamus instruct the medulla to dilate vessels in the skin so that heat can be dissipated to the environment. However, when exercise intensity exceeds 80-90% $\dot{V}O_2$ max, blood flow to the skin may again be reduced because the temperature regulation needs of the body are overridden by the requirement to maintain blood pressure (Rowell, 1993).

The major circulatory adjustment to prolonged exercise involves the vasculature of the active muscles. In a rest-to-exercise transition during human forearm or

knee extension-flexion exercise, the blood flow response is biphasic (Figure 1.1), and characterized by an immediate and substantial increase in muscle blood flow that plateaus by 5-7 s, with a second, slower adaptation to steady state initiated by ~15-20 s (MacDonald *et al.*, 1998, Shoemaker *et al.*, 1996, Shoemaker *et al.*, 1998).

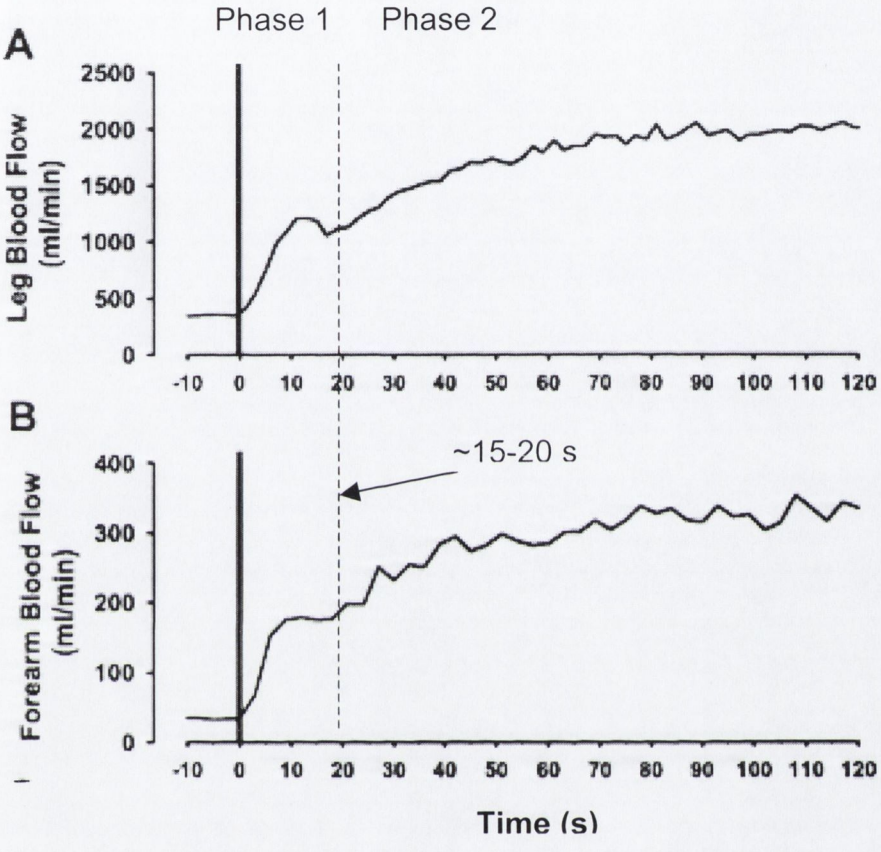


Figure 1.1: Biphasic response of blood flow during rest-to-exercise transition. **A**: human supine rhythmic knee extension/flexion exercise at 40W (adapted from (MacDonald *et al.*, 1998)). **B**: human rhythmic dynamic forearm handgrip exercise, rest to ~ 20% maximal voluntary contractions in a 1-s/2-s contractions/relaxation duty cycle (adapted from (Tschakovsky & Sheriff, 2004b)).

Depending on the work intensity the blood flow level is stabilized within 30-90 s and a minor further elevation may occur at very intense exercise (Saltin *et al.*, 1998). If the work is continued for hours then the limb blood blow and oxygen uptake are quite stable (Savard *et al.*, 1987). There is a tight coupling between power output and blood flow in certain exercise modes. For instance, during knee extension exercise 7 $l.min^{-1}$ of blood flow gives an oxygen consumption of

1 $l.min^{-1}$, and the coupling is very tight over the entire range of exercise intensities (Andersen & Saltin, 1985, Richardson *et al.*, 1993) The corresponding blood flow during ordinary bicycle exercise is 6 $l.min^{-1}$ (Savard *et al.*, 1987, Wahren *et al.*, 1974). Therefore, peak blood flow varies closely with peak power output (Saltin *et al.*, 1998).

During the second phase of blood flow in the rest-to-exercise transition (the mechanisms of the first phase of the blood flow will be discussed extensively in the last section of this introduction), vessel-dilating chemicals (i.e. adenosine, (Skinner & Marshall, 1996)) are released by the contracting muscle fibers into the tissue fluids that bathe the small arteries of the contracting muscles. These local vasodilators relax the smooth muscles in the walls of the arteries, causing the arteries to dilate and allowing more blood to perfuse the working muscles. This response is rapid and finely adjusted to the muscle's force output and metabolic needs (Bevegard & Shepherd, 1967). In addition to metabolic factors, endothelial factors also appear to regulate blood flow, which are believed to be released following an increase in blood velocity and shear stress (Rubanyi *et al.*, 1986). While local chemicals are dilating the vessels, the neural outflow from the medulla leads to constriction of blood vessels in active muscles via sympathetic activity (Thompson & Mohrman, 1983). However, this sympathetic activity appears to be minor (Strandell & Shepherd, 1967) and seems to be overridden by the powerful vasodilation induced by the local metabolites.

1.1.7 Venous return

In addition to the contribution made by sympathetically mediated constriction of the capacitance or venous vessels in both exercising and non-exercising parts of the body, venous return is aided by the working skeletal muscles and the muscles of respiration. The intermittently contracting muscles compress the vessels that course through them and, in the case of veins with their valves oriented toward the heart, pump blood back toward the right atrium. The flow of venous blood to the heart is also aided by the increase in the pressure gradient developed by the more negative intrathoracic pressure produced by deeper and

more frequent respirations. As cardiac output increases, venous tone also increases proportionally in both active and nonactive muscles. With these adjustments, a balance is maintained between cardiac output and venous return.

1.1.8 Arterial Pressure

Arterial pressure represents a balance between cardiac output and TPR (Seals & Victor, 1991). During rhythmic steady-rate exercise, dilation of the blood vessels in the active muscles decreases total peripheral resistance, thus enhancing blood flow through large portions of the peripheral vasculature. The increased blood flow causes systolic pressure to rise rapidly during the first few minutes of exercise. The blood pressure then levels off at approximately 140 to 160 mm Hg with no difference between sexes (Fagard *et al.*, 1995). As exercise continues, systolic pressure may gradually decline as the arterioles in the muscles continue to dilate and there is a reduction in peripheral resistance to blood flow. The diastolic blood pressure remains relatively unchanged during this rhythmic steady-rate exercise. During graded exercise, after the initial rapid rise from the resting level, systolic blood pressure increases linearly with exercise intensity, whereas diastolic pressure remains stable or increases slightly at the higher levels of exercise. During maximum exercise performed by healthy, fit men and women, the systolic blood pressure may increase to 200 mmHg or higher, despite significant reductions in total peripheral resistance.

1.2 The role of O_2 supply in muscle fatigue

Muscle fatigue can result as a consequence of a failure in any of the several steps in excitation-contraction coupling or force generation (for extended reviews about muscle fatigue refer to: (Allen *et al.*, 1995, Fitts, 1994, Karlsson, 1979)). However, the focus of the following section is to describe specifically evidences that support the idea that oxygen supply affect and may mediate muscle fatigue, and will not discuss the other various factors that can affect muscle fatigue.

1.2.1 How has muscle fatigue been assessed?

Several approaches have been used to study muscle fatigue. In studies concerning the influence of O_2 on fatigue. The most commonly used approaches include examinations of

a) the rate of decline in maximal voluntary contraction (MVC) during repetitive maximal contractions,

b) the rate of decline in MVC performed at intervals during constant-load submaximal exercise contractions, and

c) the time to exhaustion during exercise.

The first two approaches fit with accepted definitions of fatigue ((a response that is less than the expected or anticipated contractile response, for a given stimulation (Asmussen, 1993, Bigland-Ritchie *et al.*, 1986b)), but the later method has limitations because it is a single measurement strongly influenced by the subject's motivation to endure discomfort. However, in this respect, muscle fatigue progresses such that the maximal force production approaches the required power output (Figure 1.2, (Hepple, 2002)).

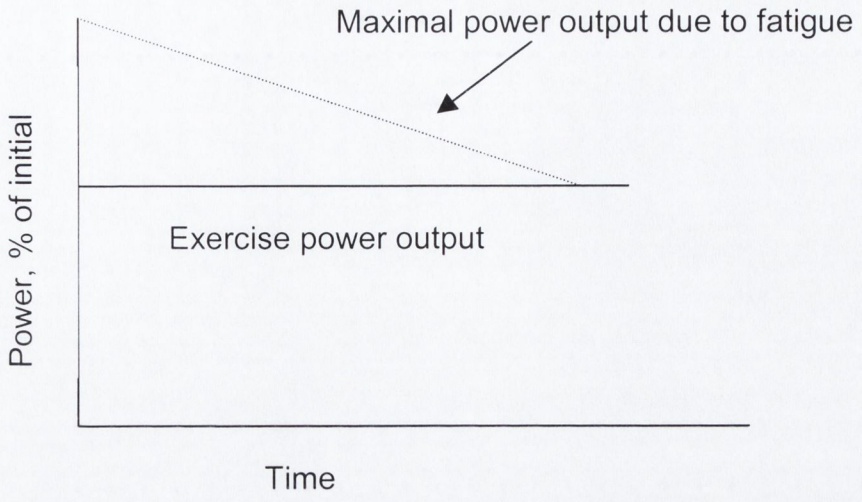


Figure 1.2: Relationship between fatigue and time to failure. During performance of constant power output exercise, muscle fatigue progresses such that the maximal voluntary contraction (MVC) approaches the force required to maintain the constant power output.

1.2.2 Evidence that O_2 and blood flow affect muscle fatigue

Skeletal muscle fatigue and muscle performance are very sensitive to O_2 supply. Hypoxia has been shown to accelerate the rate of fatigue during human knee-extensor exercise (Eiken & Tesch, 1984, Fulco *et al.*, 1996) and during human plantar-flexion exercise (Hogan *et al.*, 1999a). For instance, when 8 subjects performed 60 maximal consecutive dynamic knee extensions breathing gas mixtures containing either 11% O_2 (hypoxia) or 21% O_2 (normoxia), peak torque of individual extensions declined more rapidly during hypoxia than during normoxia (Eiken & Tesch, 1984). The authors suggested that the increase in the rate of fatigue during hypoxia was due to a faster rate of intramuscular H^+ accumulation.

Similarly, hypoxia appears to reduce the time to failure during constant-load human knee extension (Fulco *et al.*, 1996) or incremental plantar flexion (Hogan *et al.*, 1999a) exercise, and hyperoxia increases the time to exhaustion during incremental human plantar flexion exercise (Hogan *et al.*, 1999a). Fulco and co-workers (1996) compared one-leg dynamic knee extension exercise at a

frequency of 1 Hz to the point of failure at the same constant work rate (21 ± 3 W) under normoxia (barometric pressure of 758 Torr) and hypobaric hypoxia (barometric pressure of 464 Torr) conditions. These investigators observed that time to failure was 56 % shorter for hypoxia than normoxia (19 ± 5 vs. 43 ± 7 min respectively, $p<0.01$). Consistent with this, when six subjects performed incremental plantar flexion exercises to failure breathing mixtures containing either 0.1 (hypoxia), 0.21 (normoxia) or 1.0 (hyperoxia) fractions of O_2 (F_{IO2}), time to failure was significantly different ($p<0.01$) among each of the three F_{IO2} : 17.7 ± 1.3, 21.0 ± 0.9 and 24.0 ± 1.8 min respectively (Hogan *et al.*, 1999a).

In addition, the initial fall in developed force in working skeletal muscle that occurs with ischaemia has been related to O_2 availability (Hogan *et al.*, 1994). This was tested by reducing O_2 delivery to working muscle by either stopping blood flow (ischaemia) or maintaining blood flow with low arterial O_2 content (hypoxic hypoxia) in electrically stimulated canine gastrocnemius muscle. The authors noted that the rate of decline in developed force was strikingly similar between the conditions of ischaemia and hypoxia suggesting that O_2 clearly plays an important role in mediating muscle fatigue.

1.2.3 Mediation of oxygen in muscle fatigue

In assessing how oxygen availability mediates muscle fatigue, it is important to consider the influence of O_2 supply on skeletal muscle bioenergetics, as some of the bioenergetic molecules, such as P_i, are important to develop fatigue (Balog *et al.*, 2000, Bangsbo *et al.*, 1996, Sahlin *et al.*, 1998). For example, the degree of PCr hydrolysis was reduced in hyperoxia (F_{IO2}:1.0) and increased in hypoxia (F_{IO2}:0.1) compared to normoxia (F_{IO2}:0.21) during constant-load human plantar-flexion exercise (Haseler *et al.*, 1998), and the changing levels of PCr imply a corresponding change in concentrations of other proposed regulators of oxidative phosphorylation such as ADP and P_i (Binzoni *et al.*, 1997, Chance *et al.*, 1986). During voluntary contractions, as in the study by Haseler and colleagues (1998), the changes in PCr hydrolysis occur without altering $\dot{V}O_2$ and such, the normally observed coupling between $\dot{V}O_2$ and PCr hydrolysis (Bessman & Geiger, 1981, Rossiter *et al.*, 1999) is shifted with

variations in O_2 tension. In contrast, during constant-load electrically stimulated contractions *in situ,* the reduction of arterial PO_2 results in a fall in muscle tension development and thus, $\dot{V}O_2$ (Hogan *et al.*, 1999b). This difference is in part related to the neural recruitment of additional motor units (demonstrated by increased EMG activity in hypoxia, (Mateika *et al.*, 1996)) to sustain force and $\dot{V}O_2$ when O_2 availability is reduced in voluntary contractions *in vivo,* whereas typically electrically stimulated *in situ* experiments involve synchronous recruitment of all motor units.

In considering how these changes in cellular bioenergetics relate to fatigue, Hogan *et al.,* (1999a) examined the influence of altering inspired O_2 fraction on skeletal muscle bioenergetics during incremental plantar-flexion exercise in humans, and they noted that the degree of PCr hydrolysis, P_i accumulation and intracellular pH reached the same levels at exhaustion independent of O_2 supply. However, hypoxia (F_{IO2}:0.1) caused a more rapid P_i accumulation and intracellular acidosis, resulting in an earlier onset of fatigue and shortened time to failure, and hyperoxia (F_{IO2}:1.0) slowed P_i accumulation and intracellular acidosis, resulting in later onset of fatigue and an extended time to failure.

To investigate whether the force producing impairment that occurs in the working muscle during O_2 limited conditions operates through a similar excitation-contraction coupling failure mechanism as under higher O_2 availability conditions, Stary and Hogan (2000) measured the rate of fatigue and relative calcium concentration simultaneously during an incremental fatiguing protocol in frog muscle fibres at extracellular P_{O2} of either 22 Torr (hypoxia) or 159 Torr (hyperoxia). These investigators found an earlier onset of muscle fatigue ($p<0.05$) in hypoxic (237 ± 40 s) versus hyperoxic (280 ± 38 s) muscle fibers. Relative $[Ca^{2+}]$ was significantly decreased from maximal values at the fatigue time point during both, high (72 ± 4%) and low P_{O2} conditions (78 ± 4%), but no significant difference was observed between the treatments. Therefore, these later results suggest that although O_2 affects the rate of muscle fatigue, the underlying mechanisms of fatigue (e.g., diminished Ca^{2+} release) may be similar irrespective of O_2 availability (Stary & Hogan, 2000). Specifically, P_i accumulation leads to increased P_i concentration in the sarcoplasmic reticulum,

where P_i precipitates with Ca^{2+} to diminish the amount of Ca^{2+} release leading to reduced cross-bridge turnover and thus, reduced force, ATP demand and $\dot{V}O_2$ (Dahlstedt & Westerblad, 2001).

1.3 Postural effect on muscle performance and fatigue

A change in posture from supine to upright position exerts a number of changes on muscle perfusion, blood flow and cardiac output which are important to understand the postural effects on performance. Furthermore, the rate at which oxygen consumption and blood flow increase at the initial stages of the exercise appears to mediate these postural effects on performance and fatigue. Therefore, in addition to reviewing the postural changes in performance and fatigue, the present section will also review the postural changes on muscle perfusion, cardiac output and changes on the dynamics of $\dot{V}O_2$ and blood flow.

1.3.1 Effect of posture on muscle perfusion

Blood flow through resting skeletal muscle depends on the pressure gradient across the muscle (arterial luminal pressure minus venous luminal pressure) and vascular conductance. Vascular conductance is directly proportional to the radius of the vessel raised to the fourth power and is inversely proportional to the length of the vessel and to the viscosity of the fluid flowing through the vessel. At rest, when the muscle is at the heart level, the pressure gradient between the aortic and right atrial pressure creates a driving flow known as the hydrodynamic pressure, which appears to be induced by the pumping effect of the heart. In an upright posture, hydrostatic pressure is added to the hydrodynamic luminal pressure due to the effect of gravity on the fluid. The magnitude of the hydrostatic pressure can be estimated using the equation, ρgh, where ρ is the density of the fluid (i.e. blood), g is the gravitational constant, and h is the the height of the column of fluid. Therefore, the more distal is the dependent vessel from the heart, the higher is the increase in hydrostatic pressure. In arteries or resistance vessels, the increase in hydrostatic pressure happens immediately after the adoption of an upright posture because they do not contain any structures that could interrupt the column of blood in the arterial system. Veins or capacitance vessels, however, contain venous valves, which are expected to close and thus, venous valves will

interrupt the column of blood in the venous side. This effect increases the arteriovenous pressure gradient and thus, blood flow and muscle perfusion.

In addition, an increase in the hydrostatic component of luminar pressure increases the transmural pressure gradient (luminal pressure minus tissue pressure) such that additional blood volume will accumulate in the dependent regions.

1.3.2 Effect of posture on Cardiac Output (CO)

The major physiological differences between the upright and supine position concern circulatory alterations. In a supine posture the reduced hydrostatic pressure causes a shift of blood volume towards the heart, which in turn augments stroke volume (SV) and cardiac output (CO). At rest and during moderate dynamic exercise SV and CO have been reported to be lower in the upright compared to supine posture (Bevegard *et al.*, 1960, Eiken, 1988, Hughson *et al.*, 1991, Leyk *et al.*, 1992, Leyk *et al.*, 1994a, Loeppky *et al.*, 1981). At higher intensities, however, SV and CO during upright tend to approach supine values (Bevegard *et al.*, 1960, Leyk *et al.*, 1992, Leyk *et al.*, 1994a). HR at rest is higher in the upright posture, but at light to moderate exercise this effect appears to disappear (Eiken, 1988, Leyk *et al.*, 1994a, MacDonald *et al.*, 1998, Terkelsen *et al.*, 1999), and therefore a greater CO observed in the supine posture during light to moderate exercise seems to be a consequence of a greater SV.

1.3.3 Effect of posture on exercise performance

Maximal exercise performance is increased in the upright compared to supine posture during graded cycling exercise (Table 1.1). Performance time to failure (Eiken, 1988, Leyk *et al.*, 1994a, Terkelsen *et al.*, 1999), peak power output (Hughson *et al.*, 1991, Koga *et al.*, 1999, Leyk *et al.*, 1994a, Terkelsen *et al.*, 1999) and maximal or peak oxygen uptake (Astrand & Saltin, 1961, Hughson *et*

al., 1991, Koga *et al.*, 1999, Terkelsen *et al.*, 1999) have been reported to be significantly higher in the upright compared to supine graded cycling exercise.

Performance times to the point of failure have been consistently shown to be ~10-15 % higher in the upright compared to supine posture, when the majority of subjects were men (Eiken, 1988, Leyk *et al.*, 1994a) or equally men and women (Terkelsen *et al.*, 1999). For instance, in a study conducted by Leyk and co-workers (1994), 7 men and 2 women performed an incremental test to failure both in the upright and supine posture. They cycled initially from 0 to 5 min at 20 W and from 5 to 10 min at 80 W, and thereafter, the work rate was increased by 10 W every 30 s until exhaustion was reached. They pedalled at 60 rpm. It was observed that performance time during upright cycling was significantly longer than during supine (mean ± SD, 23.11 ± 2.48 min vs. 22.04 ± 2.45 min, respectively (Leyk *et al.*, 1994a). Similarly, following a protocol at which workload increased by 50 W every 3 min until exhaustion from an initial workload of 50 W pedalling at 60 rpm, 5 men and 5 women reached higher exercise times cycling in the upright (mean ± SEM, 18.4 ± 1.1 min) than cycling in the supine (16.1 ± 1.3 min) posture (Terkelsen *et al.*, 1999). However, Terkelsen and co-workers did not assess whether the longer endurance times achieved in the upright posture were different in terms of magnitude among men and women.

Author(s)	Posture	Time to failure	Peak $\dot{V}O_2$	Peak power output
Leyk *et al.*, (1994a)	Supine	22.0 ± 2.5	3.84 ± 0.76	324 ± 49
	Upright	23.1 ± 2.5 *	4.05 ± 0.71	347 ± 50 *
		(min)	($l.min^{-1}$)	(W)
Terkelsen *et al.*, (1999)	Supine	16.1 ± 1.3	35.7 ± 2.0	255 ± 60
	Upright	18.4 ± 1.1 *	39.2 ± 2.4 *	285 ± 58 *
		(min)	($ml.kg^{-1}.min^{-1}$)	(W)
Hughson *et al.*, (1991)	Supine	data not shown	44.6 ± 1.6	data not shown
	Upright		50.2 ± 1.7 *	
			($ml.kg^{-1}.min^{-1}$)	
Koga *et al.*, (1999)	Supine	data not shown	2.90 ± 0.4	245 ± 26
	Upright		3.29 ± 0.5 *	284 ± 35 *
			($l.min^{-1}$)	(W)
Eiken (1988)	Supine	93%	data not shown	data not shown
	Upright	100%*		
		(%)		
Astrand & Saltin (1961)	Supine	data not shown	86%	data not shown
	Upright		100% *	
			(%)	

Table 1.1: Effect of posture on performance parameters. All studies used the same exercise mode: graded cycling exercise to maximum.
*Significantly different from supine, $p<0.05$.

Some investigators (Eiken, 1988, Terkelsen *et al.*, 1999) hypothesized that the greater performance time observed during upright compared to supine cycling might be due to the activation of different muscle groups in the two conditions. Eiken (1988) explored this possibility applying low body negative pressure (LBNP) during supine incremental load cycling exercise. This method was used because previous reductions in CO in supine exercise with lower body suction at –6.67 kPa (-50 mmHg) at low and moderate intensities were very similar to

those observed during upright cycling, and therefore supine posture with LBNP was regarded as a model of upright exercise. During supine cycling with LBNP endurance time was improved by 9% and the rate of blood lactate accumulation ([la]) was decreased compared to supine cycling without LBNP (Eiken, 1988). The fact that LBNP reduced blood lactate accumulation can be explained by a more efficient blood perfusion of the working muscles, and this fact suggests that the primary limitation of work performance during supine cycling was set by the peripheral circulation in working muscles rather than by cardiac performance. However, the smaller improvements achieved in performance time and the lower $\dot{V}O_2$ values reached during heavy exercise during supine exercise with LBNP compared to upright exercise may in part be explained by lesser work performed by the postural muscles (Eiken, 1988).

In the cycling study conducted by Leyk *et al.*, (1994a), the lactate concentration at the end of the incremental test was not different between upright and supine postures (mean ± SD, 13.0 ± 1.6 vs. 12.9 ± 2.2 $mM.l^{-1}$ respectively). However, lactate values of 4 $mM.l^{-1}$ occurred at significantly higher work rates when subjects were upright compared to supine. Consistent with this, during a graded incremental cycling test, Hughson and co-workers (1991) observed significant reductions ($p<0.001$) in ventilatory threshold in the supine (mean ± SD, 24.9 ± 1.3 $ml.kg^{-1}.min^{-1}$) compared to upright (31.1 ± 1.6 $ml.kg^{-1}.min^{-1}$) posture. These data suggest that the demand from the anaerobic metabolism may be higher in the supine compared to upright posture.

1.3.4 Effect of posture on muscle fatigue

Muscle fatigue has been shown to be very sensitive to changes in perfusion pressure during moderately intense submaximal repeated contractions (Fitzpatrick *et al.*, 1996, Wright *et al.*, 1999). During voluntary isometric contractions of 50% MVC of the adductor pollicis muscle of the human hand a reduction in perfusion pressure induced by elevating the arm above the heart level significantly increased the rate of fatigue (Wright *et al.*, 1999). Similarly, Fitzpatrick *et al.*, (1996) showed that during electrically-induced repeated

contractions of the adductor pollicis muscle, a reduction in muscle perfusion pressure induced by elevating the arm above the heart level significantly decreased the force output whereas lowering the arm below the heart level increased the force output soon after the change in limb position.

The postural effect on fatigue has not been reported during voluntary constant load contractions involving limbs of the lower body, but Hobbs and McCloskey (1987) showed that during voluntary constant-force plantar flexion contractions at ~20% MVC, the electromyogram (EMG) of the gastrocnemius lateralis muscle was increased when the contracting muscles were raised above compared to below the heart level. This suggests that force production of active muscle fibers at a given level of activation fell when muscle perfusion pressure was lowered, and therefore, the muscle activity was augmented in order to sustain the standard contractions.

1.3.5 Effect of posture on blood flow and $\dot{V}O_2$ dynamics

The rate of increase in $\dot{V}O_2$ at the onset of submaximal exercise is slower during a supine compared to an upright cycling exercise (Convertino *et al.*, 1984, Hughson *et al.*, 1993, Karlsson *et al.*, 1975, Koga *et al.*, 1999, Leyk *et al.*, 1994a). Similarly, during leg kicking exercise the increase in $\dot{V}O_2$ is faster when the upper body is tilted upright (MacDonald *et al.*, 1998); and during handgrip exercise the increase in $\dot{V}O_2$ is more rapid when the arm is below compared to above the heart level (Hughson *et al.*, 1996).

Leg blood flow is an important component of the oxygen delivery, and has also been reported to increase more rapidly during the early period of exercise when the exercising limb is positioned below compared with above the level of the heart. Lowering the arm below from an initial heart-level position increased blood flow to the forearm muscles during handgrip exercise (Tschakovsky *et al.*, 2004a); tilting the upper body upright from a supine position increased femoral arterial flow into the thigh during a knee extension-flexion exercise (MacDonald

et al., 1998); and tilting the whole-body from a supine to inclined position increased femoral arterial blood flow during calf exercise (Leyk *et al.*, 1994b).

Interestingly Hughson *et al.*, (1996) observed a close correlation between the rate of increase in blood flow to the forearm muscles and the rate of increase in $\dot{V}O_2$. This rate of increase for both $\dot{V}O_2$ and blood flow was slower when forearm muscles were above the heart compared to below. Similarly, during knee extension and flexion exercise the slower rate of increase in $\dot{V}O_2$ at the onset of supine exercise was accompanied by a slower increase in leg blood flow (MacDonald *et al.*, 1998).

1.4 Physiological and mechanical reasons for the rapid (~0-10s) onset of hyperaemia

Blood flow through a vascular bed is proportional to the arteriovenous pressure difference across that bed and the vascular conductance of that bed. Since the mechanical activity of muscle contraction and relaxation affects this pressure gradient and imparts energy on blood flow, the muscle pump may mediate the initial hyperaemia. In addition, as vasodilatory signals determine vascular conductance, the rapid vasodilation may also contribute to the immediate exercise hyperaemia.

Thus, the following section will contrast evidence for and against the muscle pump action and rapid vasodilation

1.4.1 Effect of the muscle pump

A number of mechanisms have been proposed by which the muscle pump action may mediate rapid exercise hyperaemia. The most relevant of them is that muscle contraction empties the veins eliciting a reduction in venous pressure; and that this effect may therefore increase muscle blood flow (Laughlin, 1987). Another mechanism proposed by Laughlin (1987) is that muscle contraction can produce negative venous pressure during relaxation and consequently further increase the pressure gradient across the tissue. In addition, Tschakovsky and Sheriff (2004b) have suggested that the mechanical deformation of the vascular smooth muscle cells induced by the muscle contraction may exert a mechanically induced rapid vasodilation (Tschakovsky & Sheriff, 2004b).

Evidence that muscle pump improves muscle perfusion by increasing arteriovenous pressure gradient in dependent limbs, is well supported by experimental studies (Folkow *et al.*, 1970, Folkow *et al.*, 1971, Leyk *et al.*, 1994b, Radegran & Saltin, 1998, Sheriff & Van Bibber, 1998, Shiotani *et al.*, 2002, Tschakovsky *et al.*, 1996). An early study conducted by Folkow *et al*

(1971) showed that when the hydrostatic component was experimentally altered by tilting the body from supine to a "leg down" posture during high-intensity rhythmic calf exercise, the maximal blood flow increased by 60% and on average transmural pressure increased 65-70mmHg. More recently, Tschakovsky and co-workers (1996) simulated the rhythmic muscle pump function via repeated inflation (1s) / deflation (2s) of a forearm cuff to 100 mmHg to achieve mechanical emptying of forearm veins and measured forearm blood flow when the forearm was above and below the heart level. These investigators observed that cuff inflation increased blood flow ($p<0.05$) when the arm was below the heart level (condition where venous hydrostatic column exists) but not with the arm above, and therefore, demonstrated the existence of a functional muscle pump in the human forearm.

Studies that have tried to demonstrate that muscle pump improves perfusion in a manner other than reducing venous pressure have adopted an indirect approach. Several studies have altered the contraction frequency (treadmill speed) and contraction force (treadmill grade) at the onset of locomotion to investigate the influence of the muscle pump on blood flow in animals (Armstrong & Laughlin, 1985, Laughlin & Armstrong, 1982, Sheriff, 2003a, Sheriff & Hakeman, 2001, Sheriff *et al.*, 1993). When locomotion was initiated across a wide range of speeds and grades in rats, Sheriff *et al.,* (2001) observed that treadmill speed significantly increased blood flow as soon as the 3rd s of locomotion, but treadmill grade (contraction force) did not begin to exert a significant effect on conductance until 13 s after the onset of locomotion. Importantly, nitric oxide (NO) synthase (NOS) inhibition has been reported to slow the vasodilation in response to locomotion (Sheriff *et al.*, 2000). It is believed that the muscle pump elicits an immediate increase in blood flow and, as a consequence, there is a rise in shear stress on endothelial cells, which augments the release of NO. In this way, NO acts as an amplifier that reinforces the influence of the muscle pump on blood flow. Sheriff and coworkers (2001) sought to inhibit NOS to isolate its vasodilatory effect and evaluate muscle pump function. When nitric oxide synthase was inhibited in dogs, an approximate doubling of stride frequency (treadmill speed) lead to a doubling of the initial (2 to 3 s) rise in blood flow, but an increase in treadmill grade did not

induce an augmentation of blood flow in dogs until 10 s after the onset of locomotion (Sheriff & Hakeman, 2001). These authors suggested that muscle pump raises the initial blood flow in proportion to contraction (stride) frequency (treadmill speed). These conclusions are supported by other studies based on both, rest to work transitions (Sheriff, 2003a, Sheriff *et al.*, 1993) and recently, during exercise-exercise transition (Sheriff & Zidon, 2003b).

1.4.2 Effect of rapid vasodilation

In contrast to the muscle pump function, several studies have suggested a rapid vasodilatory response using in situ stimulated dog muscle and voluntary human forearm muscle contraction models (Corcondilas *et al.*, 1964, Hamann *et al.*, 2004, Hughson & Tschakovsky, 1999, Saunders & Tschakovsky, 2004, Shoemaker *et al.*, 1998, Tschakovsky *et al.*, 2004a, Tschakovsky *et al.*, 1996). Shoemaker *et al.,* (1998) examined the effect of contraction intensity vs. frequency in a human forearm dynamic handgripping model when the arm was positioned above and below the heart level. These investigators altered contraction intensity by raising and lowering two different weights and the frequency of stimulation by modifying the contraction/relaxation duty cycles from 1s/1s to 1s/2s. The authors observed that mean blood flow was increased 50-100% above rest following the first contraction in both arm positions ($p<0.05$) the increase being greater in the below position. However, since the positional effect on the increase in flow could not be explained entirely by the ~40 % greater blood pressure in this below position, vasodilation appears to have contributed to the increase blood flow within the initial 2-4 s of exercise. In addition, in contrast to the findings from Sheriff's lab, the greater workload resulted in greater increases in blood flow as early as the first contraction compared with the light workload ($p<0.05$), whereas the faster contraction rate did not increase blood flow until the 8th s of exercise. These data suggested that the initial rise in blood flow is related to work intensity and not work frequency in the human forearm.

More recently, Tschakovsky *et al.*, (2004a) demonstrated a similar response in that the immediate exercise hyperaemia in response to single 1 s forearm contractions performed above heart level is linearly related to muscle contraction intensity. The authors attempted to minimize the potential contribution of the muscle pump by utilizing the arm above heart position to minimize initial forearm venous volume (Laughlin & Joyner, 2003, Shiotani *et al.*, 2002) isometric contractions to minimize muscle relaxation-induced active venous opening (Laughlin, 1987) and to examine the pattern of immediate exercise hyperaemia across a range of contraction intensities since contraction intensity does not appear to enhance muscle pump function (Sheriff & Van Bibber, 1998, Sheriff & Zidon, 2003b).

In addition, in a transition from 10 % to 20 % maximal voluntary contraction in a rhythmic dynamic forearm handgrip exercise with the arm above the heart level where no muscle pump contribution was evident, forearm blood flow increased in the first relaxation following release of the first contraction. This immediate hyperaemia was of the same magnitude as in the transition from rest to 10 % maximal voluntary contraction exercise (Saunders & Tschakovsky, 2004). Therefore, these data support the existence of a rapid vasodilatory contribution to the immediate increase in muscle blood flow with increases in exercise intensity with the onset of both rest-exercise and exercise-exercise transition.

Data demonstrating a rapid vasodilation do not exclude the possibility of the existence of a muscle pump function. They indicate that rapid vasodilation is evident when the venous emptying effect of the muscle pump contribution is removed (i.e. when the arm is above the heart level).

1.4.3 Influence of the experimental model

Studies in animal locomotion models demonstrate that the muscle pump raises the immediate exercise hyperaemia in proportion to contraction (stride) frequency (treadmill speed, (Sheriff, 2003a, Sheriff *et al.*, 1993), and that the vasodilation effect of contraction intensity (treadmill grade) on the early blood flow is delayed by ~10 s (Sheriff & Hakeman, 2001, Sheriff & Zidon, 2003b).

However, human handgrip studies show that the immediate increase in blood flow depends on exercise intensity and that it is mediated at least in part by a rapid vasodilation (Saunders & Tschakovsky, 2004, Shoemaker *et al.*, 1998, Tschakovsky *et al.*, 2004a, Tschakovsky *et al.*, 1996). To date it is unclear if the differences between rodent treadmill locomotion vs. human handgrip studies are species dependent and/or muscle activation dependent Thus, it is believed that both the muscle pump and rapid vasodilation can contribute to the immediate exercise hyperaemia.

1.5 Aims

The data reviewed during this introduction suggests that the dynamics of $\dot{V}O_2$ and blood flow at the beginning of exercise may mediate the postural effect on fatigue and endurance. This is speculative, because the effect of posture on cycling endurance has only been shown during incremental graded cycling tests, whereas experiments which have investigated $\dot{V}O_2$ alterations were done during short duration constant-load cycling bouts. In addition, none of the experiments that investigated the postural effect on blood flow, $\dot{V}O_2$ and/or muscle force production in an isolated muscle group (i.e. forearm, thigh, calf), have explored in the same experiment the postural effect on the endurance of the same muscle group.

Therefore, the main aims of the present thesis were the following:

1) investigate the postural effect on the endurance time to the point of failure during constant-load cycling exercise
2) test the effect of body tilt on calf muscle strength and endurance during a graded and constant-load plantar flexion exercise
3) test the same effect (see above, aim 2) under ischaemia to explore to what extent any postural effects observed depended upon an intact peripheral circulation

4) to establish the range of intensities across which the positive effect of body tilt angle on calf muscle fatigue occurs
5) assess the effect of body tilt angle on leg blood flow during a plantar flexion exercise in which endurance and fatigue were previously assessed (aim 2 and 3).

It was hypothesised that:

a) Performance during constant-load cycling exercise would be more prolonged in an upright compared to a supine posture
b) In the absence of any effect on strength, endurance during both graded and intermittent constant-load plantar flexion exercise in an upright posture would be prolonged when compared with a supine posture.
c) The postural effects on endurance would be reduced under ischaemic conditions
d) The postural effects on endurance would be linked to a faster blood flow responses at the onset of exercise in the upright compared to the supine posture.

Chapter 2: **Effect of posture on high-intensity constant-load cycling performance in men and women**

2.1 Introduction

In humans, the endurance time sustained for a maximal graded cycle test is improved by ~10-15% with an upright posture when compared with a supine posture (Eiken, 1988, Leyk *et al.*, 1994a, Terkelsen *et al.*, 1999). Improvements of similar magnitude have also been observed during upright compared to supine cycling for peak power (Hughson *et al.*, 1991, Koga *et al.*, 1999, Leyk *et al.*, 1994a, Terkelsen *et al.*, 1999) and peak oxygen uptake (Astrand & Saltin, 1961, Hughson *et al.*, 1991, Koga *et al.*, 1999, Terkelsen *et al.*, 1999).

When the body is tilted from the supine to the upright posture, venous and arterial hydrostatic pressure increase due to the effects of gravity on the fluids between the heart and the active vessels, and the magnitude of the hydrostatic pressure is proportional to the height of the column of fluid. During the early relaxation phase of a dynamic rhythmic exercise in an upright posture, the proximal valves of the veins interrupt the vertical column of blood between the heart and the exercising limb, lowering the venous pressure towards zero while arterial hydrostatic pressure is essentially unaffected (Folkow *et al.*, 1971). As a result, a transient increase in pressure gradient increases blood flow through the active limb. Probably, due to an enhanced perfusion pressure of the active muscles in the upright posture, blood flow to active limbs at the onset of a submaximal exercise is slower in the supine posture when compared to the upright posture (Eiken, 1988, Folkow *et al.*, 1971, Leyk *et al.*, 1994b, MacDonald *et al.*, 1998, Van Leeuwen *et al.*, 1992). This effect in the upright posture in turn appears to contribute to a faster O_2 uptake ($\dot{V}O_2$) adjustment when compared to supine posture particularly during the early phase of a high-intensity constant load cycling exercise (Convertino *et al.*, 1984, Koga *et al.*, 1999, Leyk *et al.*, 1994a). Consistent with this, a slower rate of increase (i.e. kinetics) in $\dot{V}O_2$ during the early part of upright heavy cycling exercise reflects a relative inadequate perfusion and subsequent oxygen delivery to the working muscles (Gerbino *et al.*, 1996, Macdonald *et al.*, 1997). Therefore, these data imply that the dynamics in the rate of increase in $\dot{V}O_2$ at the beginning of

exercise may mediate the postural effects seen during maximal exercise performance.

However, this evidence is indirect because previous experiments which have investigated blood flow and $\dot{V}O_2$ alterations were done so during short duration constant load exercise bouts, whereas the effect of posture on exercise performance has only been shown during incremental graded exercise tests. Therefore, the main aim of the following study was to investigate exercise times to the point of failure during high-intensity constant-load cycling tests both in the upright and supine postures while simultaneously measuring changes in $\dot{V}O_2$ at the beginning of exercise.

In addition, if this reported enhanced perfusion pressure difference acting across the active muscles is indeed the mediator of the postural effect on exercise performance, this effect should be particularly large in relatively tall subjects in which venous and arterial pressure are more elevated than in shorter subjects due to a larger hydrostatic pressure change when moving from a supine to an upright posture. To explore this, the second aim of the study was to investigate the relationship between the potential postural improvements in exercise performance and the height and the subsequent estimate of the hydrostatic column of the subjects. This was facilitated by recruiting relatively tall men compared to relatively short women.

2.2 Material and methods

2.2.1 Subjects

All participants in the study were recruited by local advertisement and involvement in the study was on a voluntary basis. All subjects were free from overt cardiovascular, metabolic, pulmonary or musculoskeletal disease as assessed using a medical history questionnaire (Appendix 1), and only one subject (male) was a regular smoker. All subjects read the subject information sheet (Appendix 2) and gave written informed consent before involvement in the study (Appendix 3). All quires were answered and the risks and possible benefits of the study were clearly outlined to each participant before involvement.

2.2.2 Experimental design

2.2.2.1 Overview

The experimental procedures were conducted in accordance with the Declaration of Helsinki (2000). Initially, twenty two subjects (eleven men and eleven women) performed two incremental graded cycling tests (one in an upright and one in a supine posture). Then, of those twenty two, ten subjects (five men and five women) performed three high intensity constant load tests to the point of failure (one in upright and two in supine postures).

Subjects were tested in the department of physiology, Trinity College Dublin. All participants were tested using the same apparatus at room temperature, and there was at least 48 hours between each exercise session. To avoid potential impact of diurnal factors on exercise performance, subjects performed all the exercise sessions at the same time of the day. Subjects were requested to refrain from alcohol, caffeine and cigarettes for the twelve hours preceding the testing session as well as from any strenuous exercise for the twenty-four hours preceding the testing session.

2.2.2.2 *Graded tests*

Prior to each of the graded tests, height and weight, hip and knee angles, and the estimated distance of the hydrostatic column of blood between the heart and the arteries engaged in cycling were measured for each subject (see section2.2.3.1:anthropometric and body fat measurements). Exercise was conducted using an electrically-braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands).

For men, the exercise test began at 60 W for three minutes, and thereafter the power output was increased in a step-wise manner by 30 W each three minutes until failure occurred. The exercise protocol was the same for women except the initial power output was 30 W. The cadence required during all exercise sessions was 60 revolutions per minute (rpm). Failure was defined as the inability to maintain a minimum cadence (i.e. 50 rpm) for 3 seconds. The time to failure was recorded and used to represent cycling performance.

Prior to exercise, expired air was collected (using Douglas bags) during the last minute of two 5 minute rest periods. During the first 5 min period of rest (as a control measurement) subjects seated on a chair in the quiet testing bay, whereas during the second rest period, subjects assumed the position in which they would exercise (upright or supine) with their feet placed in the pedal straps and remained motionless. Throughout each exercise, expired air was collected during the last 30 s of each power output and during the last 30 s of maximal exercise. Heart rate was continuously recorded using a HR monitor (Polar S610TM, Finland) .

2.2.2.3 *Constant-load tests*

During the upright and one of the supine constant load tests (supine ABS), subjects cycled at a relative intensity equivalent to 80% of the maximum workload achieved during the upright graded test (80%Wload max UPR), and during the second supine test (supine REL), at 80% of the maximum workload

achieved during the supine graded test (80% Wload max SUP). Therefore, we were able to compare constant load tests at two postures working at absolute intensities (intensities for both tests were equivalent to 80%Wload max UPR), and at relative intensities (intensity for the upright posture 80%Wload max UPR, and for the supine posture, 80% Wload max SUP). Table 2.1 illustrates the experimental design of the study.

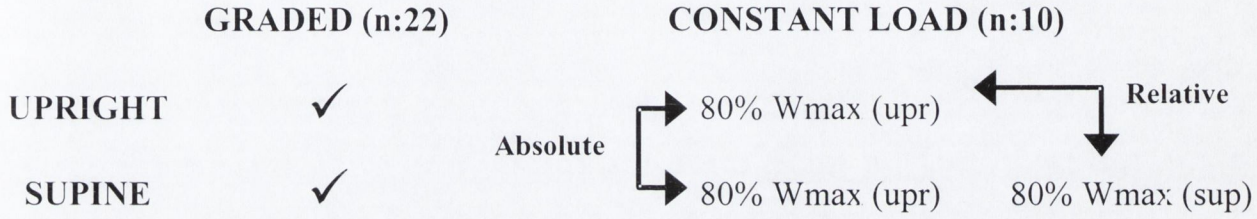


Table 2.1: Experimental design: Subjects performed initially two graded tests, one in the upright, one in the supine; and then three constant-load tests, one in the upright (at an intensity of 80% Wload max achieved in the upright graded test) and two in the supine (at intensities of 80% Wload max achieved in the upright or supine graded tests). Absolute indicates that a comparison between the two bouts selected is a comparison of absolute workloads, whereas relative indicates that a comparison between the two bouts selected is a comparison of relative workloads.

During constant-load tests expired air was collected during the last minute of the two resting periods and then over each consecutive 15 s period for the first minute of exercise (i.e. 15, 30, 45 and 60 s), and over each 30 s period during the second minute (i.e. 90 and 120 s). This was done to estimate the initial response of $\dot{V}O_2$. When $\dot{V}O_2$ values were calculated, they were plotted at the mean time points of collection (i.e. 7.5, 22.5, 32.5 and 52.5 s for the first minute, and 75 and 105 s for the second minute). Expired air was also collected during the last 30 s of maximal exercise.

All tests were carried out using a random cross-over design. such that during the graded tests half the subjects (n:11) exercised first in the supine posture and the other half (n:11) in the upright. During the constant-load tests one third of the subjects (n:3) exercised first in the upright, another third (n:3) on one of

the supine tests (i.e. 80%Wload max UPR), and the last third (n:4) on the second supine test (i.e. 80% Wload max SUP). The subsequent order of the constant-load tests was counterbalanced.

Before each graded and constant-load test subjects warmed up on the ergometer for 10 minutes at a comfortable workload. The cadence required during the warm up and all exercise sessions was 60 revolutions per minute (rpm).

2.2.3 Equipment and measurements

2.2.3.1 Anthropometric and body fat measurements

a) Weight and height

Height (cm) was measured using a Seca™ statiometer (Seca Ltd., Germany) and body mass (kg) was measured using a platform-beam scales (AVERY, England).

b) Body fat

To determine lean body mass, body fat percentage was assessed in the 10 subjects that performed the constant load tests. Skinfold thickness measurements (mm) were taken with a Harpenden skinfold calliper (Baty Ltd., UK) at four specific anatomical sites. All measurements were made while subjects were standing comfortable at the following standard sites:

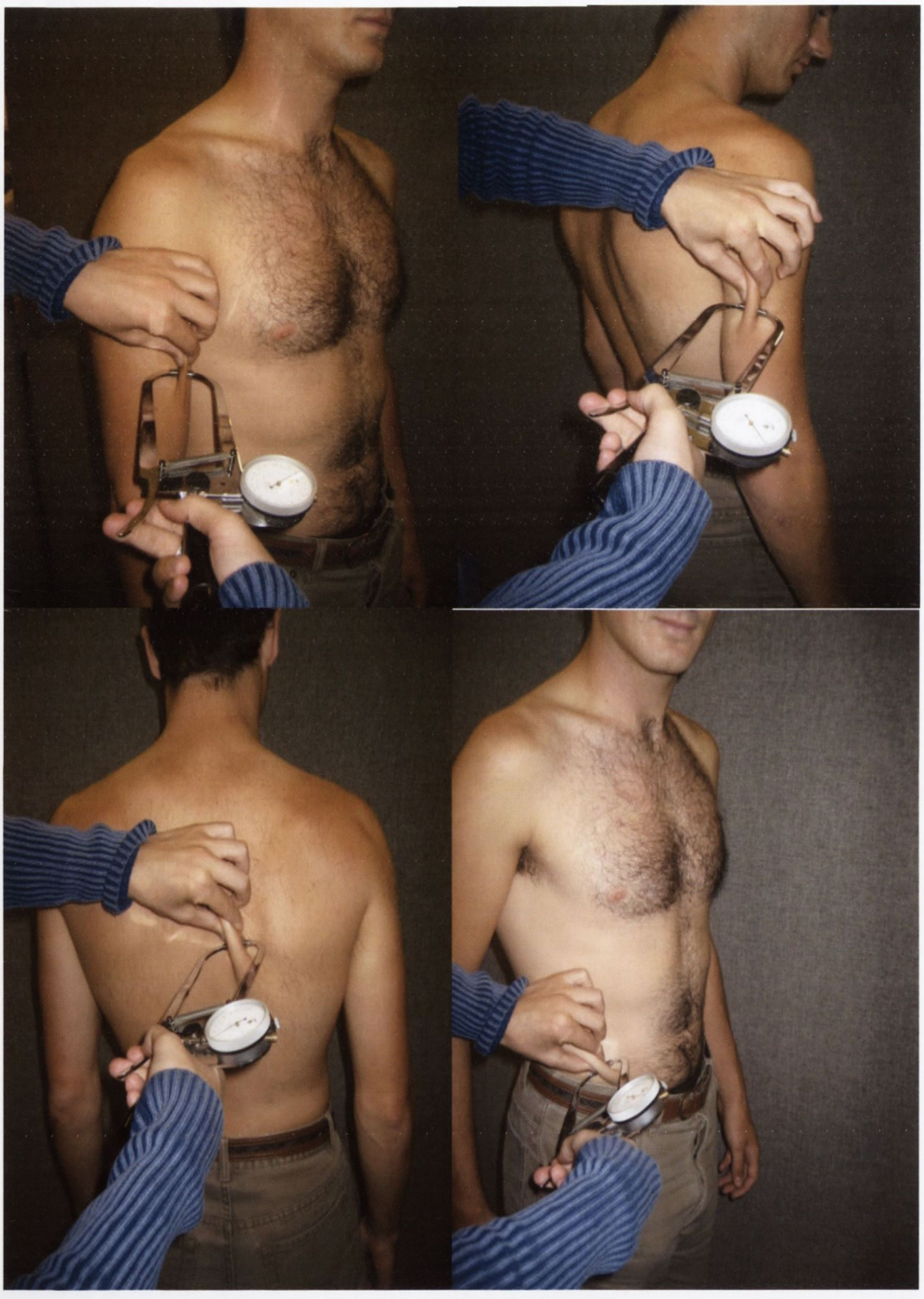
- Biceps: measured midway between the acromion process and the elbow joint, on the dominant arm, with the elbow fully extended (Figure 2.1.A).
- Triceps: measured midway between the acromion process and the elbow joint on the dominant arm, with the elbow fully extended (Figure 2.1.B).
- Sub-scapular: measured directly below the inferior angle of the scapula on the dominant side (Figure 2.1.C).

- Suprailiac: measured directly above the anterior superior iliac spine on the same side (Figure 2.1.D).

The four skinfold thickness measurements were summed and a percentage body fat was estimated using standard tables (Durnin & Womersley, 1974).

A)

B)



C)

D)

Figure 2.1: Skinfold sites. An example of the skinfold thickness technique used to measure percent body fat. Sites of measurements are shown in panels A (biceps), B (triceps), C (subscapular) and D (suprailiac).

c) Hydrostatic column length

The distance from 5 cm below the manubriosternal angle, point where the right atrium lies (landmark 1), to the midpoint of the two lateral iliac crests (landmark 2) was used to estimate the length of a hydrostatic column of blood between the heart and arteries feeding the proximal muscles engaged in cycling (Figure 2.2).

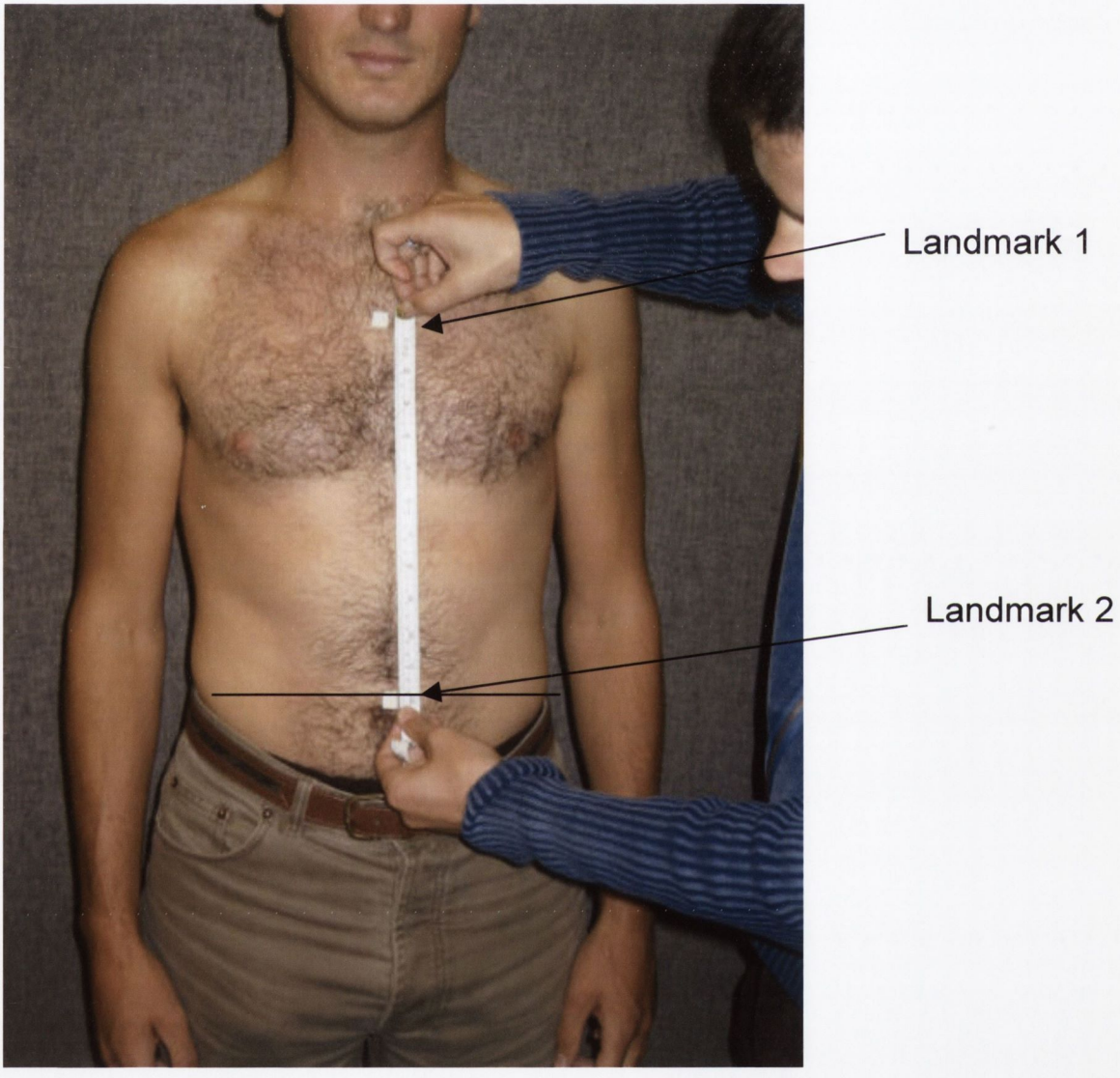


Figure 2.2: Estimated length of the hydrostatic column. Landmark 1 represents the right atrium, and landmark 2 the origin of the arteries that feed the proximal muscles engaged in cycling.

2.2.3.2 *Cycle ergometer*

Exercise was performed on an electrically-braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands) that maintained power output independent of the cadence. During the supine test, a LCD screen attached to the ergometer allowed the subjects to get feedback of the cadence. However, performance feedback (workload and time) was not given to subjects, and was hidden from the LCD screen at all times.

2.2.3.3 *Posture*

For both graded and constant load cycle tests subjects performed and were set up in the upright and supine postures according to the descriptions below.

a) *Upright posture*

During upright exercise subjects cycled with the upper body and head in the vertical plane with the arms held loosely by the side (Figure 2.3). Some verbal encouragement was given throughout the test to keep the body as vertical as possible. This was done so as to minimize muscle activation and O_2 consumption associated with postural support in the usual cycling position (i.e. gripping the handlebars).

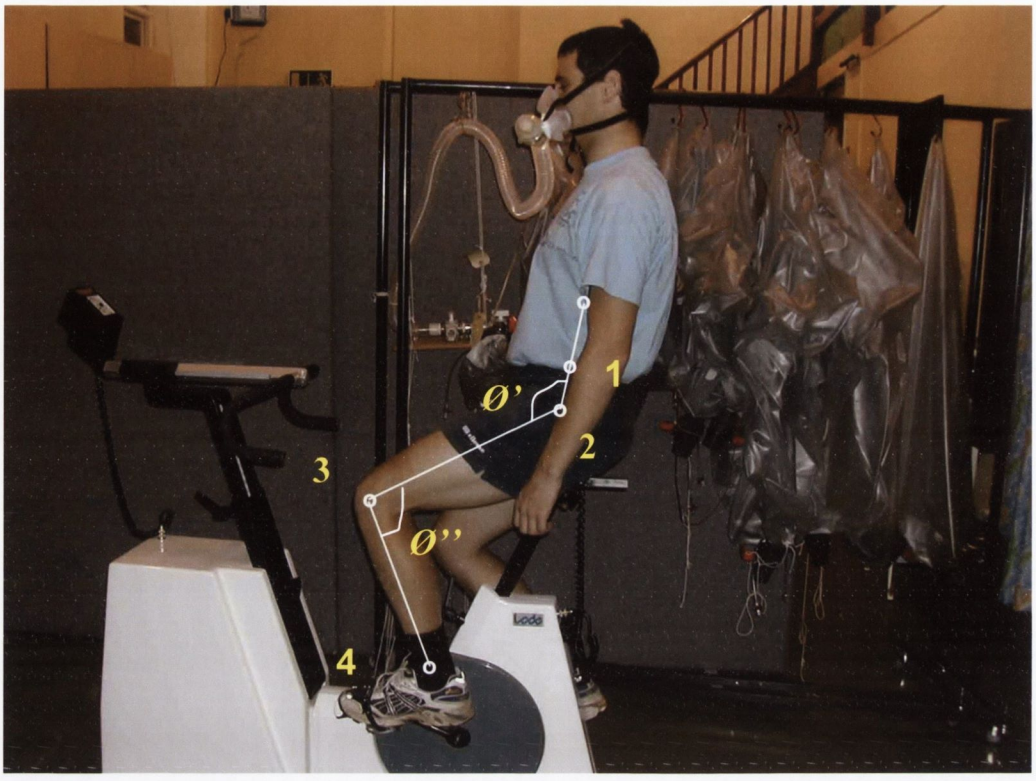


Figure 2.3: Upright posture. One subject exercises in the upright posture. The four numbers indicate the four landmarks (1: pelvis, 2: hip joint, 3: knee joint and 4: ankle joint) and Ø' represent the hip angle and Ø'' the knee angle (see below).

b) *Supine posture*

During supine exercise subjects lay comfortably horizontal behind the ergometer on an inflated lilo, arms loosely by their side (Figure 2.4). The ergometer was raised 20 cm above the floor by front and rear wooden platforms to allow sufficient foot clearance above the floor during exercise. During preliminary testing it was noticed that at higher increments in the supine position subjects tended to slip backwards which impaired maximum exercise performances. To combat this, subjects wore a harness which provided a secure attachment to the ergometer and prevented any slippage of the subjects across the floor away from the cycle ergometer. Foot clips provided by the pedals ensured that the feet did not slip out of position.

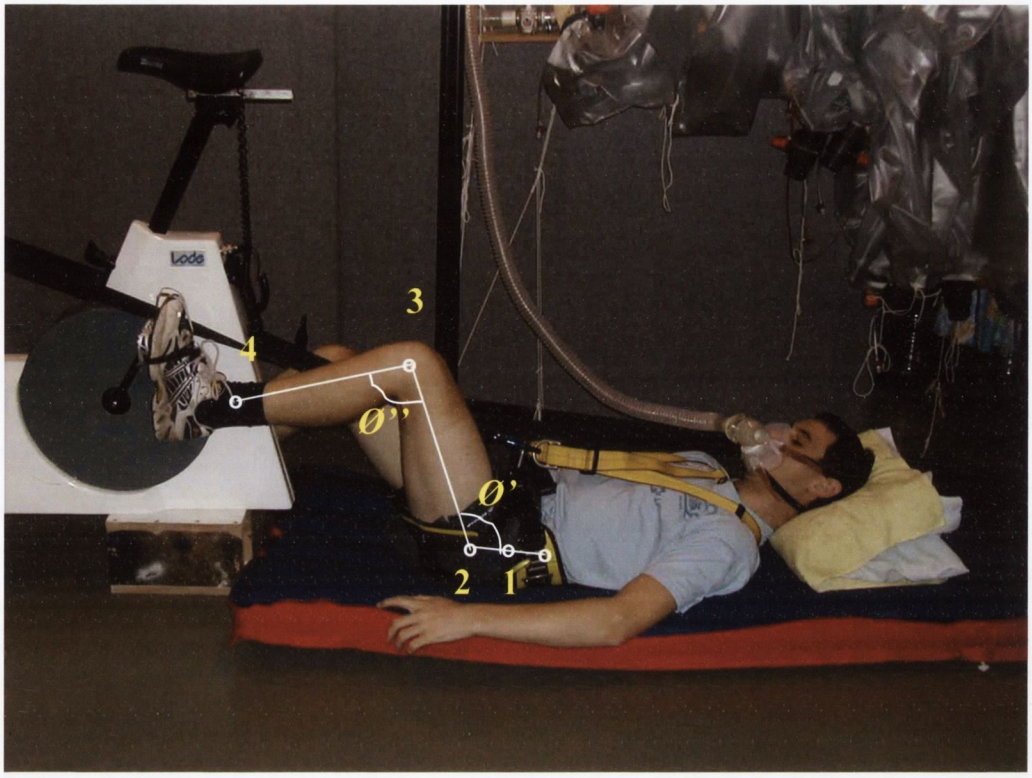


Figure 2.4: Supine posture. One subject exercises in the supine posture. The four numbers indicate the four landmarks (1: pelvis, 2: hip joint, 3: knee joint and 4: ankle joint) and Ø' represent the hip angle and Ø'' the knee angle (see below).

2.2.3.4 Hip and knee angles

Hip and knee angles were measured by a Baseline™ goniometer so that they were similar between the two positions. Four anatomical landmarks were used which guaranteed that these angles were reproducible throughout the subject population indicated by the circular white points in figures 2.3 and 2.4:

1) The anterior superior iliac spine (pelvis)
2) The greater trochanter (hip joint)
3) The midpoint between the external tibial and femoral condyl (knee joint)
4) The lateral malleolus (ankle joint)

The hip and knee angles were established first in the supine posture by subjects placing their strapped left foot proximally perpendicular to the floor

(Figure 2.4). From this position, using the anatomical landmarks, the knee and hip angles were measured. Then, during the tests in the upright posture, subjects placed their strapped left foot anterior and parallel to the floor (Figure 2.3), and adjusting the height of the saddle similar knee and hip angles were achieved. Thus, the alignment of body parts was very similar in the two postures with the essential difference being the body's orientation to the gravitational vector.

2.2.3.5 Respiratory measurements

Whole-body oxygen consumption ($\dot{v}O_2$) and minute ventilation ($\dot{v}_E$) were calculated based on standard equations (Altman *et al.*, 1958). Two different O_2 and two different CO_2 analysers (for O_2; P.K. Morgan, Kent, England; and Taylor Servomex Limited, Sussex, England; and for CO_2; Engstrom Eliza, Gambo Engstrom, Bromma, Sweden; and P.K. Morgan Ltd, Kent, England) were used. However, for all the tests performed by each subject the same respiratory equipment was used. Peak $\dot{v}O_2$ and peak $\dot{v}_E$ were taken as the highest values observed.

Prior to each test the O_2 and CO_2 analysers were calibrated using two test gases, environmental air (21% O_2, 0.04% CO_2 and balance N_2) and an alpha standard certified gas (15% O_2, 5% CO_2 and balance N_2; BOC Ltd., UK). The two different vacuum pumps used (U.G.I. Meters Ltd., England or Cranlea and company, Birmingham, England) were calibrated using fixed volumes in 10 to 100L Douglas bags, filled with room air using a 3 litre syringe (Hans Rudolf Ltd., USA). The equations obtained from the curve of nearest fit from this data was used to calculate the actual expired volume (Appendix 4 and 5). Expired air was collected using a Hans Rudolph face mask (Hans Rudolf Ltd., USA).

2.2.3.6 HR measurements

Heart rate was continuously recorded using a Polar S610TM heart rate monitor (Polar Electro, Finland). This unit consisted of a chest belt containing a transmitter unit. To enhance electrical contact, a small amount of ECG gel

(Parker Laboratories Inc., USA) was placed on the belt in contact with the skin. The transmitter used a 10kHz carrier wave upon which the ECG pulse was superimposed. A coded watch-like receiver unit displaying a digital readout of the subjects heart rate was affixed under the chest belt posterior (during the upright tests) or anterior (during supine tests), in order that heart rate could be monitored by the examiner but not by the subject. Peak heart rate (peak HR) was taken as the highest value observed.

2.2.4 Statistical analysis

A two-way (between-within) ANOVA was used to identify main effects (gender, posture) or interactions (gender by posture) for performance and all physiological variables. A repeated measures ANOVA was used to identify effects or interactions for the $\dot{V}O_2$ and HR responses during exercise. Differences were located using a Tukey's HSD test. Associations between metabolic and exercise variables were analysed using a Pearson Product Moment correlation coefficient. All data are expressed as mean ± SD. The level of significance was set to $p<0.05$.

2.3 RESULTS

2.3.1 Subjects characteristics

Men were taller and heavier than women and the estimated length of the hydrostatic column of blood between the heart and pelvis was longer in men than women (Table 2.2). Consequently, $\dot{v}O_2$ and $\dot{v}_E$ values were standardised according to body mass and therefore enabled gender comparisons using "relative" values (i.e. $\dot{v}O_2$ expressed as $ml.kg^{-1}.min^{-1}$).

Table 2.2: Subjects characteristics (mean ± SD)

	MEN		WOMEN	
	Incremental test (n=11)	Constant-load tests (n=5)	Incremental tests (n=11)	Constant-load tests (n=5)
Height (cm)	182 ± 9 *	181 ± 8 *	164 ± 8	165 ± 2
Weight (kg)	75.9 ± 13.3*	77.3 ± 7.2*	59.9 ± 9.9	62.2 ± 8.0
Age (yr)	24.0 ± 3.4	23.4 ± 3.9	26.2 ± 5.7	27.0 ± 5.9
Hydrostatic column (cm)	31.6 ± 3.0 *	31.8 ± 2.3 *	27.8 ± 3.0	26.9 ± 2.0

* significantly different from women $p < 0.05$

2.3.2 Graded tests

Exercise times and peak physiological responses for all subjects (n = 22) are shown in Table 2.3. Exercise times were longer, and maximum power achieved (peak power) and peak HR were significantly higher in the upright posture for both men and women. In contrast, peak $\dot{v}O_2$ and peak $\dot{v}_E$ were not significantly different between postures. Exercise time, peak $\dot{v}O_2$ and absolute peak power were significantly greater in men compared to women, but not relative peak power (expressed as $W.kg^{-1}$). There was no interaction between gender and posture for these variables.

Exercise times and peak physiological responses were similar for the 10 subjects that performed the constant load tests compared to the responses observed for all subjects, and are shown in Table 2.3.

Table 2.3: Graded test exercise times and peak physiological responses (mean ± SD)

	Men		Sig.	Women	
	Upright	Supine		Upright	Supine
All subjects (n=22)					
Cycle Time (min)	20.3 ± 3.7 *	18.3 ± 2.9	§	15.4 ± 3.0 *	13.9 ± 3.2
Peak Power (W)	248 ± 38 *	226 ± 34	§	169 ± 31 *	150 ± 27
Peak power ($W.kg^{-1}$)	3.31 ±0.49 *	3.02 ±0.43		2.89 ±0.73 *	2.55 ±0.58
Peak $\dot{V}O_2$ ($ml.kg^{-1}.min^{-1}$)	41.2 ± 7.7	39.7 ± 8.1	§	31.9 ±11.4	30.5 ±12.2
Peak $\dot{V}_E$ ($ml.kg^{-1}.min^{-1}$)	1054 ±459	919 ±331		845 ±335	774 ±261
Peak HR ($beats.min^{-1}$)	184 ± 19 *	172 ± 21		179 ± 11 *	164 ± 11
Subjects who performed the constant-load tests (n=10)					
Cycle Time (min)	21.2 ±3.6*	18.5 ± 3.1	§	16.4 ± 2.3 *	14.0 ± 3.1
Peak Power (W)	252 ± 46 *	228 ± 40	§	174 ±25 *	150 ± 30
Peak power ($W.kg^{-1}$)	3.3 ± 0.7*	3.0 ± 0.5	§	2.4 ± 0.4 *	2.2 ± 0.4
Peak $\dot{V}O_2$ ($ml.kg^{-1}.min^{-1}$)	42.8 ± 4.8	43.1 ± 7.3	§	33.9 ± 3.8	30.7 ± 7.4
Peak $\dot{V}O_2$ ($ml.min^{-1}.kg^{-1}$ lean)	50.6 ± 7.4	51.1 ±11.8		45.8 ± 3.2	41.5 ± 9.0
Peak $\dot{V}_E$ ($ml.kg^{-1}.min^{-1}$)	648 ± 210	647 ± 230		788 ±346	659 ±205
Peak HR ($beats.min^{-1}$)	179 ± 7 *	166 ± 13		182 ± 10 *	164 ± 11

* significantly different from supine ($p<0.05$)

§ significant difference between men and women ($p<0.05$)

2.3.3 Constant-load tests

Exercise times and peak physiological responses for the subjects that completed the constant-load tests (n=10) are shown in Figure 2.5 and Table 2.4. Exercise time was significantly longer in the upright posture than supine ABS for all subjects (main effect = posture, n=10, Figure 2.5). Furthermore, there was a significant interaction between gender and posture in time to failure, such that the difference in exercise time between upright and the supine ABS test was greater for men than for women.

When comparing upright and supine ABS exercise (ie: at 80%Wload max UPR) peak HR was significantly higher in the upright compared to supine posture in both men and women, whereas peak $\dot{v}O_2$ was not different between postures in men or women (Table 2.4). These values (peak $\dot{v}O_2$ and peak HR) during the ABS constant load tests in relation to the values achieved during the graded tests (i.e. % graded tests) were not different between men and women or between upright and supine postures (Table 2.5).

During the supine REL exercise bout (ie.: at 80%Wload max SUP), peak $\dot{v}O_2$, peak HR and peak $\dot{v}_E$ for men and women were significantly higher during upright compared with supine REL (Table 2.4). However, time to failure was not significantly different between upright and supine REL postures (Table 2.4).

In the whole group (men and women) during the ABS constant load tests, subject height was significantly and positively correlated with the absolute (i.e. expressed as minutes) and relative (i.e. expressed as percentage) difference in exercise time between the upright and supine ABS exercise bouts (Figures 2.6A and 2.6B). A significant and positive correlation was also found between the length of the hydrostatic column between the heart and the pelvis and the absolute difference (Fig. 2.6C), but not the relative difference ($r=0.53$, $p=0.12$), in exercise time between the upright and supine ABS tests. In contrast, body weight was not significantly correlated with changes in exercise time.

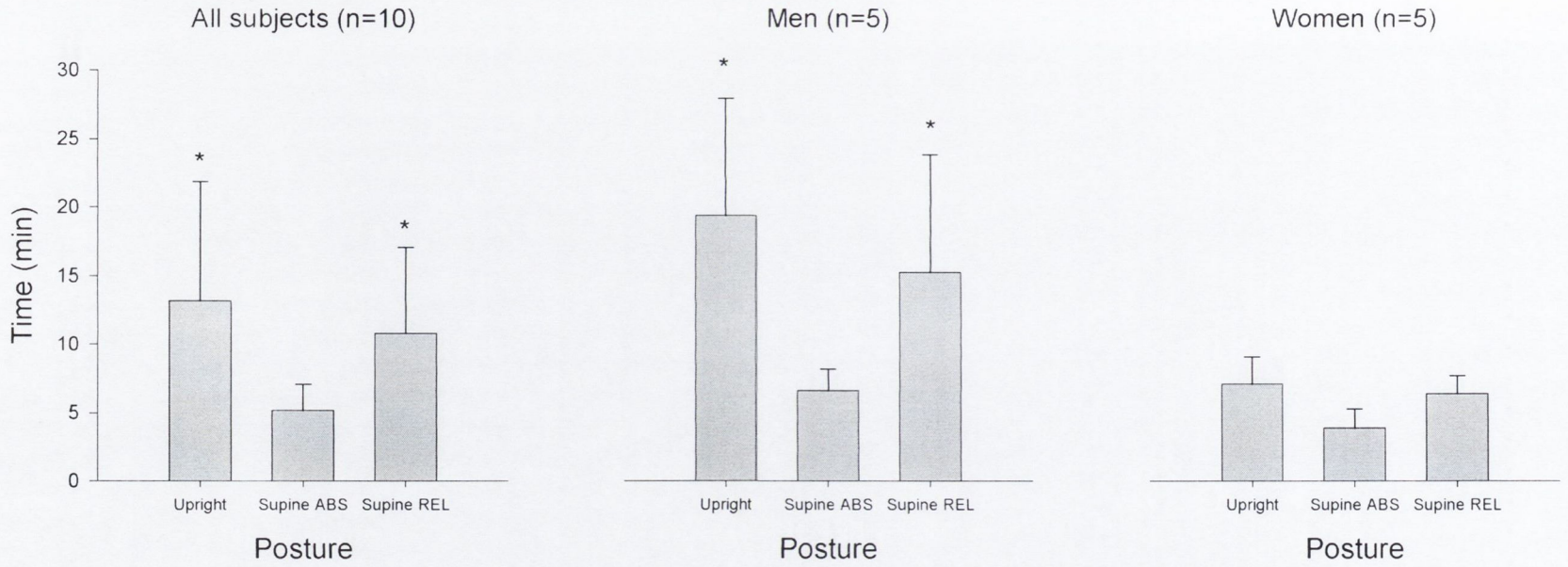


Figure 2.5: Exercise times to failure (mean ± SD) during constant-load tests in all subjects (n=10), men (n=5) and women (n=5).

* indicates significantly different from supine ABS ($p<0.05$).

Table 2.4: Constant-load exercise times and peak physiological responses (mean ± SD, n=10).

		Men (n=5)	Sig.	Women (n=5)
Cycle Time (min)	Upright	19.4 ± 8.5 *	§	7.1 ± 2
	Supine (ABS)	6.6 ± 1.6		3.9 ± 1.4
	Supine (REL)	15.3 ± 8.6 ‡	§	6.4 ± 1.3
Peak $\dot{V}O_2$ ($ml.kg^{-1}.min^{-1}$)	Upright	33.8 ± 5.1 †		28.3 ± 4.5 †
	Supine (ABS)	32.1 ± 7.7		25.3 ± 4.9
	Supine (REL)	30.1 ± 5.6		23.5 ± 5.3
Peak HR ($beats.min^{-1}$)	Upright	177 ± 7 * †		175 ± 6 * †
	Supine (ABS)	158 ± 10		162 ± 10
	Supine (REL)	157 ± 6		149 ± 13
Peak $\dot{V}_E$ ($ml.kg^{-1}.min^{-1}$)	Upright	1236 ±154 †		1035 ±159 †
	Supine (ABS)	1174 ± 34 ‡		900 ±222 ‡
	Supine (REL)	909 ±167		831 ±150
Peak RER	Upright	1.04 ±0.04		1.05 ±0.12
	Supine (ABS)	1.22 ±0.13		1.09 ±0.12
	Supine (REL)	1.12 ±0.11		1.06 ±0.07

* upright significantly different from supine ABS $p < 0.05$

† upright significantly different from supine REL $p < 0.05$

‡ supine ABS significantly different from supine REL $p < 0.05$

§ men significantly different from women $p < 0.05$

Table 2.5: Constant-load (80% Wload max UPR) values for peak $\dot{V}O_2$ and peak HR (mean ± SD) in relation to the graded tests (n=10).

	Men (n=5)		Sig.	Women (n=5)	
	Upright	Supine ABS		Upright	Supine ABS
Peak $\dot{V}O_2$ ($ml.kg^{-1}.min^{-1}$)					
Graded test	42.8 ± 4.8	43.1 ± 7.3		33.9 ± 3.8	30.7 ± 7.4
Constant-load test	33.8 ± 5.1	32.1 ± 7.7		28.3 ± 4.5	25.3 ± 4.9
As a % of graded test	83 ± 22	80 ± 31		88 ± 16	89 ± 22
Peak HR ($beats.min^{-1}$)					
Graded test	179 ± 7 *	166 ± 13		182 ± 10 *	164 ± 11
Constant-load test	180 ± 7 *	158 ± 10		175 ± 6 *	162 ± 10
As a % of graded test	101 ± 1	96 ± 8		96 ± 5	98 ± 4

* significantly different from supine ($p<0.05$)

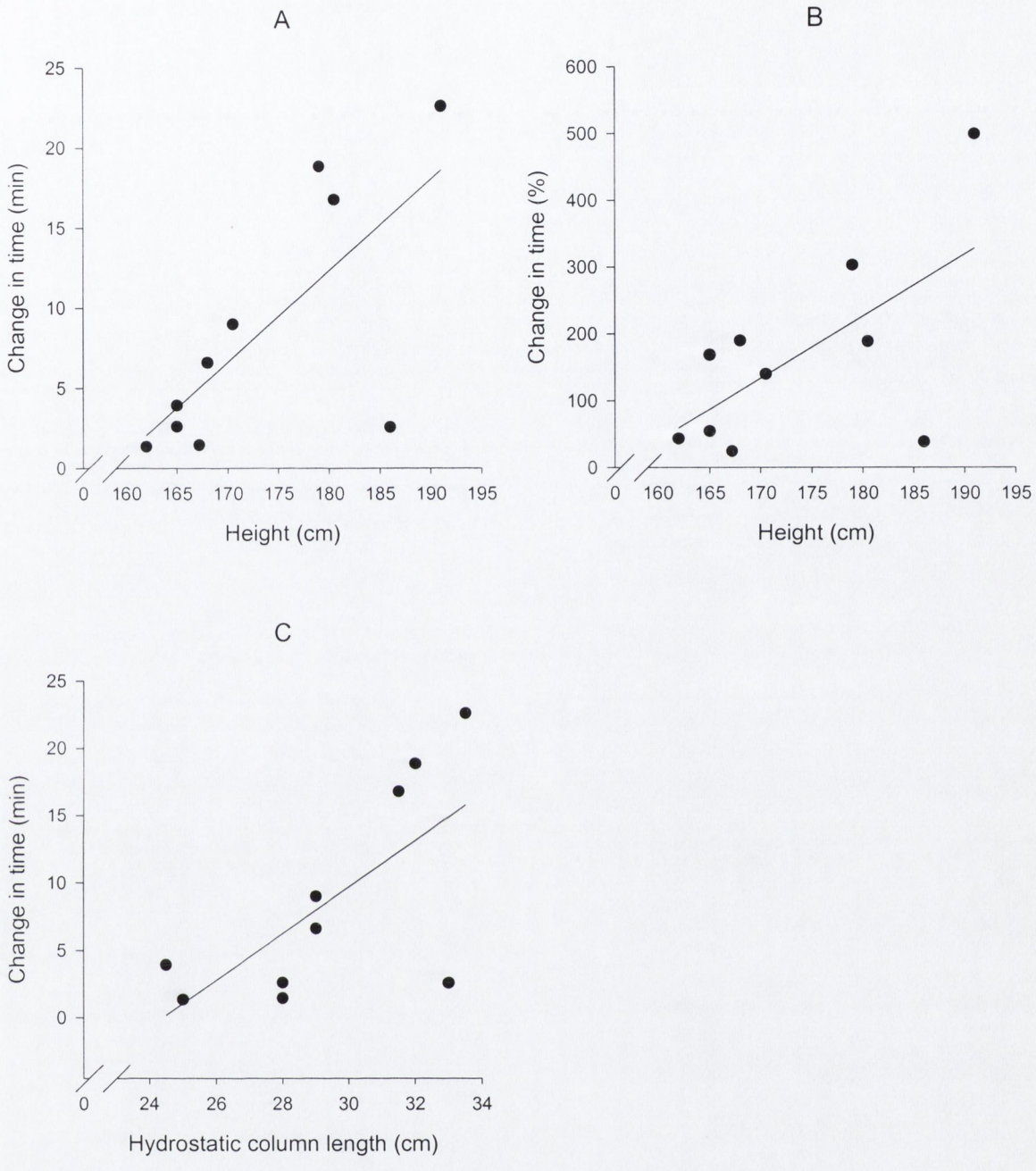


Figure 2.6: Correlations between exercise variables and anthropometry. **A**: Height versus the change in time to failure (upright minus supine ABS) during the constant-load tests; $r=0.72$, $p<0.05$. **B**: Height versus the percent change in time to failure (upright minus supine ABS) during the constant-load tests; $r=0.63$, $p<0.05$. **C**: Length of the hydrostatic column versus the change in time to failure (upright minus supine ABS) during the constant-load tests; $r=0.68$, $p<0.05$.

2.3.4 Initial $\dot{V}O_2$ and HR responses (upright vs supine ABS)

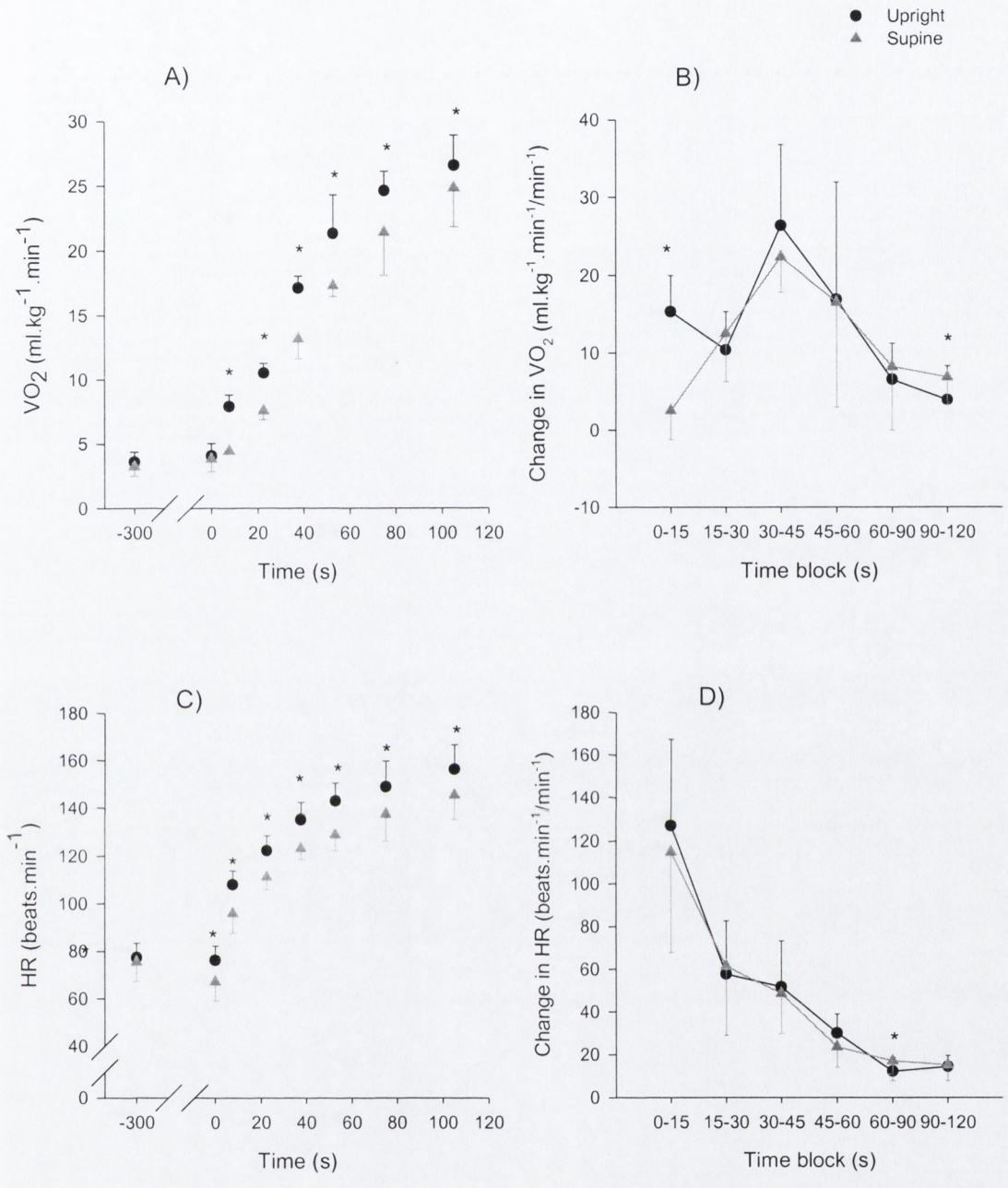
2.3.4.1 Initial $\dot{V}O_2$ responses

Resting $\dot{V}O_2$ was similar in the upright and supine posture. Despite this, $\dot{V}O_2$ was significantly higher in the upright posture compared with the supine posture at all data points during the first two minutes of exercise (Figure 2.7A). The change in $\dot{V}O_2$ was significantly higher in the upright compared to supine posture from 0 to 15 s, whereas changes in $\dot{V}O_2$ during subsequent periods in the first minute were not affected by posture (Figure 2.7B). There were no significant interactions between men and women, such that the responses between upright and supine were similar for both groups. End-exercise $\dot{V}O_2$ (i.e. peak $\dot{V}O_2$) was not significantly different between postures in either men or women (Table 2.4).

The difference in $\dot{V}O_2$ (both, expressed as $ml.kg^{-1}.min^{-1}$ and as ml. hydrostatic column $cm^{-1}.min^{-1}$) over the first minute (i.e. $\dot{V}O_2$ at 60 s minus pre-exercise $\dot{V}O_2$) between upright and supine ABS posture was significantly correlated with the absolute change in the final time to failure between the upright and supine absolute intensity constant load tests (Figure 2.8).

2.3.4.2 Initial HR responses

Resting HR (on the chair) was similar between the two postures. However, resting HR (on the bike) was significantly higher in the upright posture compared with the supine posture (Figure 2.7C). HR during the first two minutes of exercise was significantly higher in the upright posture at all data points (Figure 2.7C). The change in HR over the four 15 s periods in the first minute of exercise was not affected by posture in men and women (Figure 2.7D). There were no significant interactions between men and women such that the responses between upright and supine were similar for both groups. End-exercise HR (i.e. peak HR) was significantly higher in the upright posture for men and women (Table 2.4).



* indicates significantly different between upright and supine ABS posture ($p<0.05$)

Figure 2.7: Initial $\dot{V}O_2$ and HR responses. **A**: $\dot{V}O_2$ response (mean ± SD) at the beginning of exercise (0 –120 s). **B**: Change in $\dot{V}O_2$ (mean ± SD) at time blocks of 15 s during min 1 and time blocks of 30 s during min 2. **C**: HR response (mean ± SD) at the beginning of exercise (0 –120 s). **D**: Change in HR (mean ± SD) at time blocks of 15 s during min 1 and time blocks of 30 s during min 2.

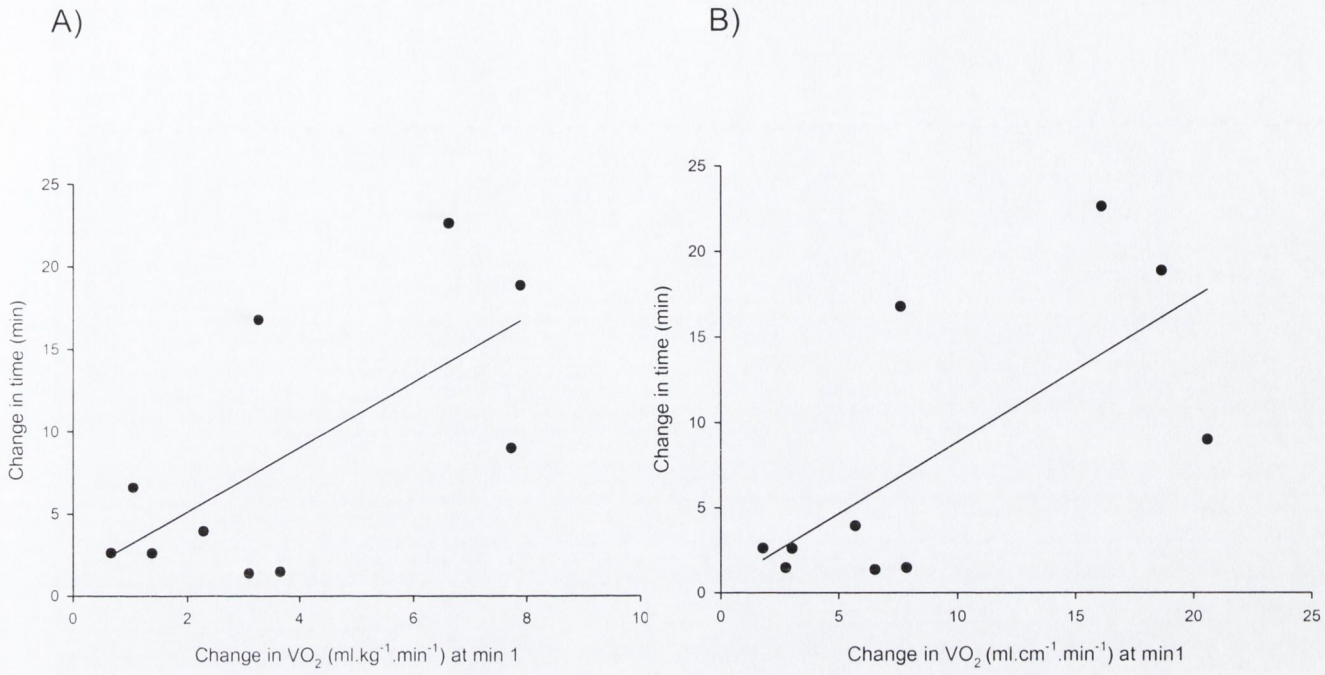


Figure 2.8: Correlation between metabolic and exercise variables. The difference between upright and supine ABS for the change in $\dot{v}O_2$ over the first 60s versus the change in time to failure (upright minus supine ABS) during constant load tests. A: $\dot{v}O_{2v}$ is expressed as ml/kg/min; r=0.67, $p<0.05$, and B: $\dot{v}O_2$ is expressed as ml/hydrostatic column cm/min; r=0.7, $p<0.05$.

2.4 Discussion

The main findings of the present study show that:

1) performance time is significantly longer in an upright posture compared to a supine posture during both incremental graded exercise and absolute constant-load cycling exercise.
2) that this effect is 10-fold larger for constant-load than graded exercise.
3) that this effect is similar in both men and women during graded exercise, but of a significantly greater magnitude for men compared to women during the absolute constant load exercise; and
4) that this effect during the absolute constant load exercise is correlated with the change in $\dot{V}O_2$ between the two postures during the early period of exercise, and also with height and the estimated length of the hydrostatic column of blood between the heart and the pelvis of the subjects.

2.4.1 Graded tests

During the graded tests, exercise time significantly increased by 10% in both men and women in the upright posture (table 2.3). These effects are comparable to the increases (~10-15%) in cycling time observed under similar exercise conditions in studies where the majority of subjects were men (Eiken, 1988, Leyk *et al.*, 1994a) or equally men and women (Terkelsen *et al.*, 1999). However, Terkelsen and coworkers did not assess gender differences with respect to the relative changes in exercise time between upright and supine postures. Therefore, the present study adds the fact that the relative increase in exercise time from supine to upright posture during incremental graded exercise is similar in both men and women.

2.4.2 Constant load tests

This is the first study to investigate exercise time to the point of failure in both upright and supine postures during high intensity constant-load cycling exercise. Time to failure was greater by up to 160% (n=10) in the upright compared to supine posture during absolute constant load exercise (Table 2.4). This effect was 10-fold larger than that observed during the incremental graded exercise in the same subjects (~16%, n=10). Moreover, there was a significant interaction between gender and posture, such that the postural effect on exercise time was significantly larger in men (mean increase = 194%) than women (mean increase = 82%, Table 2.4, Figure 2.5). Unlike the small postural effect on graded exercise performance, these data demonstrate that changes in posture can exert a very large effect on exercise performance when it is conducted at a constant and high intensity.

Exercise time to failure during high-intensity cycling exercise in the upright posture has been reported to be very sensitive to oxygen availability (Adams & Welch, 1980). These investigators assessed exercise cycling time to exhaustion at approximately 90% $\dot{V}O_2$ max in six subjects on three occasions breathing oxygen concentrations of either 17% (hypoxia), 21% (normoxia) or 60% (hyperoxia). They observed that performance times were longer ($p<0.05$) with hyperoxia than with normoxia or hypoxia, and that hypoxic exercise was associated with increased lactate levels. Consistent with this, the slower rate of increase (i.e kinetics) in $\dot{V}O_2$ during upright heavy exercise reflects a relative inadequate perfusion and oxygen delivery to the working muscles (Gerbino *et al.*, 1996, Macdonald *et al.*, 1997). Macdonald and colleagues (1997) measured the kinetics of $\dot{V}O_2$ at the beginning of a heavy exercise during normoxic and hyperoxic gas breathing conditions, and observed that $\dot{V}O_2$ kinetics were faster during hyperoxia than normoxia. In addition, Casaburi and coworkers (1987) observed that training-induced improvements in $\dot{V}O_2$ kinetics (i.e. faster kinetics) positively correlate with a decrease in end-exercise blood lactate demonstrating that the slower rate of increase in $\dot{V}O_2$ during heavy cycling exercise is linked to a higher blood lactate concentration (Casaburi *et al.*, 1987). These data suggest

that cycling performance during high-intensity constant-load exercise is sensitive to changes in oxygen supply and that slower $\dot{V}O_2$ kinetics are associated with an inadequate oxygen supply and higher blood lactate levels.

In a direct comparison between upright and supine $\dot{V}O_2$ kinetics during high-intensity constant-load cycling tests, $\dot{V}O_2$ adjustment during the initial stages of exercise in the upright posture has been reported to be faster than during supine exercise (Convertino *et al.*, 1984, Koga *et al.*, 1999, Leyk *et al.*, 1994a). In addition, Leyk and coworkers (1994a) noted that lactate values of 4 $mM.l^{-1}$ occurred at significantly higher work rates when subjects were upright compared to supine. Therefore, these data show that faster $\dot{V}O_2$ kinetics observed in the upright posture appear to increase oxygen availability to active muscle, delay the rise in lactate, and consequently prolong performance time to failure which is similar to what we observed in the present study.

In the present study there was a significantly higher $\dot{V}O_2$ at the beginning of exercise (i.e. 0 to 15 s) in the upright compared to supine ABS posture (Figure 2.7B). The magnitude of this effect entirely explained the higher $\dot{V}O_2$ that was sustained during the first two minutes of exercise (Fig 2.7A). Furthermore, the postural effect on the change in $\dot{V}O_2$ over the first minute of exercise was positively correlated with the simultaneous effect on the absolute improvement in exercise time in the upright compared with the supine ABS posture (Fig 2.6D). These data are consistent with the idea that the postural effect on exercise performance is mediated by a faster response of oxygen uptake, and probably blood flow, to the working muscles.

The HR response for all data points during the first 2 min of exercise was significantly higher in the upright compared to supine posture (Figure 2.7C). However, these differences in HR were established at rest (on bike, Fig 2.7C) and the changes in HR over the four 15 s periods in the first minute of exercise, unlike the changes in $\dot{V}O_2$, were not affected by posture (Figure 2.7D). Therefore, these data suggest that HR does not appear to have contributed

primarily to the greater $\dot{V}O_2$ values observed during the first 15 s of exercise in the upright posture.

However, the main limitations of the usage of Douglas Bags in this study were:

a) During the onset of exercise expired air was collected over short time periods (i.e. every 15 sec over the first minute) rather than over a specific number of respiratory cycles. Thus, it was possible that expired gas from part or all of a single breath cycle was excluded from collection during changing of the Douglas bags from one collection period to another.

b) Usually oxygen uptake is measured using Douglas Bags under steady-state conditions. Subjects in this study performed the submaximal cycling tests at relatively high intensities, and therefore, it was unlikely that they reached steady-state values before the first minute of exercise.

2.4.3 Interaction effect (gender x posture)

The relatively larger postural effect on performance observed in men during the constant-load exercise might be attributed to a relatively greater effect on muscle perfusion and oxygen uptake. Men were significantly taller than women, and the estimated hydrostatic column of blood between the heart and arteries feeding proximal muscles engaged in cycling (i.e. gluteals) was also longer in men than women (Table 2.2). When the body is tilted from the supine to the upright posture the magnitude of the gain in venous and arterial hydrostatic pressure due to the effect of gravity on the fluids is proportional to the distance between the heart and the active vessels (Folkow *et al.*, 1971). Therefore, when the venous valves interrupt the vertical column of blood during the relaxation phase of a dynamic rhythmic exercise and the venous pressure falls towards zero, the transient increase in the pressure gradient across the active capillary bed and consequently blood flow, are proportional to the distance between the heart and the active limbs. This effect may explain the greater endurance times achieved by men. Consistent with this, we observed positive correlations

between height and both the absolute and relative differences in cycle time between the upright and supine position (Fig 2.6A and 2.6B). In addition, the estimated distance of the hydrostatic column operating between the heart and the proximal muscles engaged in cycling was also correlated with the absolute differences in cycle time between the upright and supine position (Figure 2.6C). These data raise the possibility that the magnitude of the postural effect on cycle performance induced by tilting the body upright is linked to the magnitude of the increase in perfusion pressure, and thus blood flow.

2.5 Conclusion

In conclusion, inclining the body from a supine position significantly improves endurance during constant-load high-intensity cycle test. This improvement is an order of magnitude larger than the postural effect seen for graded performance. Moreover, the postural effect on performance is larger for men than women and is related to height, the estimated distance of the hydrostatic column operating between the heart and the active limbs, and the early exercise response of oxygen uptake.

Chapter 3: **Effect of posture on the strength, endurance and fatigue of the plantar flexors**

3.1 Introduction

As demonstrated in the previous chapter (chapter 2) and also by other research groups, tilting the body from a horizontal to an upright position increases the maximal time to failure both during incremental (Eiken, 1988, Leyk *et al.*, 1994a, Terkelsen *et al.*, 1999) and high-intensity constant load (chapter 2) cycling exercise, which suggests that endurance during whole-body exercise is particularly sensitive to changes in perfusion pressure.

The postural effect on endurance during exercise of individual muscle groups is unknown, but there is evidence that muscle endurance is sensitive to changes in oxygen supply. It has been demonstrated that hypoxia reduces the time to failure during constant-load knee extension exercise in humans (Fulco *et al.*, 1996) or incremental plantar flexion exercise (Hogan *et al.*, 1999a), whereas hyperoxia increases the time to exhaustion during incremental plantar flexion exercise in humans (Hogan *et al.*, 1999a).

Muscle fatigue during exercise of individual muscle groups has been reported to be sensitive to changes in oxygen availability, perfusion pressure and blood flow. Hypoxia has been reported to accelerate the rate of fatigue during human knee-extensor exercise (Eiken & Tesch, 1984, Fulco *et al.*, 1996) and during human plantar-flexion exercise (Hogan *et al.*, 1999a). Consistent with this, increases in perfusion pressure have also been shown to increase muscle force production in the adductor pollicis muscle (Fitzpatrick *et al.*, 1996, Wright *et al.*, 1999). For instance, during electrically-induced submaximal contractions (40 %MVC) of the adductor pollicis muscle in humans, a sudden elevation in perfusion pressure induced by rapidly lowering the arm below heart level significantly increased muscle force production, whereas elevating the arm above the heart level significantly decreased muscle force production (Fitzpatrick *et al.*, 1996). Similarly, during electrically-induced contractions of the human triceps surae muscle (Cole & Brown, 2000) and in a canine hindlimb muscles (Hogan *et al.*, 1994) a reduction in blood flow significantly reduced muscle force production within a few seconds These data demonstrate that

muscle fatigue during moderately intense exercise is very sensitive to changes in perfusion pressure and muscle blood flow.

However, the effect of posture on the rate of fatigue or endurance time to failure during a voluntary intermittent constant-force or incremental exercise has never been studied in an isolated muscle group. The present study explored these effects on the human calf muscle, and therefore, the aims of the present study were to test the effect of body tilt,

1) on calf muscle strength and endurance during a plantar flexion graded test, and
2) on the strength, fatigue and endurance during a plantar flexion constant-force exercise.

In addition, to investigate if blood flow mediates the postural effect on performance, we tested the effect of posture on muscle fatigue and endurance time during incremental and constant-load tests with the blood flow to the legs occluded (i.e. ischaemia).

3.2 Material and methods

3.2.1 Subjects

In total nine subjects (8 male and 1 female) participated in these experiments (mean ± SD: age = 27 ± 4.3 yr; height = 179 ± 5 cm; weight = 76.2 ± 10.1 kg), 6 in experiment 1 and 6 in experiment 2; so, 3 subjects performed the 2 experiments. All subjects read the subject information sheet (Appendix 6) and provided informed written consent (see Chapter 2, Appendix 3) in accordance with the Ethical Committee of Trinity College Dublin. Subjects were non-smokers and free of any clinically significant disease that could affect their exercise performance. The procedures were conducted in accordance with the standards set by the Declaration of Helsinki (2000)

3.2.2 Experimental design

3.2.2.1 Overview

Two experiments were performed.

- Experiment 1: tested the effect of body tilt on muscle strength and endurance time during incremental calf exercise in five men and one women.
- Experiment 2: tested the effect of body tilt on muscle strength, endurance time and fatigue during constant-force calf exercise in six men.

In addition, in both experiments four subjects performed two further tests (in the horizontal and in one of the tilt angles) with the blood flow to the legs occluded with a thigh cuff (i.e. ischaemia), to explore the role of the peripheral circulation in the postural effects observed.

To avoid potential impact of diurnal factors on exercise performance, subjects performed all the exercise sessions at the same time of the day. Subjects were

requested to refrain from alcohol and caffeine for the 12 hours preceding the testing session as well as from any strenuous exercise for the 24 hours preceding the testing session.

3.2.2.2 Graded exercise (Experiment 1)

In Experiment 1, muscle strength and maximal intermittent graded muscle performance was assessed at three tilt angles (0°, 47° and 90°). After two familiarisation sessions (Appendix 7), the maximum force (F_{max}) at the three tilt angles was determined on one day and then, on three subsequent days separated by at least 72 hours, time to failure during a graded test was determined at the same tilt angles. The order of the angles at which strength and endurance tests were performed was randomised using a cross-over design, so that one third of the subjects (n=2) performed their graded tests first at 0°, the second third (n=2) at 47° and the last third (n=2) at 90° . Then, in subsequent tests, the order was counterbalanced.

During the graded test, subjects performed isometric muscle contractions intermittently so that the calf contracted for 3 s and relaxed for 3 s. The initial force to be sustained in each contraction was 100N, and thereafter, the force was increased by 100N every 3 minutes. The test was stopped when the subject was unable to sustain the required force for two consecutive contractions (Figure 3.1). At inclined positions, the graded test was started within 15 s of tilting the subject from the horizontal. All exercise tests were performed on a custom built calf ergometer, to be described later (see section 3.2.3.2).

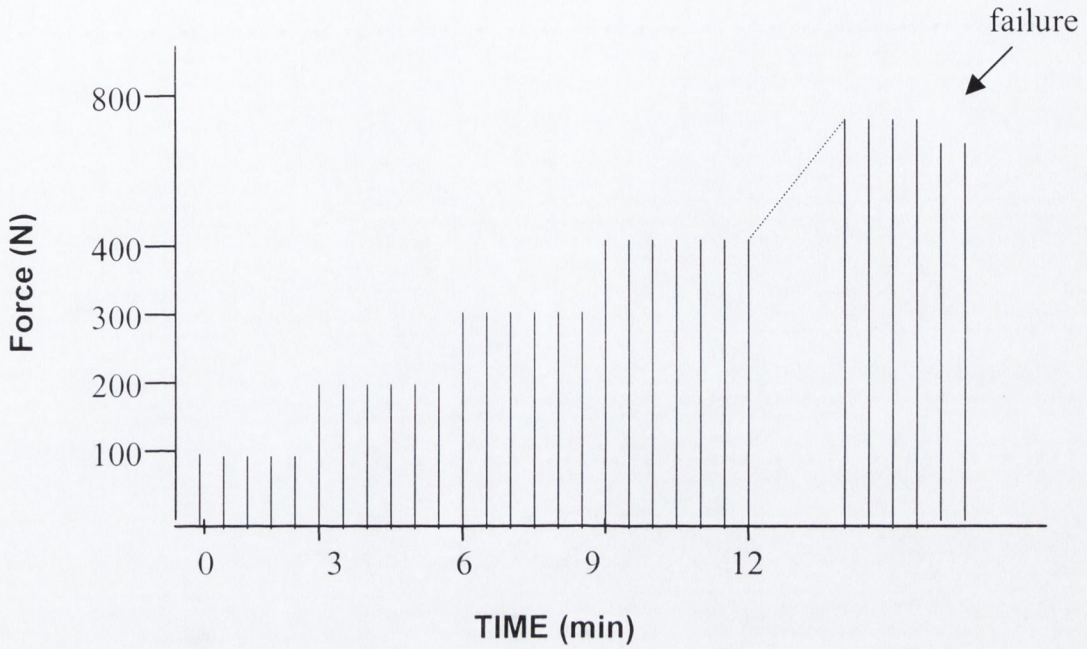


Figure 3.1: Diagram of the graded test. During the progressive incremental test, the intermittently performed force (3 s on, 3 s off) is increases by 100N every 3 min (i.e. every 30 contractions) until the subject fails to sustain the required force during 2 consecutive contractions (800N in this example).

3.2.2.3 Constant-force exercise (Experiment 2)

In Experiment 2, muscle strength, endurance time to failure and the rate of fatigue during constant-force exercise was assessed at four tilt angles (0°, 32°, 47° and 67°). After two familiarisation sessions (Appendix 7), F_{max} at the four tilt angles was determined during a strength testing session. Then, four constant-force tests were performed separated by at least 72 hours at 70 % F_{max}, where F_{max} was the highest force achieved at any of the four tilt angles during the strength session. The order of the angles at which strength and endurance tests were performed was also randomised using a cross-over design.

Prior to each constant-force test, F_{max} was determined as the highest of three maximum voluntary efforts. During the constant-force test, isometric muscle actions were performed intermittently so that the calf contracted for 2 s and

relaxed for 4 s. During a pilot test it was observed that the relative activation of the calf muscle was on average one third of the total gait duration during walking at different speeds and inclinations (Appendix 8), and therefore, the contraction-relaxation ratio of 1:2 was chosen. The long relaxation phase (4 s) was chosen to enable blood flow to be measured using plethysmography in future experiments. In order to measure the rate of fatigue, during each fifth contraction, or every 30 s, a maximum effort was sustained for 2 s. The decline in force during these maximum efforts was described using a linear function ($y = a + b \cdot x$), where y is force, x is time, parameter *a* or intercept represents force at t = 0 (i.e. F_{max}) and parameter *b* represents the rate of fatigue. The test was terminated when the subject was unable to sustain the required contraction force for 2 consecutive contractions (Figure 3.2).

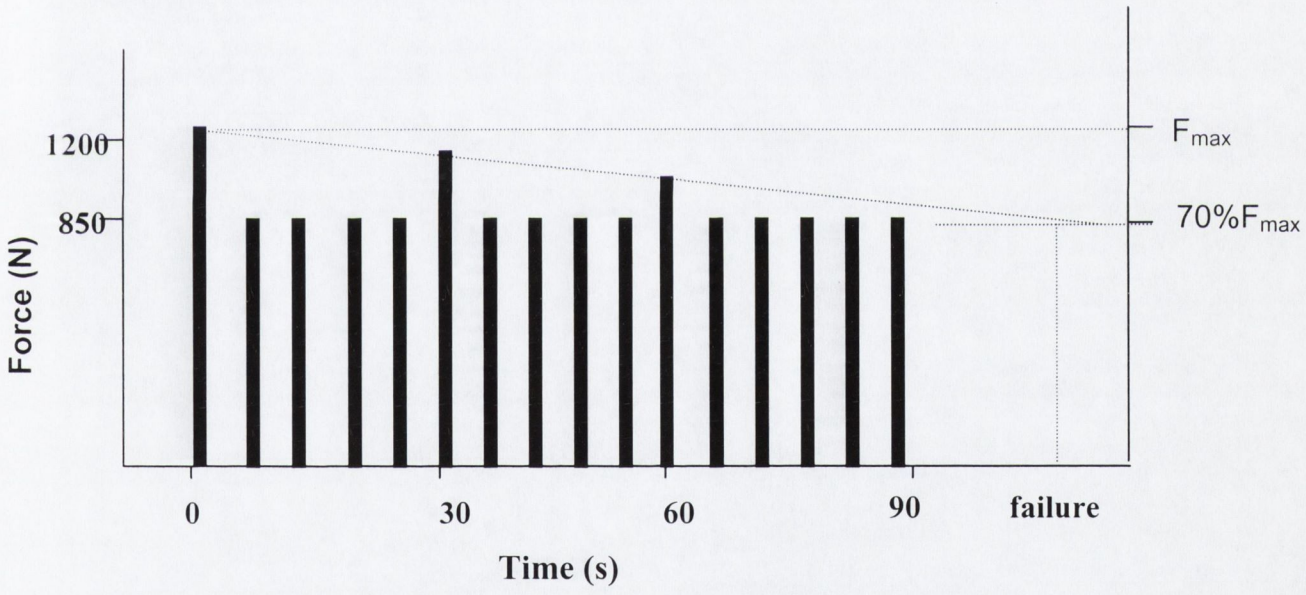
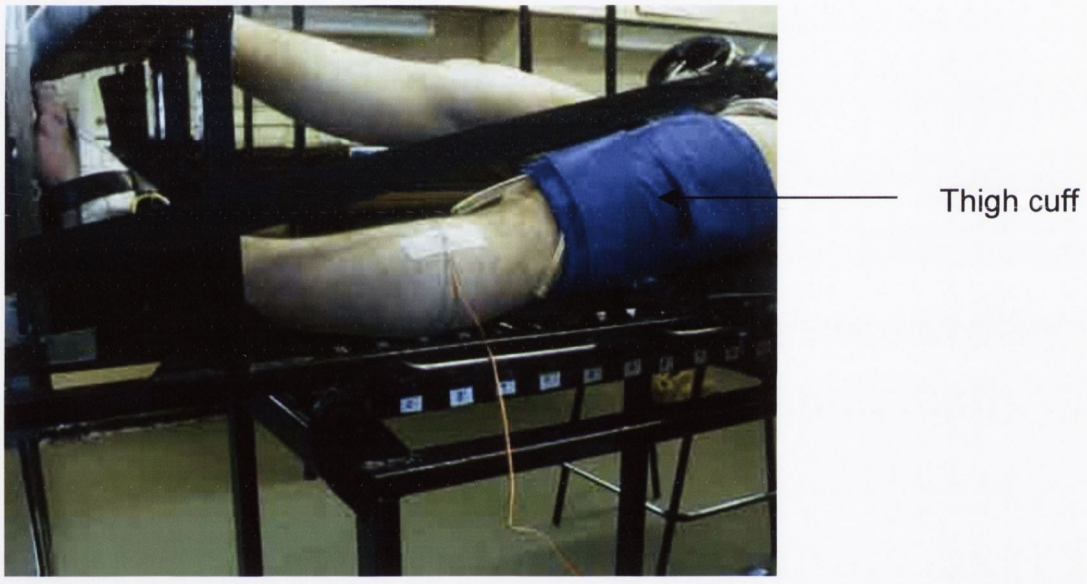


Figure 3.2: Diagram of the constant-load test. During the performance of the constant-load test, muscle fatigue progresses such that the maximum force (i.e. F_{max}, 1200N in this example) approaches the force required to maintain the constant power output (i.e. 70 % F_{max}, 850 N). Failure is reached when the subject is unable to sustain the constant power output for 2 consecutive contractions.

3.2.2.4 Ischaemic tests

When the postural effects on strength, endurance and fatigue during graded and constant-load exercise were known, four subjects repeated the graded test at 0° and 47° and another four subjects the constant-load test at 0° and 67° on separate days with the arterial flow into the leg stopped by inflating a thigh cuff to 250 mmHg. (Figure 3.3). During the ischaemic tests the cuff was rapidly inflated (within 0.5 s) immediately prior to the graded and constant load tests.

Figure 3.3: Thigh cuff to create ischaemic condition.



3.2.3 Equipment and measurements

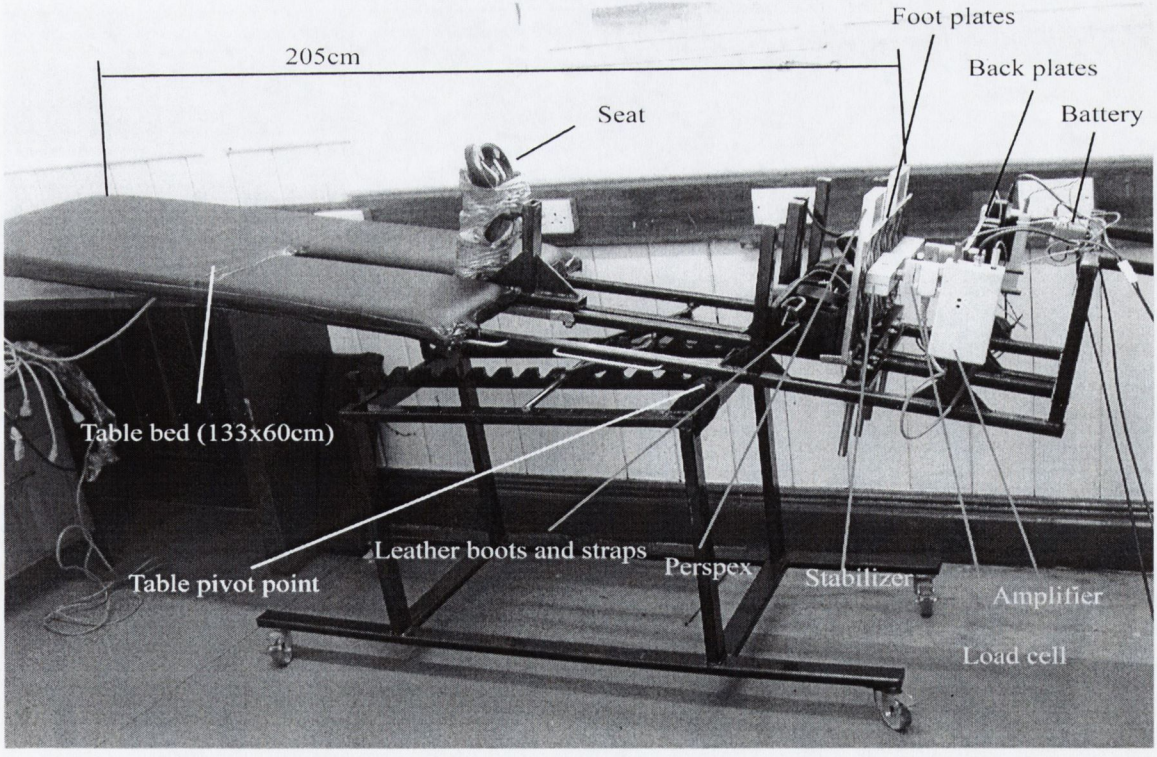
3.2.3.1 Anthropometric measurements

Height (cm) was measured using a Seca™ statiometer (Seca Ltd., Germany) and body mass (kg) was measured using a platform-beam scales (AVERY, England).

3.2.3.2 *Calf Egometer and force measurement*

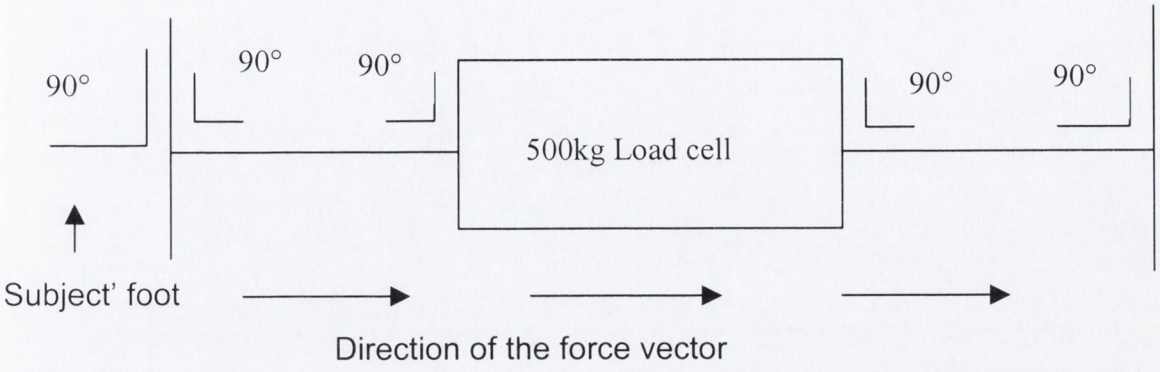
Calf exercise was performed on an ergometer that could tilt subjects to several angles between the horizontal (0°) and upright positions (90°) (Fig. 3.4). In all the different postures the position of the body was straight and subjects were unable to bend their knees. An adjustable padded seat supported the weight of the body. The feet of the subjects were placed on two individual perspex plates and into a leather boot, and were tightly cross-strapped with leather straps to minimize any heel lifting. A pilot study showed that lifting the heel up to 2cm did not change the maximum force the subjects could apply to the foot plate (Appendix 9). Underneath the Perspex plates, the ergometer has two immobile footplates, each of which is connected to its own strain gauge and against which force can be applied through trying to plantar flex the foot about the ankle. The lower end of the load cells are fixed to an aluminium back plate.

Figure 3.4: Calf exercise ergometer on the horizontal position



Since the positioning of the load cells is always at 90° to the fixed back plate and the front footplates, most of the force is distributed to the load cell. (Fig 3.5).

Figure 3.5 Positioning of the load cell.



The load cell was placed for all the subjects 16 cm below the top of the perspex plate. It was very important that the foot of the subject was placed at the same place for all the exercise tests, as in a pilot study it was observed that small changes in the position of the foot, exerted substantial effects on the force production (Appendix 10).

The calf ergometer was calibrated every testing day using precisely measured free weights ranging between 1 and 72 kg. An expanded description of the calibration and the stability of the system are shown in Appendix 11.

The force was amplified by two amplifiers (RS stock no. 846-171). The power source for the amplifiers was obtained from a dryfit battery (A 500, 12V, 1.2 Ah, Germany), which was maintained by the Optimate III battery charger (12V, Germany). The amplified force was then sampled at 40 Hz before being processed by a PowerLab (ML 795, AD Instruments) analog-digital converter and displayed on a screen for the subjects to see clearly (Chart v4.12, AD Instruments). Prior to testing the computer software was programmed to automatically convert the voltage (mV) measurements to force (N) measurements using the linear relationship between mV and N established from the calibration routine. Therefore, subjects used this visual display to regulate their own muscular effort (Figure 3.6).

Figure 3.6: A subject clearly observing the performance on the screen of the computer.



3.2.3.3 Calf exercise model

Single-limb (i.e. right leg) isometric exercise was performed in this study. The subject lay on the table bed in the fully extended position. The right foot was tightly and comfortably strapped into a leather boot fixed to the footplate. The leather boots were fitted with padding to ensure maximal comfort for the subjects (Figure 3.7A). Details of strapping and padding were noted for each subject and the same arrangement was used for each test. The left (non-exercising) foot was supported on a padded platform and was kept immobile throughout the tests (Figure 3.7B). This was done because in preliminary experiments it was observed that when the left leg was fixed to the left boot, subjects tended to dorsiflex that leg producing a "lever-effect" that resulted in additional increase of maximum force of 100-200N (Appendix 12). A padded seat supported the majority of the subjects weight (>80 %) when inclined. All subjects wore a harness (Figure 3.8) which provided a secure attachment to the ergometer and prevented any slippage of the subjects across the table bed that could result in lower maximum forces particularly during exercise on the horizontal posture. A pilot experiment established that the tension of the

harness had to be kept "comfortably tight" (Appendix 13). Subjects were positioned and harnessed first in the horizontal position and the force transmitted to the footplate by compression of the body (i.e. residual force) was recorded. The residual force recorded in the horizontal posture was maintained constant for all tests. Then, the subject was tilted up to the predetermined tilt angle without changing the tension of the harness. The residual force at tilted angles was slightly higher than the horizontal, but for all test conditions the residual force was converted in 0 N force so that the absolute force applied in all conditions was the same.

Figure 3.7 A: Strapping and padding arrangement of the footplates. **B**: The non-exercising foot is supported on a padded platform.

A) B)

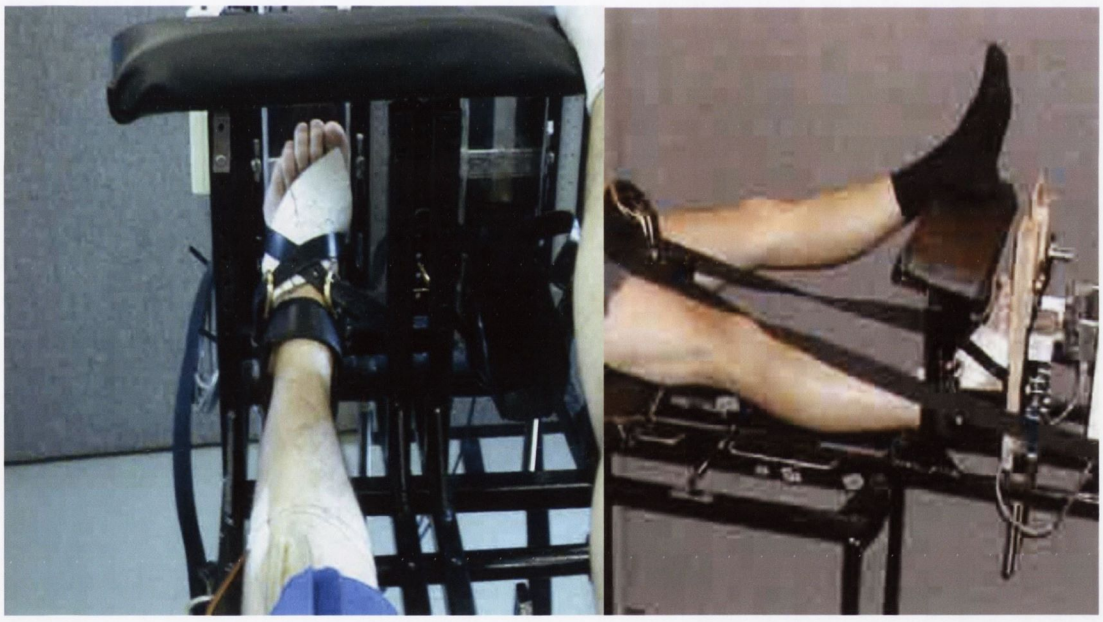


Figure 3.8: A subject wearing the harness



3.2.3.4 Maximum Force (F_{max}) measurement

In each of the strength sessions subjects performed 3-4 maximal voluntary efforts in each of the body tilt angles separated by 1 min. The highest force recorded was taken as the maximum force (F_{max}) at that angle. Importantly, a pilot experiment showed that when subjects were passively kept in the incline posture for up to 20 min, the maximal force (F_{max}) was not different from the one achieved immediately after tilting the body upright (Appendix 14). Despite this, to avoid passive venous pooling and expansion of calf volume, at all inclined positions, maximum efforts were performed within 15 s of being tilted from the horizontal and after each effort subjects were tilted back to the horizontal position and rested for one minute before being tilted up again.

Under ischaemic conditions, the F_{max} test was immediately preceded by rapid inflation of the cuff (within 0.5 s) up to 250 mmHg. During the rest periods between maximum efforts the cuff was deflated and the ergometer returned to the horizontal position in the case of inclined positions.

3.2.4 Statistical Analysis

Effects of body tilt angle on F_{max}, time to failure and rate of fatigue were identified using repeated-measures ANOVA and differences were then located using Tukey's HSD test. Comparisons of performance variables under ischaemic conditions (Experiments 1 and 2) at two angles were made using a paired t-test. Comparisons of performance variable between non-ischaemic and ischaemic conditions were identified using one-way ANOVA. The level of significance was set at $p<0.05$. All results are shown as means $\pm$ standard deviations

3.3 Results

3.3.1 Strength and endurance during graded exercise

Maximum force (F_{max}) values determined during the specific strength testing session at 0°, 47° and 90° are shown in Figure 3.9A. There was no significant difference between these values.

Times to failure on the graded test at these same three angles are shown in Figure 3.9B. There was a significant difference between time to failure at 0° and the other two angles; but no significant difference between 47° and 90°.

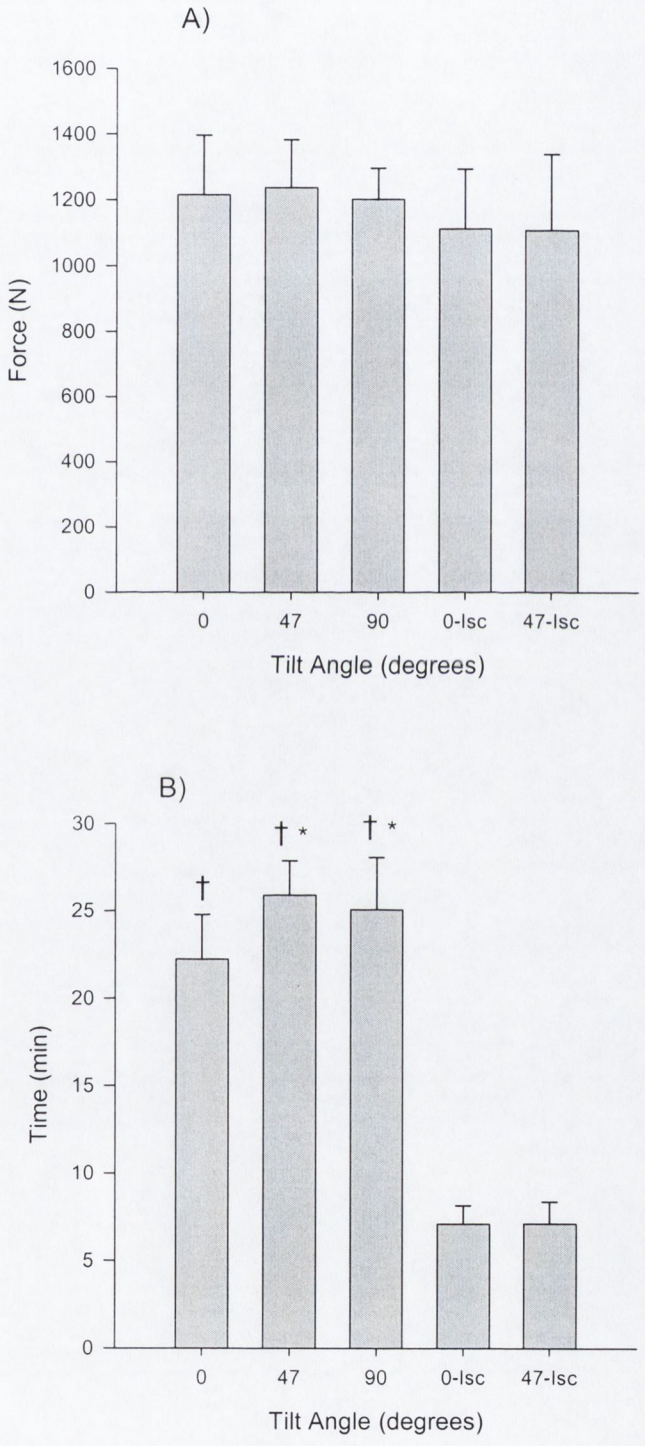
F_{max} and time to failure under ischaemic conditions at 0° and 47° are shown in Figure 3.9A and 3.9B. There was no effect of body tilt angle on F_{max} or time to failure under ischaemia. In addition, F_{max} values under ischaemia were not different than the ones under non-ischaemia, but times to failure during each of the three non-ischaemic conditions (0°, 47° and 90°) were significantly longer than during the two ischaemic conditions.

The mean relative forces achieved at the end of the graded tests were not significantly different among the three tilt angles (62, 70 and 70% F_{max} at 0°, 47° and 90°, respectively, Table 3.1).

Table 3.1: Mean relative force (% Fmax) achieved at the end of the graded test for all subjects.

	Tilt angle (degrees)		
	0	47	90
% F_{max}			
Subject 1	68.3	65.9	77.6
Subject 2	54.1	65.7	58.7
Subject 3	60.6	68.8	72.7
Subject 4	48.6	62.5	69.6
Subject 5	76.5	79.8	78.1
Subject 6	62.8	79.6	61.7
MEAN	61.8	70.4	69.7
SD	10.0	7.5	8.1

Figure 3.9. Graded exercise. **A:** Maximum force (mean ± SD) under non-ischaemic (n = 6) and ischaemic (i.e. "0-Isc" and "47-Isc"; n = 4) conditions. **B:** Time to failure (mean ± SD) during graded exercise under non-ischaemic (n = 6) and ischaemic (i.e. "0-Isc" and "47-Isc"; n = 4) conditions.



* significantly different ($P < 0.05$) from 0°.

† significantly different ($P < 0.05$) from ischaemic conditions.

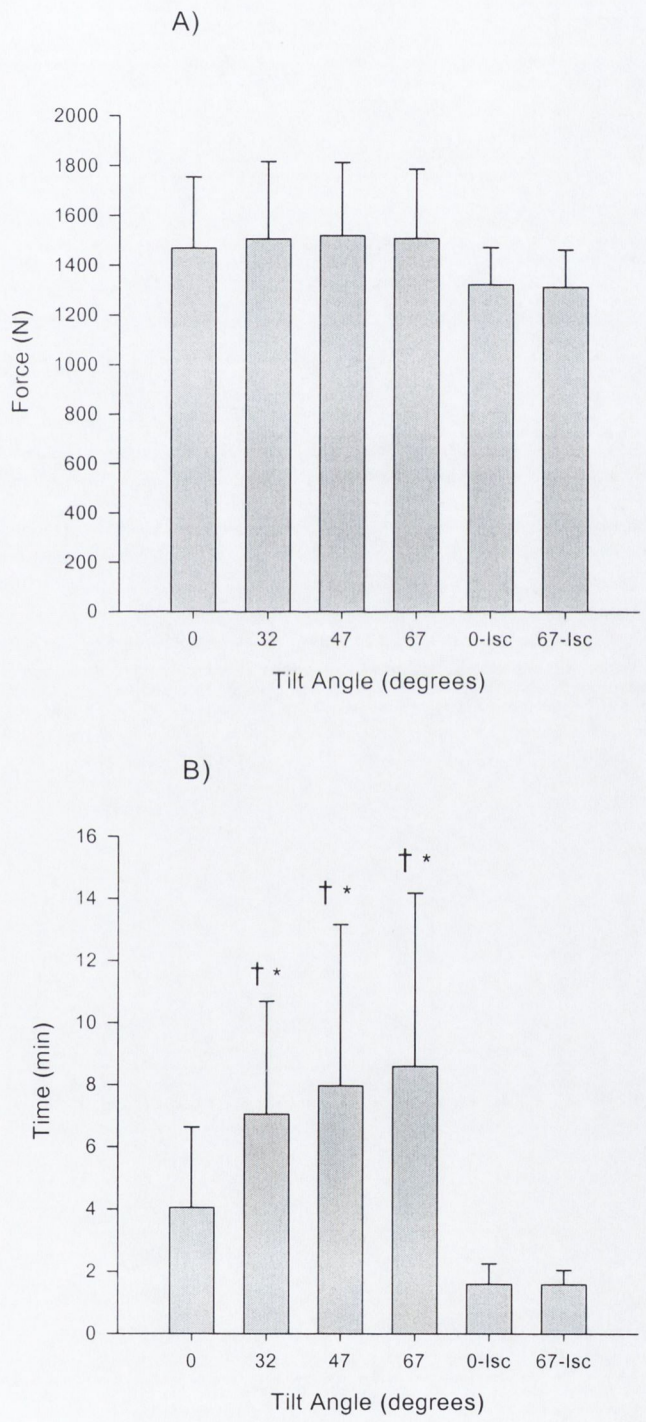
3.3.2 Strength and endurance during constant-force exercise

F_{max} values determined during the strength testing session at 0°, 32°, 47° and 67° are shown in Figure 3.10A. There was no significant difference between these values. F_{max} values prior to each of the constant-force tests were also unaffected by tilt angle. These F_{max} values were also not different from those F_{max} values measured during the strength testing session.

Times to failure for the constant-force test at these same four angles are shown in Figure 3.10B. There was a significant difference between time to failure at 0° and the other three angles; but no significant difference between 32°, 47° and 67°.

F_{max} and time to failure under ischaemic conditions at 0° and 67° are shown in Figure 3.10A and 3.10B. There was no effect of body tilt angle on F_{max}, or time to failure under ischaemia. In addition, F_{max} values were not different between non-ischaemic and ischaemic conditions but times to failure were significantly longer at 32°, 47° and 67° during non-ischaemia than the two ischaemic conditions.

Figure 3.10. Constant-force exercise. **A:** Maximum force (mean ± SD) under non-ischaemic (n = 7) and ischaemic (i.e. "0-Isc" and "67-Isc"; n = 4) conditions. **B**: Time to failure (mean ± SD) during constant-force exercise under non-ischaemic (n = 7) and ischaemic (i.e. "0-Isc" and "67-Isc"; n = 4) conditions.



* significantly different ($P < 0.05$) from 0°.

† significantly different ($P < 0.05$) from ischaemic conditions.

3.3.3 Rate of Fatigue during constant-force exercise

Fatigue during the four constant-force tests for the seven subjects are shown in Figure 3.11.

The predicted F_{max} values (parameter *a*) and the rates of fatigue (parameter *b*) at the four angles are shown in Table 3.2. Body tilt angle had no significant effect on the predicted F_{max}; but the rate of fatigue was significantly higher at 0° compared with the other angles.

The predicted F_{max} values (parameter *a*) and the rates of fatigue (parameter *b*) under ischaemic conditions at 0° and 67° are shown in Table 3.2. There was no effect of body tilt angle on predicted F_{max}, or on the rate of fatigue under ischaemia. In addition, the predicted F_{max} values were not different between non-ischaemic and ischaemic conditions, but the rate of fatigue was significantly lower at 32°, 47° and 67° during non-ischaemia than the two ischaemic conditions.

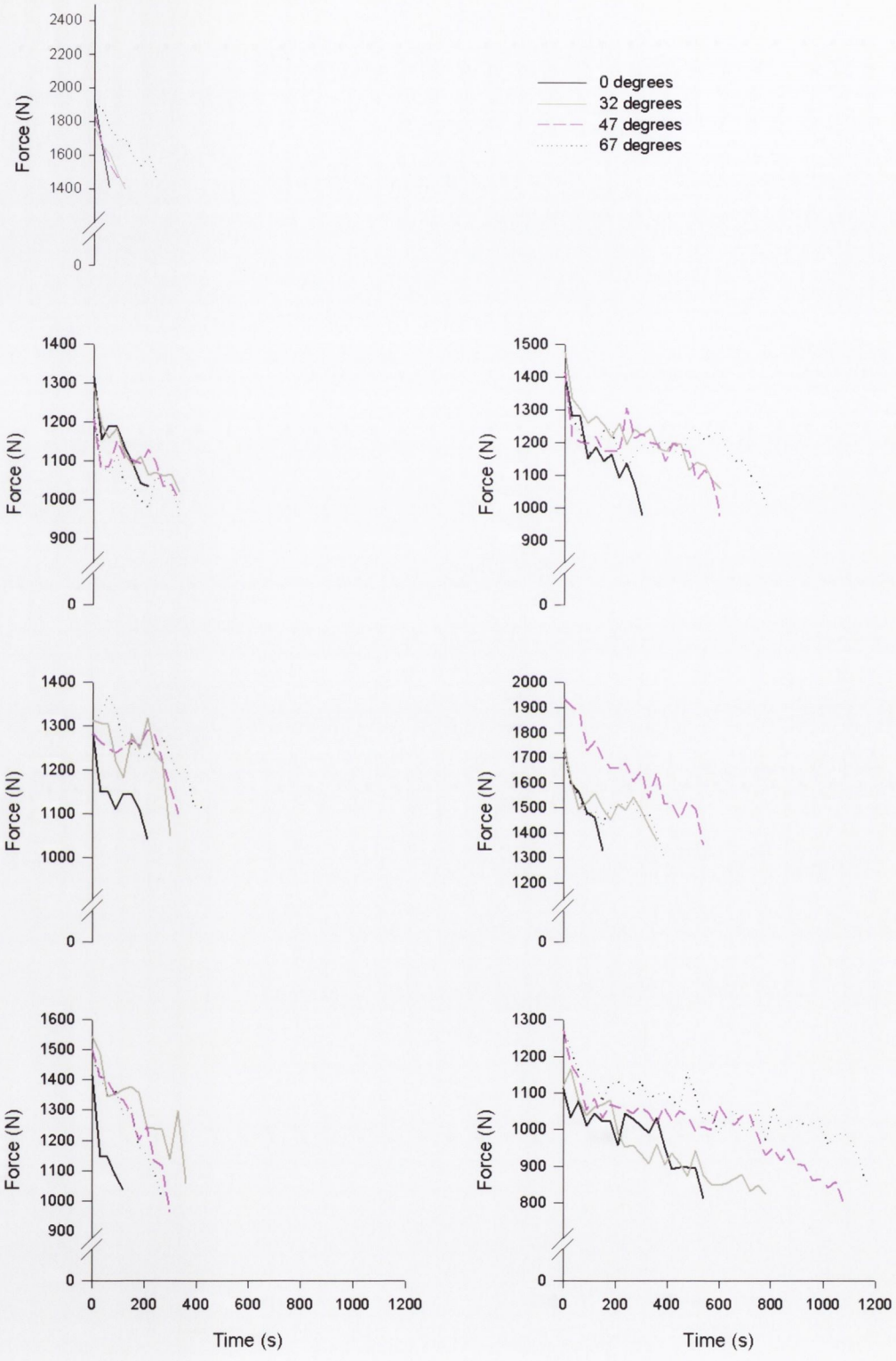


Figure 3.11: Constant-force exercise. The decline in peak force during constant-force exercise in seven subjects.

Table 3.2. Constant-force test (Exp. 2). Mean (± SD) values for parameter *a* (predicted F_{max} at t = 0 s) and *b* (rate of fatigue) obtained from fitting a linear equation (y = *a* + *b*x) to the decline in maximum force during the constant-force test. See Fig. 3.11 for individual responses.

		Non-ischaemia (n = 7)				Ischaemia (n = 4)	
	Tilt angle	0°	32°	47°	67°	0°	67°
Parameter							
a (N)		1410 ± 296	1407 ± 236	1449 ± 286	1415 ± 269	1294 ± 167	1315 ± 166
b (N s^{-1})		-2.46 ± 2.86	-0.92 ± 0.96 *†	-1.04 ± 1.14 *†	-0.76 ± 0.66 *†	-2.50 ± 1.22	-2.38 ± 0.79

* significantly different (P < 0.05) from 0° (non-ischaemia).

† significantly different (P < 0.05) from ischaemic conditions.

3.4 Discussion

The main findings of the present study were that the performance of graded exercise was prolonged significantly when the body was inclined from the horizontal (0°) to 47° and 90°, and that fatigue during submaximal constant-force exercise was diminished with a subsequent increase in time to failure when the body was inclined from the horizontal to 32°, 47° and 67°. In addition, these effects occurred in the absence of a postural effect on muscle strength and were abolished when the leg blood flow was interrupted.

3.4.1 Endurance during graded exercise

We (chapter 2) and others have demonstrated that during a maximal graded cycle tests endurance is ~10-15% longer when the body is tilted upright from a supine position (Eiken, 1988, Leyk *et al.*, 1994a, Terkelsen *et al.*, 1999). In the present study, subjects completed a similar graded test to the one performed during cycling exercise (see chapter 2); it began at a low effort, the effort was increased in a stepwise manner each 3 minutes, and the total duration was of the order of 15-30 minutes. Several pilot experiments were carried out to identify the initial workload (100 N), the contraction-relaxation ratio (3:3) and the subsequent change in workload (100 N every 3 min), so that the times to failure during isometric plantar flexion graded tests would be similar to the times to failure observed during cycling graded tests. In the present study, times to failure during the two inclined positions (47° and 90°) were ~17% longer compared with the horizontal position (Fig. 3.9B). These relative improvements in the incline position are similar to the changes observed during graded cycling exercise, and therefore, these data demonstrate that the postural effects on the endurance of the graded exercise are similar for exercises involving the whole-body or individual muscle groups.

3.4.2 Endurance and muscle fatigue during constant-force exercise

The intensity of the constant-force test in the present study was relatively high (70% F_{max}), and was close to the highest achieved during the graded test (i.e. 62-70% F_{max}, Table 3.1). Preliminary tests showed that some subjects did not reach failure after exercising for over 1 h 30 min at moderate intensities (40-50% F_{max}), but most subjects reached failure within the first 20 min of exercise at high intensities (70-80% F_{max}). Since one of the aims of the present study was to assess the effect of tilt angle on endurance time to failure, we decided to use the relatively high intensity of 70% F_{max}. Time to failure on this test was increased on average by up to 100% when the tilt angle was increased from the horizontal (Fig. 3.10B), and the rate of fatigue was decreased on average ~40% (Fig. 3.11, Table 3.2). These effects were much larger than those observed for graded exercise (Fig 3.9B). Previously, we demonstrated in Chapter 2 that performance during high-intensity constant-load cycling exercise is largely improved (~160%) in the upright compared to supine posture and that this improvement in the upright posture during constant-load exercise is larger than during graded cycling exercise (~16 %) for the same subjects. Therefore, these data suggest that the postural effect on performance time during high-intensity constant-load exercise is in an order of magnitude greater than the postural effect seen for graded tests for both, whole-body dynamic cycling exercise and isolated muscle isometric exercise.

3.4.3 Incline tilt angles

The lack of differences in times to failure between 47 and 90° (25.9 ± 2.0 vs. 25.1± 3.0 min respectively) could have been due to the fact that some subjects complained of discomfort (i.e. testicular compression) during parts of the test at 90° which may have limited performance. For this reason, during the subsequent constant-force tests the highest tilt angle was limited to 67°, during which subjects did not complain of discomfort. Also, in order to investigate if there was any postural effect in between the horizontal and 47° one more tilt angle (32°) was added. During the constant-load tests endurance was increased and the rate of fatigue diminished when the body was inclined from 0° to 32°, but no significant differences in exercise times or rates of fatigue were observed between the three inclined positions. Therefore, these data rise the

possibility that the postural effect on muscle fatigue and endurance during plantar flexion exercise may reach a plateau at a range of about 30-50 degrees.

3.4.4 Strength

In the present study it is unlikely that strength affected the rates of fatigue or endurance times at different tilt angles because maximum forces recorded in the specific strength sessions prior to the graded and constant-load exercises were not different at any of the tilt angles (Fig. 3.9A and 3.10A). F_{max} values prior to each constant-force test and the predicted values of muscle strength (i.e. parameter *a*) were also unaffected by tilt angle.

3.4.5 Incremental versus constant-load exercise

Studies carried out in individual muscle groups show that endurance time to failure is very sensitive to oxygen supply both during constant-load (Fulco *et al.*, 1996) and incremental (Hogan *et al.*, 1999a) exercise. Fulco and coworkers (1996) observed that time to failure was 56% shorter during hypobaric hypoxia than normoxia when 8 subjects performed one-leg dynamic knee extension exercise at a constant force equivalent to ~60% of the maximum work rate achieved during a normoxic incremental test. Similarly, when 6 subjects performed incremental plantar flexion exercises to the point of failure under hypoxia (0.1 F_{IO2}) and normoxia (0.21 F_{IO2}), time to failure was 18 % shorter during hypoxia than normoxia (Hogan *et al.*, 1999a). These data suggest that a reduced O_2 supply to the active muscle may exert greater reductions in endurance times of individual muscle groups when the overall intensity of the exercise is higher (i.e. moderate to high intensity constant load exercise) rather than lower (i.e. incremental exercise). Therefore, since the postural effect on performance is likely to be mediated, at least in part, by its effects on leg blood flow and oxygen supply (as demonstrated in Chapter 2), in the present study the relatively larger postural effects observed for high-intensity constant-load exercise might be attributed to the relatively stronger influence of oxygen and blood flow on performance of this type of exercise.

3.4.6 Ischaemia

The initial fall in muscle force that occurs with ischaemia in working skeletal muscle has been closely related to oxygen availability (Hogan *et al.*, 1994). Then, we reasoned that if oxygen supply and blood flow play an important role mediating the postural effects on fatigue and performance, preventing arterial blood flow into the leg (i.e. ischaemia) should reduce these postural effects. In the present study, during the non-ischaemic graded tests times to failure at 47° and 90° tilt angles were identical, but at 90° some subjects complained of postural discomfort. Consequently the ischaemic tests during graded exercise were performed at 0° and 47° tilt angles. During the non-ischaemic constant-force tests endurance and fatigue were not different among the 3 incline tilt angles (32°, 47° and 67°), but the average time to failure at 67° was relatively higher (i.e. 21 %) than that observed at 32°, and therefore, the tests under ischaemia were performed at 0° and 67° tilt angles.

In the present study, strength and endurance were both unaffected by tilt angle during graded or constant-force exercise under ischaemia (Fig. 3.9B and 3.10B). It could be argued that the lack of difference in times to failure under ischaemia was due to a lesser temporal resolution as a result of significantly shorter times compared to non-ischaemic conditions. However, this is unlikely because in a different experiment (described later in Chapter 4) times to failure in the horizontal posture at 90% F_{max} were similar to the ones we observed in the present study under ischaemia. However, the times to failure at 67° at 90% F_{max} were almost 100% longer compared to 0° (see Chapter 4, Experiment 2). Therefore, this data demonstrates that the postural effects observed under conditions of normal blood flow were completely abolished under ischaemic conditions, suggesting that arterial blood flow into the leg mediates the postural effect on calf muscle endurance.

3.5 Conclusion

In conclusion, this study has demonstrated that inclining the body from a supine position significantly prolongs endurance during graded and constant-load calf muscle exercise. This improvement for constant-load tests is an order of magnitude larger than the postural effect seen for graded exercise. In addition, during the constant-load test muscle fatigue is diminished as the body is tilted from the horizontal to an inclined head-up position. This effect occurs in the absence of an effect on strength, and it depends on an intact peripheral circulation.

Chapter 4: **Intensity-dependent effect of body tilt angle on calf muscle performance in humans**

4.1 Introduction

Muscle fatigue has been shown to be very sensitive to changes in perfusion pressure during both voluntary (Wright *et al.*, 1999) and involuntary (Fitzpatrick *et al.*, 1996) exercise of moderate to high submaximal intensities in humans. For instance, in a study conducted by Wright and co-workers (1999), subjects made repeated voluntary isometric contractions of the adductor pollicis muscle of the human hand at 50% of the maximal voluntary contraction in a 6 s on, 4 s off cycle. Fatigue was assessed by eliciting a maximal isometric twitch during each "off" period by electrical stimulation. The authors observed that a reduction in perfusion pressure induced by elevating the arm above the heart-level from an initial position located at the level with the heart significantly decreased the force production ($p<0.01$) within the initial 4 minutes of exercise (Wright *et al.*, 1999). Consistent with this, during constant-load voluntary plantar flexion exercise at a relatively high intensity (70 % F_{max}), we have shown that tilting the whole-body from the horizontal position decreases the rate of calf muscle fatigue on average by 40% (see Chapter 3, Exp. 2). Similarly, Fitzpatrick *et al* (1996) showed that during electrically-induced repeated contractions of the adductor pollicis muscle at frequencies of 3-9 stimulii each second, a reduction in muscle perfusion pressure induced by elevating the arm above the heart level significantly decreased the force output within seconds after the change in limb position, whereas, when contractions were induced less frequently, it had no effect on muscle force production, suggesting that fatigue also depends on the frequency of stimulation.

Muscle activity has also been reported to be very sensitive to changes in perfusion pressure. For instance, Hobbs and McCloskey (1987) showed that during voluntary constant-force plantar flexion contractions equivalent to ~20 % MVC, the electromyogram (EMG) was increased when the contracting muscles were raised above compared to below the heart level. This suggests that force production of active muscle fibers at a given level of activation fell when muscle perfusion pressure was lowered, and therefore, the muscle activity was augmented in order to sustain force.

These data suggest that the postural effect on muscle fatigue is observed at a large range of intensities, but it seems likely that the postural effect may not occur when the exercise is performed at a very low intensity at least during electrically stimulated contractions (Fitzpatrick *et al.*, 1996). Furthermore, it is unknown if there is an upper intensity threshold at which the postural effect on fatigue disappears. Therefore, the aim of the present study was to establish the range of intensities across which the positive effect of body tilt angle on muscle fatigue occurs during voluntary plantar flexion constant-load exercise.

4.2 Material and methods

4.2.1 Subjects

Eleven subjects participated in this study (mean ± SD: age = 26.4 ± 1.8 yr; height = 178 ± 4 cm; weight = 78.8 ± 7.5 kg). They were non-smokers and free of any clinically significant disease that could affect their exercise performance. All subjects read the subject information sheet (Appendix 15) and gave written informed consent before involvement in the study (see Chapter 2, appendix 3) and these procedures were approved by the ethics committee of Trinity College Dublin. The procedures were conducted in accordance with the standards set by the Declaration of Helsinki (2000).

4.2.2 Experimental design

4.2.2.1 Overview

Two experiments were performed. Experiment 1 tested the effect of body tilt on muscle strength and fatigue during constant-force calf exercise at low to moderate intensities in five men; whereas Experiment 2 tested the effect of body tilt on muscle strength, performance, and fatigue during constant-force calf exercise at high intensities in seven men.

To avoid potential impact of diurnal factors on exercise performance, subjects performed all exercise sessions at the same time of the day. Subjects were requested to refrain from alcohol and caffeine for the 12 hours preceding the testing session as well as from any strenuous exercise for the 24 hours preceding the testing session.

4.2.2.2 *Muscle fatigue during constant-force low to moderate exercise (Exp.1)*

In Experiment 1, calf muscle strength and fatigue during constant-force exercise was assessed at two tilt angles (0° and 67°) and at four low to moderate intensities equivalent of 30, 40, 50 and 60 % F_{max}. After two familiarisation sessions (Appendix 16), F_{max} at the two tilt angles was determined as the highest of four maximum voluntary efforts during a strength testing session. Then, on 8 other occasions separated by at least 72 hours, a constant-force test was performed. The order of the angles and intensities at which the strength and constant tests were performed was randomised using a cross-over design.

Prior to each constant-force test, F_{max} was determined as the highest of three maximum voluntary efforts. During the constant-force test, isometric muscle actions were performed intermittently so that the calf contracted for 2 s and relaxed for 4 s. Every tenth contraction (or every minute) a maximum effort was required to be sustained for 2 s. A plot of the decline in F_{max} over time was used to measure the rate of fatigue and a prediction of maximal force (as in Chapter 3, Exp.2). The duration of the test was limited to a maximum of 20 min.

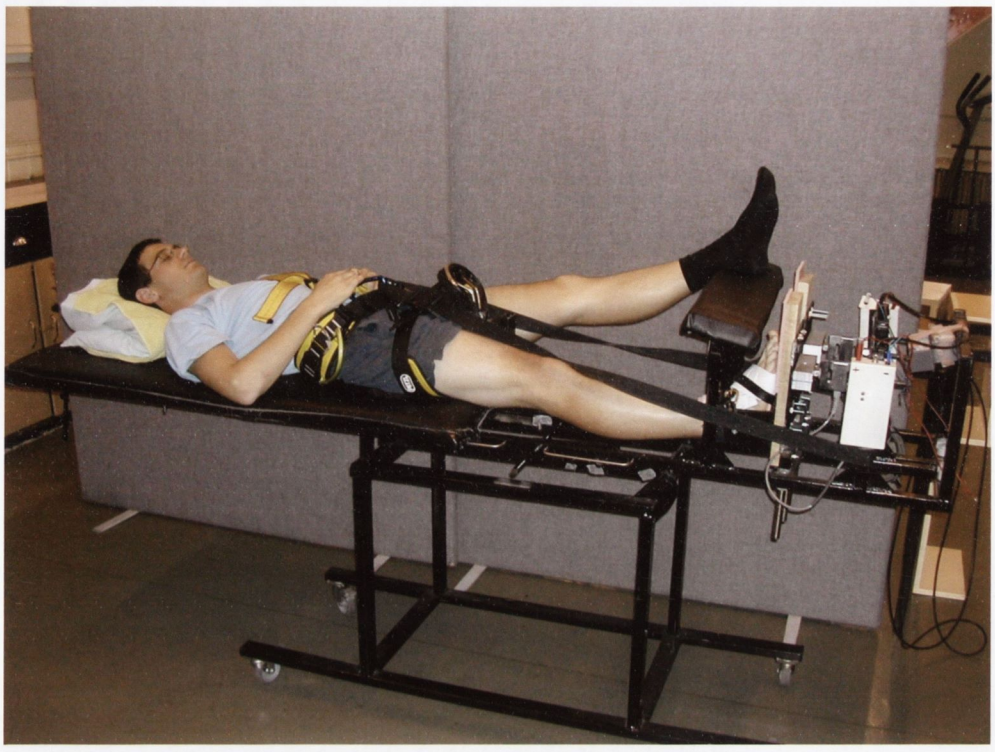
4.2.2.3 *Muscle fatigue and endurance during constant-force high intensity exercise (Exp.2)*

In Experiment 2, calf muscle strength, fatigue and endurance during constant-force exercise was assessed at two tilt angles (0° and 67°) and two high intensities equivalent to 80 and 90 % F_{max}. After two familiarisation sessions (Appendix 16), F_{max} at the two tilt angles was determined on one day and then, on 4 other occasions separated by at least 72 hours, fatigue and time to failure during constant-force test were determined.

Prior to each constant-force test F_{max} was recorded. Then, during the tests subjects exercised until failure and performed a maximum effort every fifth contraction (i.e. every 30 s).

Figure 4.1: a subject exercising in the horizontal (0°, A) and inclined (67°, B) posture.

A)



B)



4.2.3 Equipment and measurements

4.2.3.1 *Anthropometric measurements* (see Chapter 3, section 3.2.3.1)

4.2.3.2 *Calf ergometer and force measurement* (see Chapter 3, section 3.2.3.2)

4.2.3.3 *Calf exercise model* (see Chapter 3, section 3.2.3.3)

4.2.3.4 *Maximum Force (F_{max}) measurement* (see Chapter 3, section 3.2.3.4)

4.2.4 Statistical analysis

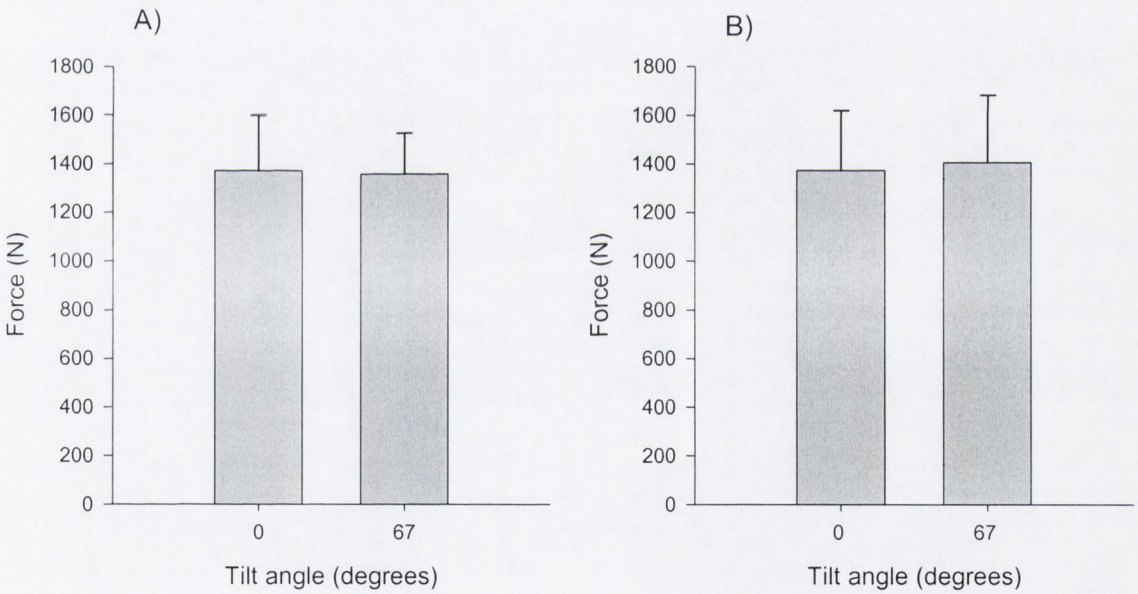
Effects of posture and intensity on F_{max}, time to failure and rate of fatigue were identified using a two-way repeated-measures ANOVA and differences were then located using Tukey's HSD test. The level of significance was set at $P<0.05$. All results are shown as means ± standard deviations.

4.3 Results

4.3.1 Maximum force (F_{max}) during Experiments 1 and 2

F_{max} values determined during the strength testing session at 0° and 67° in Experiments 1 and 2 are shown in Figure 4.2. There was no significant difference between these values both for Exp. 1 (Fig. 4.2A) or Exp. 2 (Fig. 4.2B). F_{max} values prior to each of the constant-force tests were also unaffected by tilt angle in both Experiments. These F_{max} values were also not different from those F_{max} values measured during the strength testing session.

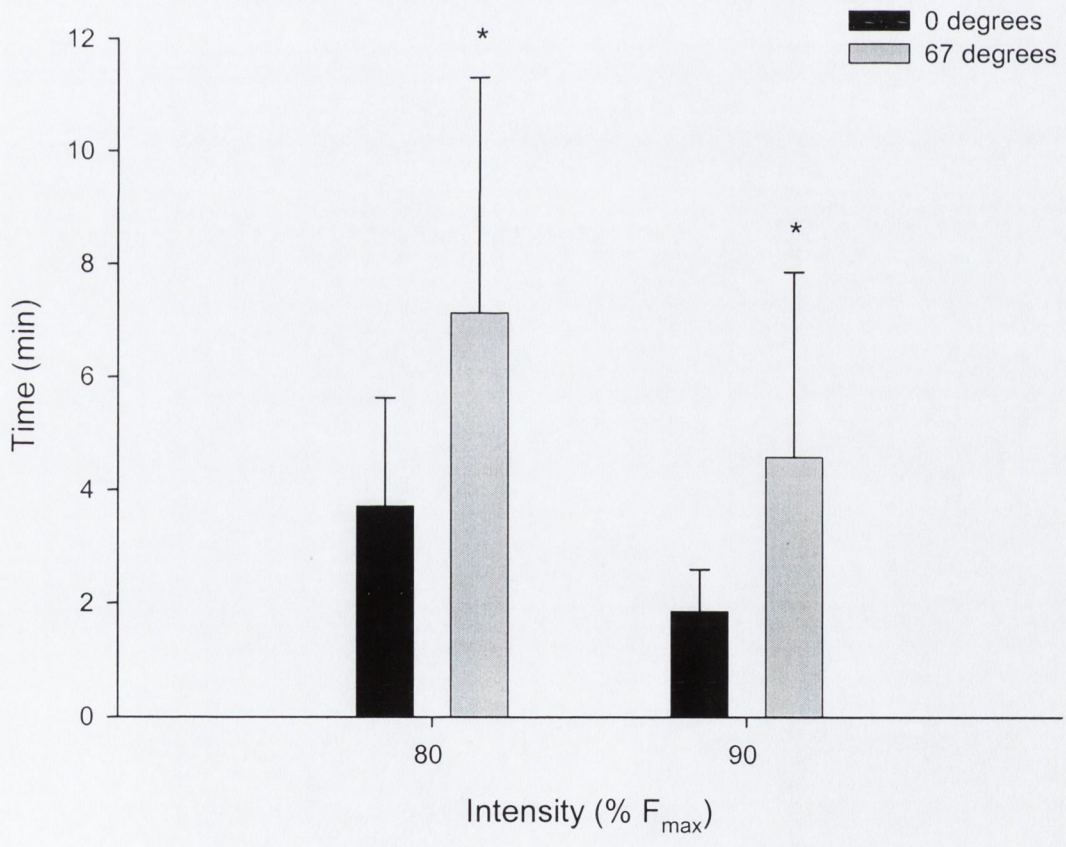
Figure 4.2: Fmax data for both Experiments. A: Maximum force during Exp.1(n=5), B: Maximum for during Exp. 2 (n=7).



4.3.2 Time to failure during Experiment 2

During Experiment 1, the duration of the tests was limited to 20 min, and since most of the subjects reached this duration, time to failure was not assessed. In Experiment 2, the time to failure was significantly longer at 80 and 90 % F_{max} in the inclined compared to horizontal position (Figure 4.3)

Figure 4.3: Time to failure during high intensity exercise (Exp. 2)



4.3.2 Fatigue during Experiments 1 and 2

The predicted F_{max} (i.e. y-intercepts) and the rates of fatigue for Experiments 1 and 2 are shown in Figure 4.4. Although the subjects who took part in Exp 1 (n=5) were different from the ones in Exp 2 (n=7), for a better overall understanding of these results, the data from the 2 experiments are plotted together.

In Experiment 1 and 2 tilt angle had no effect on the predicted F_{max} (i.e. y-intercept) in any intensity (Figure 4.4 A).

In all intensities the rate of fatigue was significantly greater than zero. The rate of fatigue was lower in the inclined than supine position (main effect = posture: $P < 0.05$) for Experiment 1 and 2. In Experiment 1 only, there was a significant interaction (intensity × posture) for rate of fatigue, such that significant differences between inclined and supine postures were observed at all intensities between 40 and 90 % F_{max}; but not at 30 % F_{max} (Figure 4.4 B). All individual fatigue responses are shown in Appendix 17.

Exercise intensity (i.e. % Fmax) was significantly correlated with the absolute ($p<0.05$) but not relative ($p=0.43$) change in the rate of fatigue between the incline and supine posture (Figure 4.5).

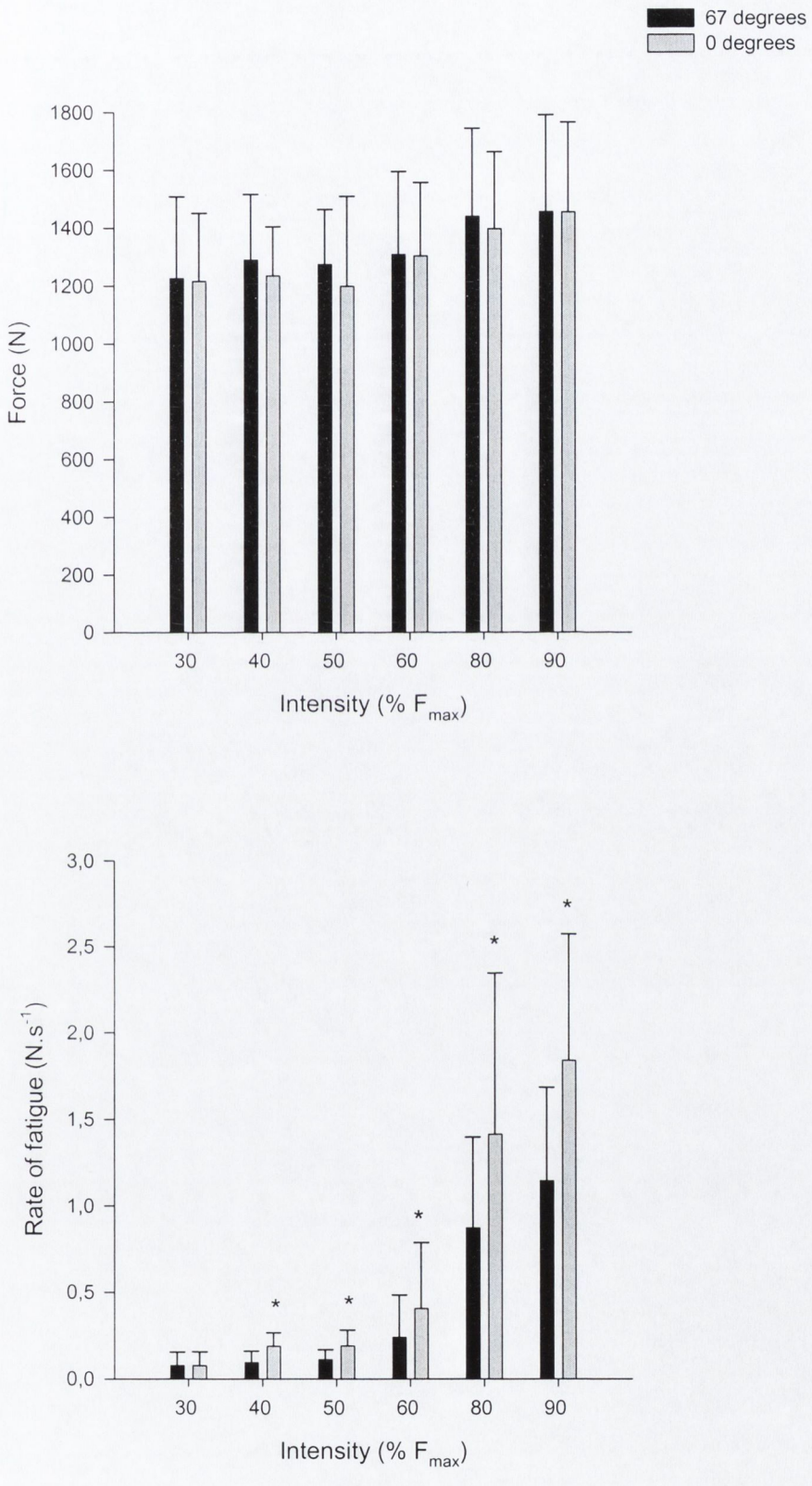


Figure 4.4: A: Predicted F_{max} (y-intercept) at different intensities. B: Rate of fatigue at different intensities.

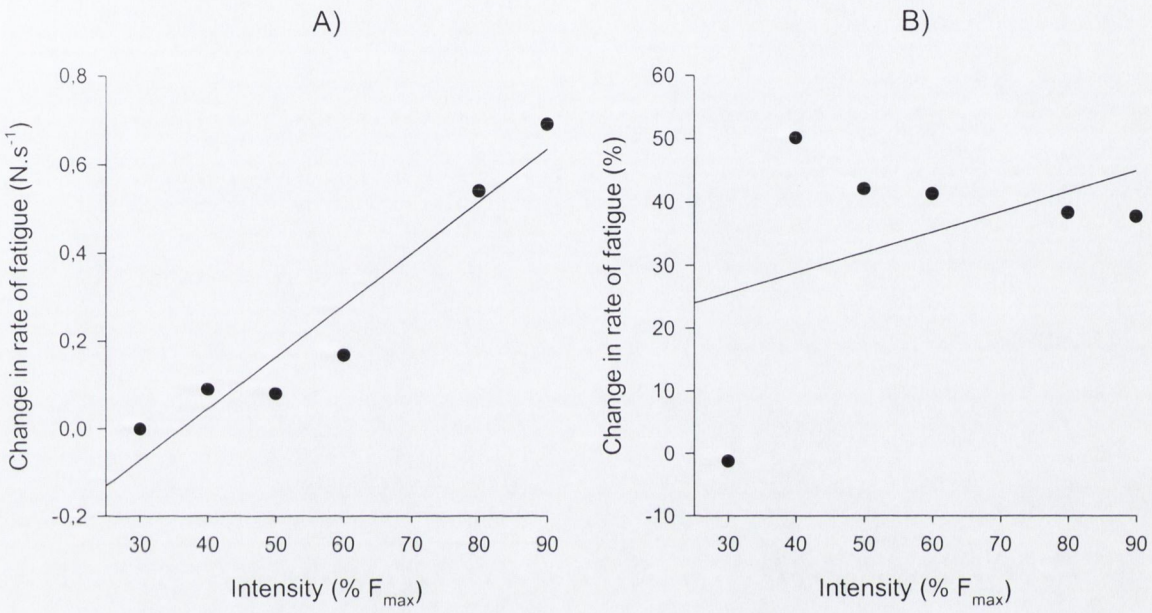


Figure 4.5: Correlations between fatigue and intensity. **A:** The intensity of contraction (% F_{max}) versus the absolute change in the rate of fatigue (67° minus 0°) during constant load plantar flexion exercise; r=0.960, $p<0.05$. **B:** The intensity of contraction (% F_{max}) versus the relative change in the rate of fatigue (67° minus 0°) during constant load plantar flexion exercise; r=0.408, $p>0.05$.

4.4 Discussion

The main findings of the present study were that fatigue at intensities between 40 and 90 % F_{max} was significantly diminished at 67° when compared to 0°, but was not significantly different between 0° and 67° at 30 % F_{max}. In addition, endurance during the high intensity tests was prolonged at 67° compared to 0°. These data extend our previous findings of a postural effect on fatigue during high intensity (70 %F_{max}) calf exercise (Chapter 3, Exp 2) and demonstrate that there is a wide range of intensities across which posture affects calf muscle fatigue and endurance.

In Chapter 3 during constant-force submaximal exercise at relatively high intensities (i.e. 70% F_{max}, Exp.2) exercise times or rates of fatigue were not significantly different between the three inclined positions (32°, 47° and 67°). However, the average time to failure at 67° was relatively longer (i.e. 21 %) than at 32° and thus, in the present study the tilt angles of 0° and 67° were used

4.4.1 Intensity thresholds

This is the first study showing an intensity-dependent effect of posture on muscle performance during voluntary exercise. Previously, Fitzpatrick and coworkers (1996) showed during involuntary exercise involving the adductor pollicis muscle, that raising the arm above the level of the heart resulted in a reduction of muscle force production during electrically-induced tetanic contractions at frequencies of 3-9 stimuli at 25 Hz each second. These authors also showed that the relative decline in muscle force increased as the number of stimuli increased, so that force declined by 17, 22, 32 and 36 % for 3, 5, 7 and 9 stimuli respectively (Fitzpatrick *et al.*, 1996). However, when contractions were induced less frequently (i.e. single twitches) elevating the arm above the heart level had no effect on muscle force production. The results of the present study support the idea that there is a lower intensity threshold below which posture does not affect calf muscle fatigue, and therefore, for voluntary contractions or electrically stimulated contractions the effect of posture on

muscle force production depends on the intensity of contraction and/or frequency of stimulation.

Based on the results from the study by Fitzpatrick and colleagues (1996), the decline in force production induced by a reduction in perfusion pressure (i.e. tilting the body from incline to supine) during a voluntary exercise should increase as contraction intensity increases. The findings of the present study support this idea in part since the absolute change on the rate of fatigue between postures was linearly correlated with exercise intensity (Figure 4.5). However, the relative difference on the rate of fatigue between 0° to 67° did not change as contractile force increased (on average 50, 42, 41, 38, 38 % decrease in rate of fatigue at intensities of 40, 50, 60, 80 and 90 % F_{max} respectively, Figure 4.5). This could be because we measured the difference on the rate of fatigue while Fitzpatrick *et al*, (1996) calculated just the decline in maximal force.

However, it is worth noting that there was a considerable variation in the postural effect on muscle fatigue mainly amongst the subjects who performed the low to moderate intensity exercises (see Appendix 17 for individual responses). The present study is unable to explain the reasons of this variation, with some subjects reaching lower rates of fatigue at 50% F_{max} compared to 40% F_{max}, and this deserves further attention.

In the present study, the times to failure at intensities equivalent to 80 and 90 % F_{max} were increased on average by ~ 85 % (Fig 4.3) when subjects were tilted from 0° to 67°. These findings were similar to those previously observed (Chapter 3, Exp 2), when different subjects exercised at 70 % F_{max} and identical exercise conditions as in the present study. Therefore, these data suggest that the effect of posture on the endurance of the plantar flexors is still present at near maximal intensities during intermittent exercise.

Muscle fatigue and endurance are very sensitive to changes in perfusion pressure (Eiken, 1988, Fitzpatrick *et al.*, 1996, Leyk *et al.*, 1994a, Terkelsen *et al.*, 1999, Wright *et al.*, 1999) and oxygen supply (Eiken & Tesch, 1984, Fulco *et*

al., 1996, Haseler *et al.*, 1998, Hogan *et al.*, 1999a). During plantar flexion exercise a reduction in O_2 availability causes a more rapid P_i accumulation and intracellular acidosis, which results in an earlier onset of fatigue and shorter time to failure (Hogan *et al.*, 1999a). Previously we demonstrated that at relatively high-intensity (70 $\%F_{max}$) tilting the body from 0° to 67° reduces the rate of fatigue and increases the time to failure during a plantar flexion exercise and that this effect depends on an intact peripheral circulation (Chapter 3, Exp 2). Therefore, these data, suggest that the reduction in muscle fatigue and the improvement in the endurance time in the incline position observed in the present study at moderate to very high intensities are due to a higher blood flow and oxygen supply to the active muscles.

The present findings can not explain the lack of postural effect on fatigue at 30 $\%F_{max}$. This could be attributed to a lack of faster and/or larger increase in blood flow during the upright versus supine postures at this intensity, or alternatively, to an insensitivity of muscle force production to an increase in leg blood flow at 30 $\%F_{max}$.

4.4.2 Strength

The effect of posture on muscle endurance and fatigue observed in the present study, could also have been mediated by effects on muscle strength. To explore this we tested the effect of posture on calf muscle strength in a specific strength session prior to the tests, and no differences were found in maximum forces between 0° and 67° (Figure 4.2). In addition, the predicted F_{max} during the exercise (i.e. y-intercept, Figure 4.4 A) was also unaffected by body tilt. Therefore, the postural effect on muscle fatigue and endurance observed in the present study is unrelated to any effect on muscle strength.

4.5 Conclusion

In conclusion, this study has shown that there is an exercise intensity-dependant effect of body tilt angle on calf muscle fatigue during voluntary exercise. At low intensities (approximately < 40 % F_{max}) this effect is not observed. However, at moderate to very high intensities (approximately > 40 % F_{max}) the rate of fatigue at 0° tilt angle is an estimated 40 % greater compared to 67° tilt angle, and is consistent at all intensities up to 90 % F_{max}.

Chapter 5: **Effect of body tilt angle on calf muscle blood flow**

5.1 Introduction

Modest but consistent improvements in endurance time (~ 10-15 %) have been reported for graded cycling exercise when the body is tilted upright from a horizontal position (Eiken, 1988, Leyk *et al.*, 1994a, Terkelsen *et al.*, 1999). This improvement in performance is similar (~17 %) when the body is inclined from the horizontal position during plantar-flexion graded exercise (Chapter 3, Experiment 1). In contrast, relatively larger improvements in endurance time have been reported in the inclined posture during both, high-intensity constant-load cycling (~ 160 %, Chapter 2), and plantar flexion (~ 100 %, Chapter 3, Exp 2) exercise. Similarly muscle fatigue is reduced during electrical stimulation of the abductor pollicis muscle when the arm is lowered from the level of the heart (Fitzpatrick *et al.*, 1996, Wright *et al.*, 1999), and when the body is inclined from the horizontal posture during plantar flexion exercise (Chapter 3, Exp 2; Chapter 4). These findings suggest that fatigue of working skeletal muscle is reduced and endurance increased when the exercising limb is lowered below the level of the heart from an above-heart or heart-level posture.

Blood flow to the active limb has been shown to increase more quickly during the first minute of submaximal exercise when the exercising limb was placed below compared with above the heart level (Leyk *et al.*, 1994b, MacDonald *et al.*, 1998, Tschakovsky *et al.*, 2004a). MacDonald and coworkers (1998) observed a significantly faster rate of increase of femoral blood flow during upright compared to supine knee extension and flexion exercise. Similarly, Leyk *et al.*, (1994) noted higher blood flow values measured at the femoral artery in the upright posture during calf plantar flexion exercise. These data suggest that a faster rate of blood flow and thus, oxygen supply, may mediate the postural effect in muscle fatigue and endurance.

However, the mediation of blood flow on the postural effect in performance is speculative because muscle endurance and/or fatigue and blood flow in response to a postural change have not been assessed within the same study. Therefore, the objective of the present study was to assess the effect of body tilt

angle on leg blood flow during an exercise in which endurance and fatigue were previously assessed.

In our previous studies we found that at 70 % F_{max} the rate of fatigue was significantly diminished and endurance time significantly prolonged when the body was tilted from the horizontal to an incline position and that this postural effect depended on the peripheral circulation. So, our first aim was to explore whether the postural effects on fatigue and endurance observed at 70 % F_{max} are mediated by the rate of increase in blood flow. In addition, we also observed that at 30% F_{max} the rate of fatigue was not different between horizontal and incline postures during exercise limited to 20 min. Therefore, the second aim of the present study was to investigate whether the lack of postural effect on fatigue observed at 30 %F_{max} is either due to an unaffected blood flow or an insensitivity of muscle force production to an increase in leg blood flow. In order to achieve these aims, in the present study we measured the blood flow responses at intensities of 30 % and 70 % Fmax.

5.2 Material and methods

5.2.1 Subjects

The 6 subjects that participated in this experiment were male, young (mean ± SD: age = 26.2 ± 2 yr; height = 181 ± 6 cm; weight = 83 ± 6.2 kg)., non-smokers and free of any clinically significant disease that could affect their exercise performance. All subjects read the subject information sheet (Appendix 18) and gave written informed consent prior to their participation in this study (see Chapter 2, Appendix 3). The procedures were conducted in accordance with the standards set by the Declaration of Helsinki (2000) .

5.2.2 Experimental design

5.2.2.1 Overview

The present experiment investigated the effect of body tilt angle on leg blood flow during isometric exercise of the plantar flexors at high and low intensities.

To avoid potential impact of diurnal factors on exercise performance, subjects performed their exercise sessions at the same time of the day. Subjects refrained from alcohol and caffeine for the 12 hours preceding the testing session as well as from any strenuous exercise for the 24 hours preceding the testing session.

5.2.2.2 Exercise sessions

After two familiarisation sessions (Appendix 19), F_{max} was determined at 0° and 67° body tilt angles during a strength testing session. Then, on four subsequent days separated by at least 72 hours, four intermittent constant-force tests were performed. Two of the tests were conducted at 0°, one at 30 % F_{max} and the other at 70 % F_{max} and the other two tests were conducted at 67° body tilt angle, again one at 30 % F_{max} and the other at 70 % $F_{max.}$ The order of the tests was randomised for all subjects using a cross-over design. The constant-force tests

consisted of isometric contractions of the plantar flexors using 2 s contraction / 4 s relaxation duty cycle. No maximal efforts were performed during constant-force tests. For each test, three exercise trials, separated by 20-30 min of recovery, were completed so that in total each subject performed 12 trials. The duration of each exercise trial at 30 $\%F_{max}$ was 6 min; whereas it ranged between 2-6 min at 70 $\%F_{max}$, because three subjects failed to complete six minutes of exercise at 70 $\%F_{max}$ in the supine condition. As such, only blood flow data collected over the same period (i.e. < 6 min) during the supine and inclined positions were analysed.

5.2.3 Equipment and measurements

5.2.3.1 *Anthropometric measurements* (see Chapter 3, section 3.2.3.1)

5.2.3.2 *Calf Egometer and force measurement* (see Chapter 3, section 3.2.3.2)

5.2.3.3 *Calf exercise model* (see Chapter 3, section 3.2.3.3)

5.2.3.4 *Maximum Force (F_{max}) measurement* (see Chapter 3, section 3.2.3.4)

5.2.3.5 *Venous occlusion plethysmography*

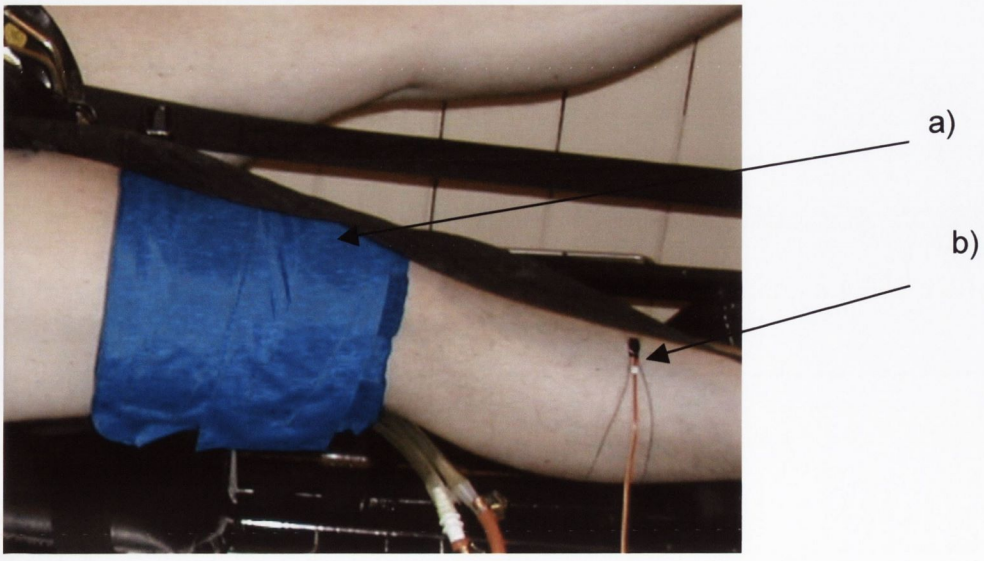
Leg blood flow was assessed using venous occlusion plethysmography (Wilkinson & Webb, 2001). Throughout each exercise session subjects wore a thigh cuff that was inflated to a pressure of 55 mmHg. The pressure of 55 mmHg was chosen because in a pilot experiment (n = 9) it was observed that 55 mmHg was the lowest pressure that prevented venous leakage at very high blood flows (i.e. 70 % F_{max}) from a range of cuff occlusive pressures between 0 and 100 mmHg. Leg blood flow was assessed by measuring the change in leg volume using a mercury-in-elastic strain gauge, placed around the widest girth of the calf (Figure 5.1) and a Hokanson EC-6 plethysmograph (Figure 5.5). Blood flow was measured during each 4 s relaxation period between contractions. Thus, leg blood flow was calculated from the increase in leg

volume over time during each relaxed state after a contraction (see Figure 5.8 in results).

We measured the average slope during the relaxation phase (usually across 2-3 complete cardiac cycles) rather than the peak response over a single cardiac cycle. This was done because this entire response probably affects most O_2 availability and fatigue and also this approach over a longer period will probably reduce measurement error

The change in leg volume was only assessed if the force had returned to within 10 N of a stable minimal value, and only if this stable force value was maintained for at least 3 s (Figure 5.2). In a pilot experiment it was observed that blood starts flowing into the leg only when force falls below ~100-150 N (~10 $\%F_{max}$, Appendix 20). The time spent between this force (~100-150 N) and zero as the muscle is relaxing is very short (~0.1-0.2 s), and therefore, relatively little of the change in leg volume due to arterial inflow is likely to be missed. Consequently, the vast majority of the arterial blood flow into the leg occurs during the relaxed state, and so an estimate of leg blood flow based on the change in leg volume during this period leads to only a slight underestimate of leg blood flow.

Figure 5.1: Tight cuff and strain gauge. a) the tight cuff inflated at 55 mmHg and b) the strain gauge around the widest girth of the calf.



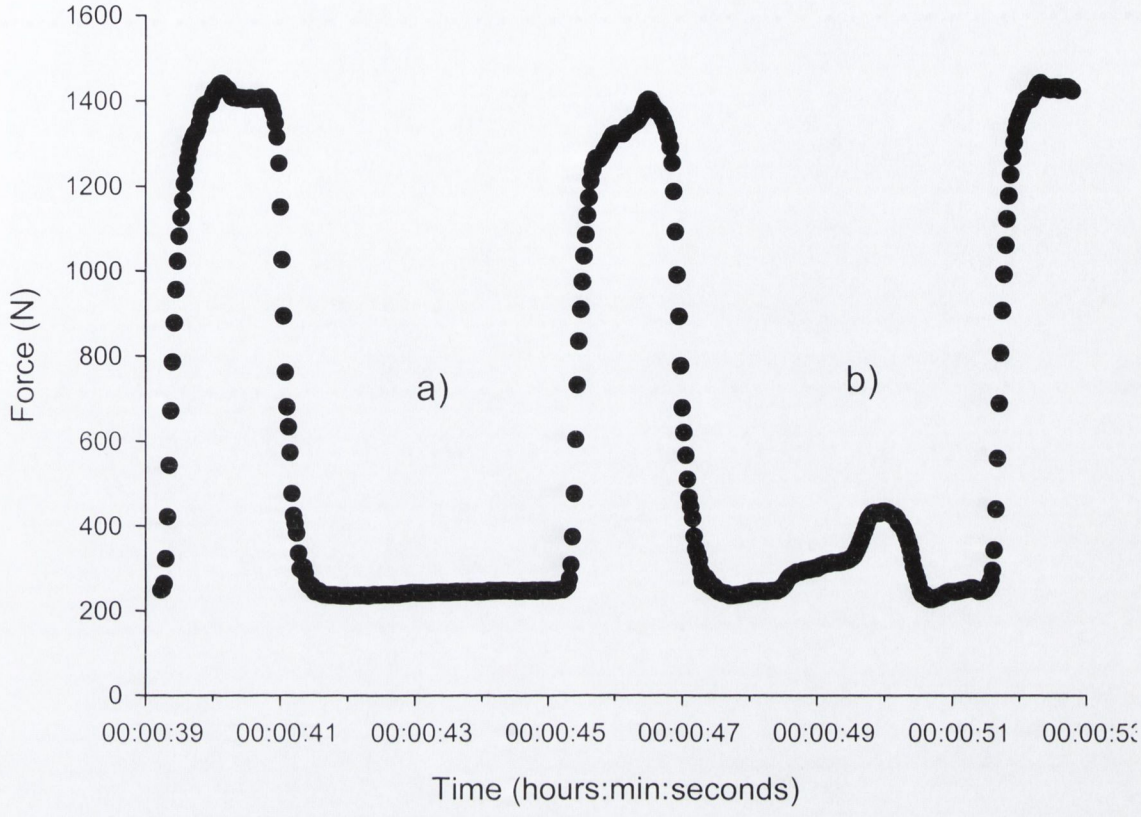


Figure 5.2: A valid and invalid force trace during exercise at 70 % F_{max}. a) is a valid trace because the force during relaxation is stable and is maintained for at least 3 s within 10N. b) is an invalid trace because the force during relaxation is unstable and is not maintained for at least 3 s.

5.2.3.6 Blood flow analysis

The time course data for blood flow collected from the three trials in each condition were averaged to produce a single data for each subject in each test condition. The time course of changes in blood flow were analysed by fitting an exponential curve to the average results of the trials. A two-component (i.e. fast and slow) or biexponential equation was chosen because in over 90 % of cases it fitted better, based on the adjusted R^2, than a monoexponential or triexponential equation. The curve fitting analysis was performed using the software Table Curve (Systat software, Inc. USA).

The equation fitted was:

$$y(t) = a + A1 \cdot (1-e^{-(t-TD1/\tau1)}) + A2 \cdot (1-e^{-(t-TD2/\tau2)}):$$

where y is blood flow at time t; *a* is the y-intercept and approximates resting blood flow; parameters A1 and A2 represent the amplitudes of the two components, TD1 and TD2 are the time delays for the appearance of the two components, and $\tau1$ and $\tau2$ are the time constants of the two components respectively (Figure 5.3). The change in blood flow over the exercise period (i.e. total amplitude) was estimated using the biexponential equation.

An indicator of the rate of change of each of these variables for each subject and condition was obtained by calculation of the mean response time (MRT). The MRT is the time required to achieve ~63 % of the difference between baseline and the exercise plateau, and it was calculated using the equation:

$$MRT = [A1/(A1+A2)] \cdot (TD1 + \tau1) + [A2/(A1+A2)] \cdot (TD2 + \tau2),$$

as described elsewhere (MacDonald *et al.*, 1998).

At the intensity equivalent to 70 $\%F_{max}$ in the supine position three subjects failed to complete six minutes of exercise. In these cases, blood flow data beyond these exercise times to failure collected in the inclined position were eliminated from the curve fitting of the blood flow responses.

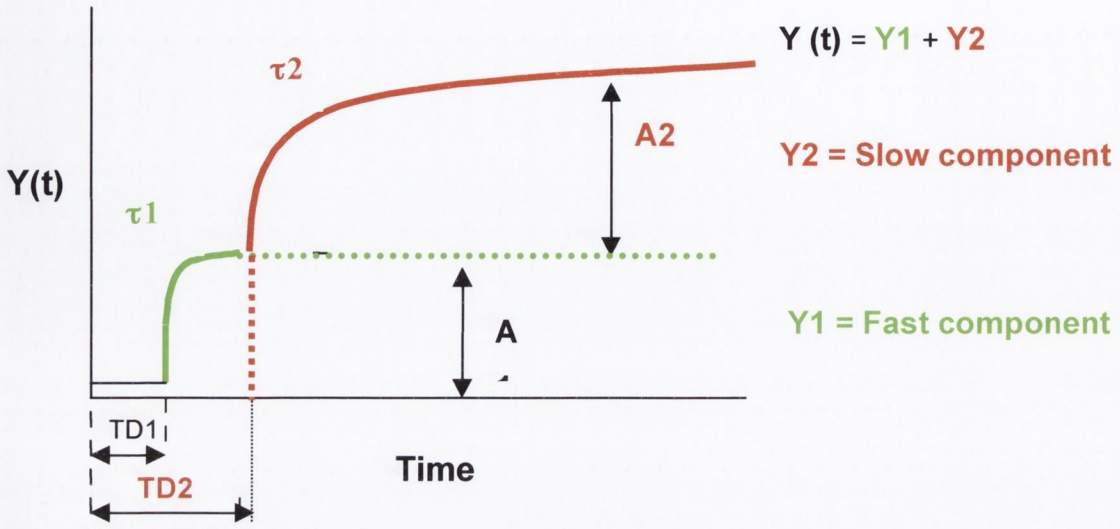


Figure 5.3: An example of a biexponential model. TD1 and TD2 are the time delays of the two components, $\tau 1$ and $\tau 2$ the time constants and A1 and A2 the amplitudes of the two components. Y(t) represent blood flow at time t, and is the result of the superimposition of the two components (i.e. the fast and slow components).

5.2.3.7 Arterial pressure

Mean arterial pressure and heart rate were measured continuously during exercise (COLIN CBM7000; Colin Corp., Komaki City, Japan). The monitor was placed on the right arm of the subject. The arm was fully extended and lay at the heart level of the subjects (Figure 5.4).



Figure 5.4: Blood pressure measurement during exercising in the inclined posture.

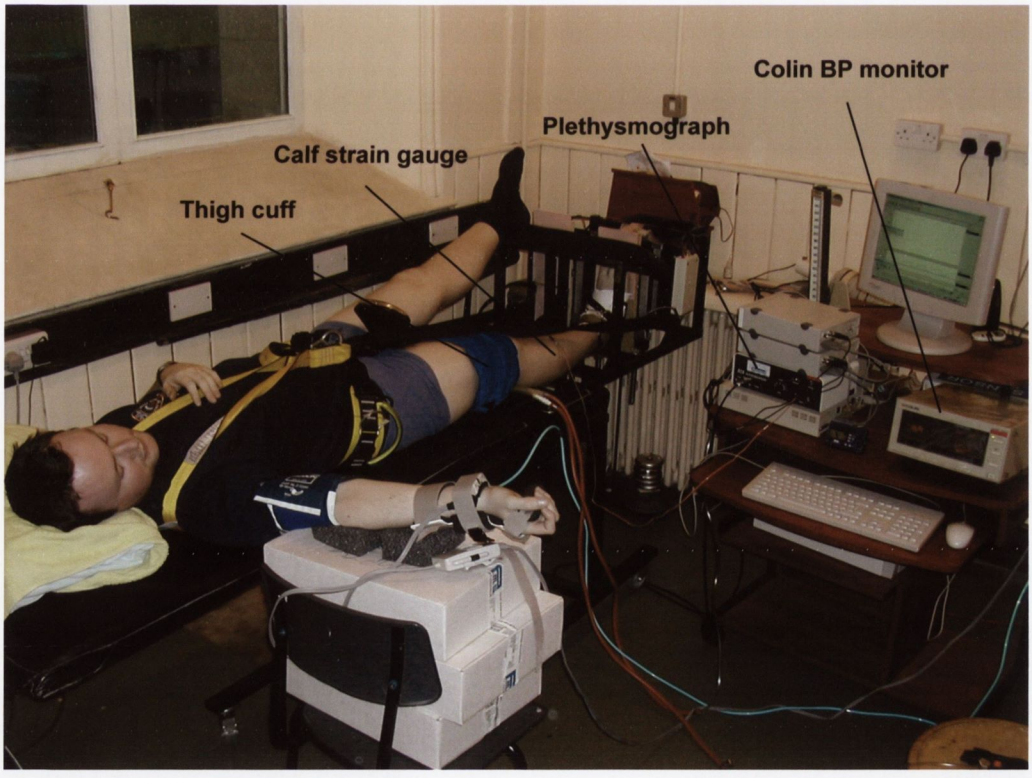


Figure 5.5: Complete setting of the experiment in the supine posture. The figure shows a subject exercising in the horizontal posture. The thigh cuff, strain gauge, plethysmograph and blood pressure monitor are indicated.

5.2.3.8 Leg volume measurements

Leg volume immediately after each contraction was calculated as a percentage of resting volume (100 %) and was measured at the start of the pulsatile increase in leg volume during the relaxed state (Figure 5.6).

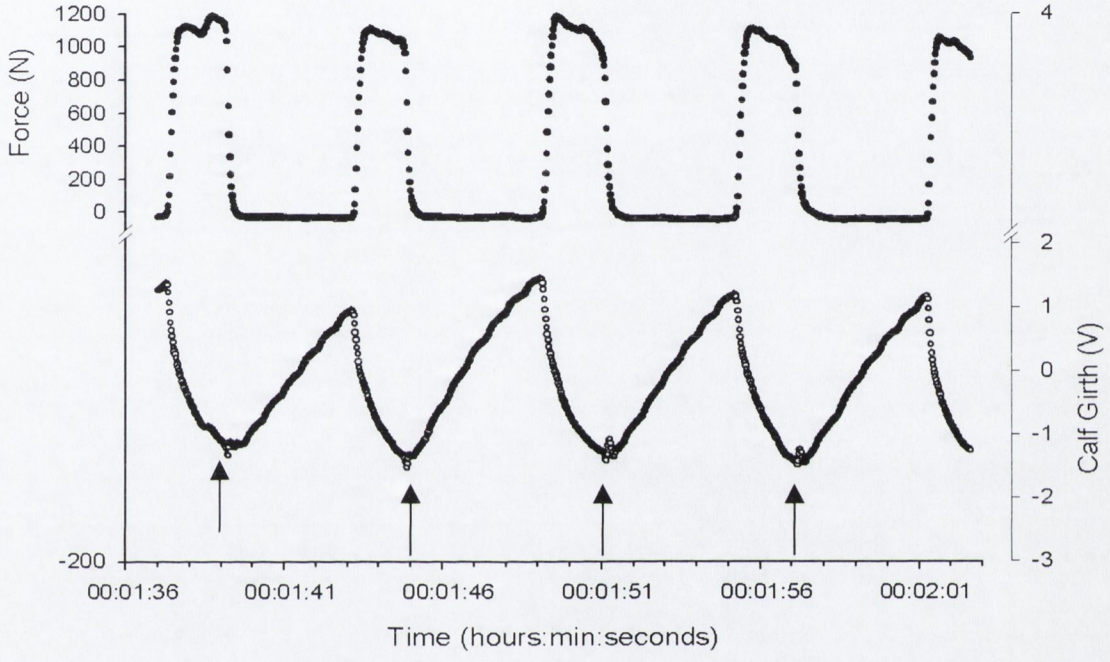


Figure 5.6: Leg volume was measured at the start of the pulsatile increase in leg volume during the relaxed state. The arrows in the figure indicate the measurement points.

Note that the lack of increase in girth volume during the contraction phases shown in this figure did not result in all the subjects; in contrast, some subjects showed a steep increase in girth during contractions. A possible reason of the shape of the trace during muscle contraction in this figure may have been that during contraction one part (probably the lower part) of the mercury strain gauge may have been loosened, consequently reducing the resistance of the gauge and so, decreasing the trace of the calf girth. However, it is unlikely that this distension of the strain gauge during contraction affected blood flow

measurements because in all cases the calf girth started to increase at the very beginning of the relaxation phase.

5.2.3.9 Calculation of leg volumes of the subjects

Lower leg volumes (i.e. right leg) were calculated following the regression equations for the prediction of tissue masses of the leg ((skin, adipose tissue, muscle and bone (Clarys & Marfell-Jones, 1986)). The following measurements were taken and applied in the equations:

1. Lower leg (tibiale-malleolare) length
2. Maximal calf girth
3. Maximal calf skinfold
4. Bi-malleolare breadth

5.2.3.10 Vascular conductance

Vascular conductance was calculated using the following formula:

Vascular conductance= Blood flow / Mean Arterial Pressure

5.2.4 Statistical Analyses

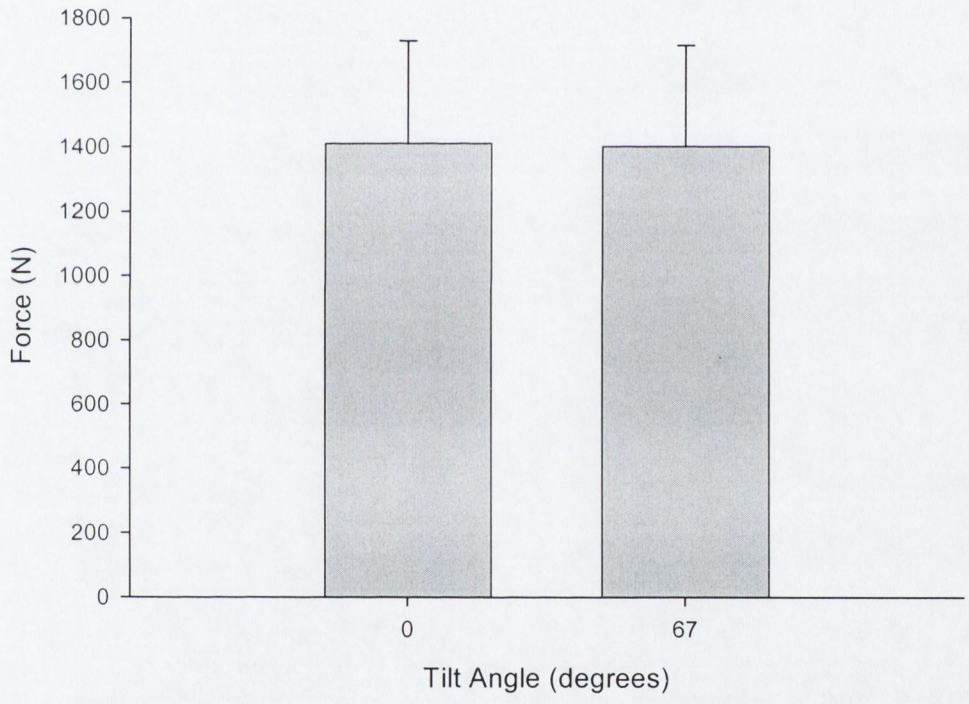
In this experiment a two-way repeated measures ANOVA was performed (main effects = intensity and body tilt angle). Effects of body tilt angle on F_{max}, were identified using one-way repeated-measures ANOVA. The differences were located using a Tukey's HSD test or, for non-normal data, a Wilcoxon's Rank test. The level of significance was set at $P<0.05$. All results are shown as means ± standard deviations.

5.3 Results

5.3.1 Maximum force (F_{max}).

F_{max} was assessed during a specific strength session prior to the constant load tests and was not different between supine and inclined positions (Figure 5.7)

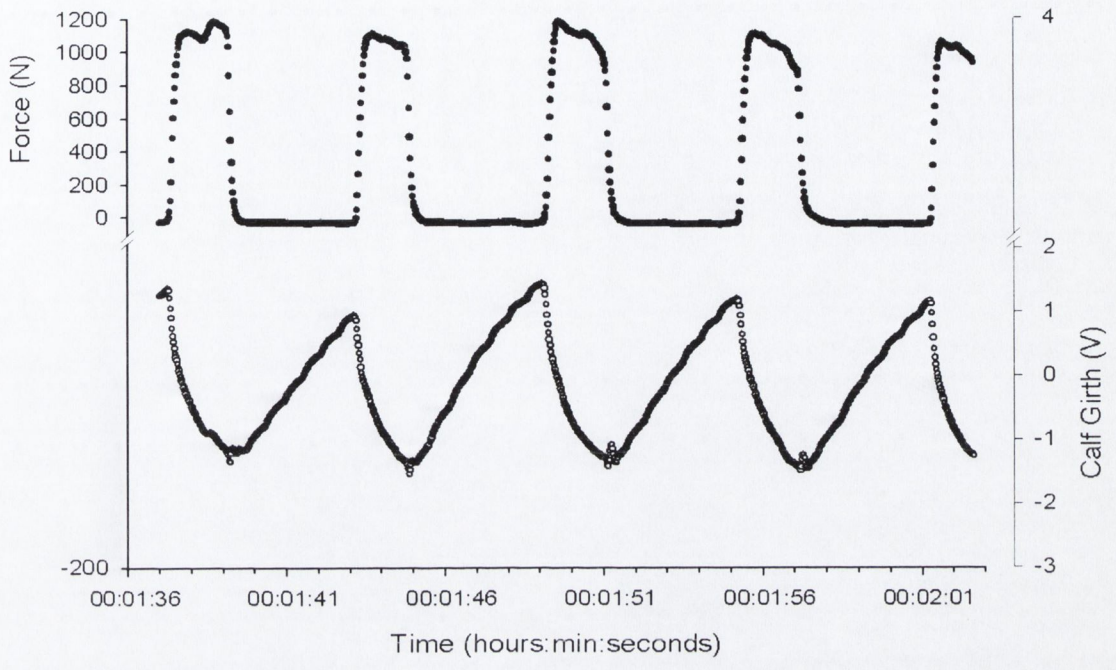
Figure 5.7: Fmax data for supine and incline positions



5.3.2 Force and leg volume

Force and leg volume ('calf girth') recordings during single exercise trials at 70 $\%F_{max}$ at 0° and 67° are shown in Figures 5.8A and 5.8B respectively. Leg blood flow was calculated from the increase in leg volume over time during each relaxed state after a contraction.

A)



B)

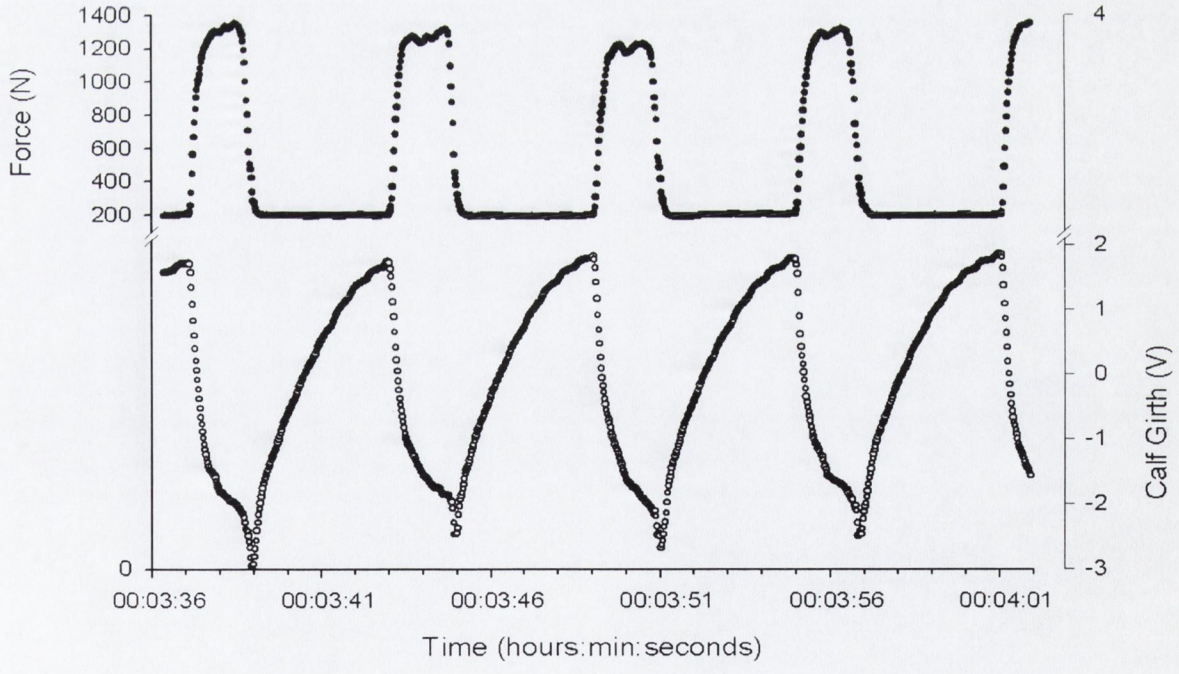


Figure 5.8. Force and calf girth traces during the last 30 s of exercise at 70 $\%F_{max}$ at body tilt angles of 0° (A) and 67° (B) in one subject. Note that the higher residual force at 67° (~ 200N) is added to the force to be sustained during the test. Also note that the changes on girth are approximately 2-fold larger at 67° compared to 0°.

5.3.3 Leg blood flow

The mean blood flow data for the same subject is shown in Figure 5.9. Throughout the 3 exercise trials of each of the exercise conditions no systematic changes in blood flow responses were observed. An example of the leg blood flow for each of the 3 exercise trials at each of the 4 conditions for the same subject are shown in Figure 5.10.

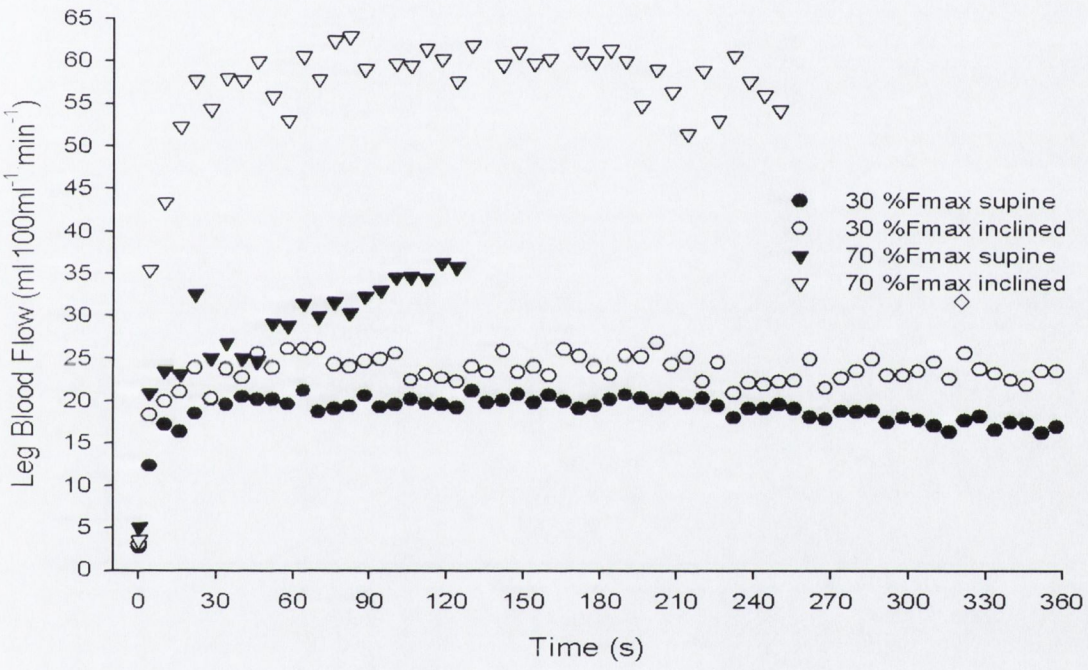


Figure 5.9. Average leg blood flows during exercise at two tilt angles (0° and 67°) at two intensities (30 and 70 $\%F_{max}$) in one subject.

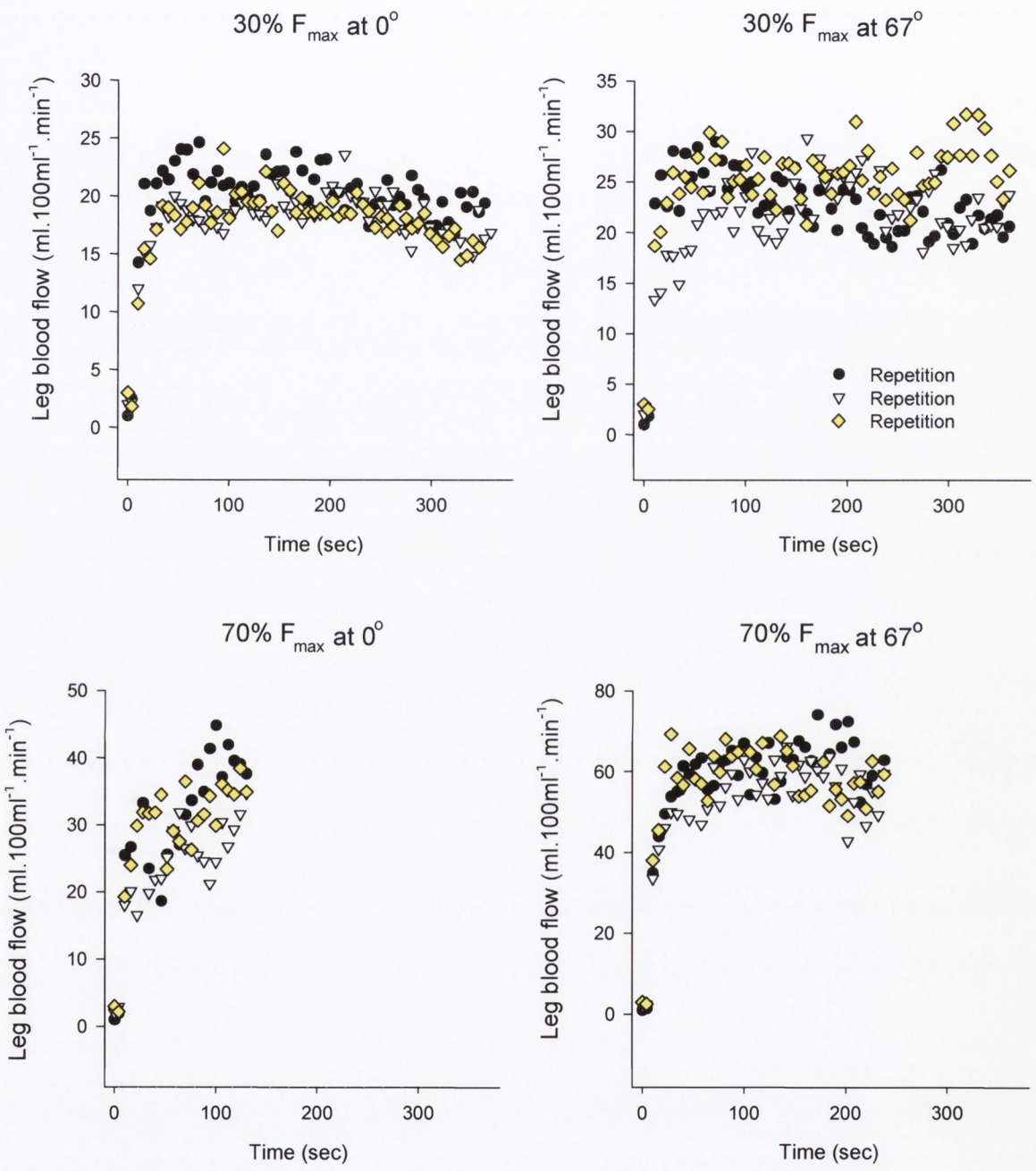


Figure 5.10: Leg blood flow responses for the 3 exercise trials during exercise at two tilt angles (0° and 67°) at two intensities (30 and 70 %F_{max}) in one subject.

5.3.4 Blood flow parameters

The parameter estimates, total amplitudes and mean response times describing the blood flow responses under the four conditions are shown in Table 5.1. The total amplitude of leg blood flow was two-fold higher ($P < 0.05$) at 70 versus 30 $\%F_{max}$ in both the horizontal and inclined positions. In addition, the amplitudes of the fast (parameter A1) and slow (parameter A2) phases were also significantly higher at 70 versus 30 % F_{max}. Increasing the body tilt angle significantly increased the total amplitude at 30 $\%F_{max}$; but not significantly at 70 % F_{max} ($P = 0.19$). However, at both intensities, increasing body tilt resulted in a significantly higher amplitude of the fast phase (A1). As a proportion of the total amplitude, the amplitude of the fast phase (i.e. A1/total amplitude) was significantly greater in the inclined than the supine position at 30 $\%F_{max}$ (69.5 ± 7.5 vs 48.7 ± 10.9 %, respectively) and 70 $\%F_{max}$ (70.1 ± 17.3 vs 52.7 ± 12.1 %, respectively); and the amplitude of slow phase (A2) as a proportion of the total amplitude was significantly lower in the inclined than the supine position at 30 $\%F_{max}$ (31.2 ± 8.2 vs 51.3 ± 10.9 %, respectively) and 70 $\%F_{max}$ (30.6 ± 17.2 vs 55.0 ± 19.8 %, respectively).

Table 5.1. Parameter estimates, total amplitudes and mean response times (MRT) of the blood flow responses under the four exercise conditions.

Force / Tilt Angle: Condition	30 %F_{max} 0^0	30 %F_{max} 67^0	70 %F_{max} 0^0	70 %F_{max} 67^0
a (ml 100 ml^{-1} min^{-1})	3.16 ± 0.21	3.20 ± 0.13	3.39 ± 0.60	3.06 ± 0.38
A1 (ml 100 ml^{-1} min^{-1})	6.17 ± 2.58* †	12.72 ± 4.25	13.59 ± 5.21 †	23.63 ± 13.68
A2 (ml 100 ml^{-1} min^{-1})	6.47 ± 2.46*	5.84 ± 3.26	14.24 ± 6.28	10.67 ± 8.81
Total Amplitude (ml 100 ml^{-1} min^{-1})	12.65 ± 4.02*†	18.43 ± 6.75*	25.95 ± 8.71	34.16 ± 18.55
$\tau 1$ (s)	2.3 ± 1.5	2.9 ± 3.7	1.8 ± 2.0	1.8 ± 1.9
$\tau 2$ (s)	24.0 ± 10.6	67.2 ± 41.5	82.2 ± 98.0	52.9 ± 38.4
MRT (s)	17.1 ± 7.2	27.4 ± 19.6	57.9 ± 81.8	19.1 ± 18.6

* significantly different (P 0.05) from higher intensity at same tilt angle

† significantly different (P 0.05) from inclined position (67^0) at same intensity

5.3.5 Blood pressure, heart rate, blood flow, vascular conductance and change in leg volume

The responses of mean arterial pressure, heart rate, blood flow and relative change in leg volume to the first, middle and last contraction during exercise under the four conditions are shown in Table 5.2. Mean arterial pressures were not affected by tilt angle at rest or during exercise at both 30 or 70%F_{max}. Heart rate during rest was significantly higher in the inclined than supine position at 30%F_{max} only; but the change in heart rate (i.e. increase from this resting value) was not affected by tilt angle at either 30 or 70 %F_{max}.

On average, leg volume (percent of resting value) was significantly lower ($P < 0.001$) during the first two minutes of exercise at 70 %F_{max} in the inclined (99.2 ± 0.5 %) than the supine position (99.8 ± 0.3 %). The duration of two minutes was chosen to include all subjects in the analysis, since two subjects reached failure at minute two in the supine posture. At 30 % F_{max} leg volume was also, on average, lower ($P < 0.001$) throughout the six minutes of exercise (all subjects reached 6 minutes at 30 % F_{max}) in the inclined (99.0 ± 0.2 %) versus the supine position (99.3 ± 0.4 %).

Blood flow at rest was not different between postures or intensities, but after the first contraction and at the end of exercise it was significantly higher in the inclined compared to supine posture both at 30 and 70 %$F_{max.}$ In addition, blood flow during exercise was higher at 70 % compared to 30 %$F_{max.}$

Vascular conductance responses were very similar to the blood flow responses.

Table 5.2. Mean arterial pressure, heart rate, blood flow, vascular conductance and leg volume (percent of rest) prior to and during exercise at two intensities and two tilt angles. "1st", "Mid" and "End" refer to the first, middle and last contractions across the exercise period.

		MAP (mmHg)		HR (bpm)		Blood flow ($ml.min^{-1}$)		Vascular conductance ($ml.min^{-1}.mmHg^{-1}$)		Leg Volume (%)	
	Tilt Angle	0°	67°	0°	67°	0°	67°	0°	67°	0°	67°
0% $_{max}$	Rest	92 ± 7	87 ± 4	66 ± 8*	80 ± 8	77 ± 17	64±23	0.83±0.19	0.74±0.24	100	100
	1st	92 ± 8	89 ± 8	69 ± 8*	81 ± 10	280 ± 109*t	530 ± 152 t	3.03±1.20* t	6.03±1.83 t	98.7 ± 1.1	98.7 ± 0.7
	Mid	93 ± 7	88 ± 7	72 ± 7*	83 ± 10	502 ± 145t	678 ±286 t	5.48±1.68*	7.83±3.54	98.9 ± 2.0	99.0 ± 1.0
	End	96 ± 8	91 ± 7	73 ± 5*	86 ± 5	494 ± 140*t	732 ± 281 t	5.20±1.51* t	8.00±2.76 t	100.0 ± 1.2	99.0 ± 1.3
0% $_{max}$	Rest	89 ± 5	88 ± 8	71 ± 9	76 ± 12	75 ± 16	79 ± 21	0.85±0.18	0.90±0.29	100	100
	1st	87 ± 7	89 ± 9	77 ± 10	83 ± 13	481 ± 163*	752 ± 319	5.50±1.71*	8.32±3.02	99.2 ± 0.5	98.1 ± 1.2
	Mid	90 ± 3	91 ± 9	85 ± 10	87 ± 11	902 ± 383	1198 ± 628	9.56±3.56*	12.71±5.56	100.1 ± 1.5	99.2 ± 2.0
	End	94 ± 4	92 ± 5	88 ± 11	94 ± 15	925 ± 375*	1272 ± 548	9.80±3.87*	13.66±5.46	100.2 ± 1.9	99.6 ± 2.8

*- significantly different ($P < 0.05$) from inclined position (67^0) at same intensity

†- significantly different ($P < 0.05$) from higher intensity at same tilt angle

5.4 Discussion

The main findings of the present study were that the total amplitude of leg blood flow was significantly higher at 70% F_{max} compared to 30% F_{max} in both postures. At both intensities the amplitude of the fast phase (A_1) was significantly higher in the incline compared to supine posture. In addition, on average, the leg volume during exercise was significantly higher at 0° compared to 67° at both intensities.

5.4.1 Mediation of blood flow

That endurance is mediated by changes in perfusion pressure has been shown in several studies that have compared graded cycling performance when the exercising limb was positioned below the heart level (upright) and at the same level of the heart (supine). These studies have shown a small but consistent increase in endurance time (~10-15 %) during upright compared to supine posture (Eiken, 1988, Leyk *et al.*, 1994a, Terkelsen *et al.*, 1999). We have also demonstrated that changes from supine to upright posture increase endurance time by 160 % during high-intensity constant-load cycling exercise (Chapter 2) and by 100 % during high-intensity constant-load plantar-flexion exercise (Chapter 3 and 4). Similarly, muscle fatigue has been shown to be very sensitive to changes in perfusion pressure and presumably blood flow. For instance, during electrically-induced submaximal contractions of the adductor pollicis muscle in humans, a reduction in muscle perfusion pressure induced by elevating the arm above the heart level significantly decreased the force output within seconds (Fitzpatrick *et al.*, 1996, Wright *et al.*, 1999), and during moderate to high intensity constant-load plantar flexion exercise in humans, tilting the body from the supine posture reduced the rate of fatigue by around 40% (Chapter 3 and 4).

Several studies have attempted to investigate the physiological causes to the changes in endurance and fatigue seen in the abovementioned studies without necessarily measuring endurance and/or fatigue parameters. These studies

have suggested that leg blood flow mediates the postural effect on fatigue and probably endurance such that a more rapid hyperaemia occurs during the early period of exercise when the exercising limb is positioned below compared with above the level of the heart. For instance, tilting the upper body upright from a supine position increases quadriceps muscle blood flow during knee extension exercise (MacDonald *et al.*, 1998) and tilting the whole-body from a supine to inclined position increases femoral arterial blood flow during calf exercise (Leyk *et al.*, 1994b). These effects occurred following either the first contraction or throughout the first minute of exercise.

In agreement with the abovementioned studies, the findings of the present study showed that during high-intensity exercise (70% F_{max}) the amplitude of the fast component was significantly higher in the inclined than the supine position (Table 5.1); whereas the slow phase tended to be lower ($P = 0.17$). As a proportion of the total amplitude, the amplitude of the fast phase (i.e. A1/total amplitude) was significantly greater in the inclined than the supine posture, suggesting that over the initial minutes of exercise blood flow was higher in the inclined posture. Therefore, in response to our first aim, these data demonstrate that the postural effect on fatigue and endurance during a high-intensity plantar flexion exercise is linked to blood flow.

Our second aim was to explore the causes of the lack of postural effect observed at low intensity exercise (Chapter 4, Exp 1). The present data showed higher increases in leg blood flow in the inclined compared to the supine posture during the low-intensity exercise (30% F_{max} : Table 5.1). Consequently, a likely explanation for the intensity-dependent effect of posture on fatigue is that, at low intensities, muscle force production and fatigue are insensitive to changes in blood flow and O_2 delivery; whereas at moderate to high intensities this is not the case.

5.4.2 Cardiovascular responses at the onset of exercise

The mechanical and physiological factors determining the early hyperaemic response at the onset of exercise are not clear, but presently, most of the focus

centers on the relative contributions of the muscle pump and vasodilation (Hamann *et al.*, 2004, Hamann *et al.*, 2003, Laughlin & Schrage, 1999, Naik *et al*, 1999, Sheriff, 2003a, Sheriff & Hakeman, 2001, Sheriff *et al.*, 1993, Sheriff & Van Bibber, 1998, Sheriff & Zidon, 2003b, Tschakovsky & Hughson, 2000, Tschakovsky *et al.*, 2004a, Tschakovsky *et al.*, 1996). Studies in animal locomotion models demonstrate that muscle pump raises the immediate exercise hyperaemia in proportion to contraction (stride) frequency ((i.e. treadmill speed, (Sheriff, 2003a, Sheriff *et al.*, 1993)). However, it has been suggested that muscle pump contribution to hyperaemia is unaffected by an increase in contractile force (Laughlin & Schrage, 1999, Sheriff & Hakeman, 2001, Tschakovsky *et al.*, 2004a) whereas it has been shown in human handgrip studies that muscle contraction intensity is linearly proportional to an increase in vasodilation (Saunders & Tschakovsky, 2004, Tschakovsky *et al.*, 2004a). In the present study the amplitude of the fast phase of leg blood flow was intensity-dependent as it was two-fold higher at 70 % F_{max} compared to 30 % F_{max} in both postures (Table 5.1). Therefore, the present data suggest that the early hyperaemic response during plantar flexion exercise is mediated at least in part by local vasodilation.

A clear evidence of local vasodilation can be seen, for instance, during supine exercise at the intensity relative to 70% F_{max}, where the 640% increase in blood flow from rest to the first contraction was clearly mediated by vascular conductance (see Table 5.2), since perfusion pressure only increased by 25% from 70 to 88 mmHg, assuming that venous pressures at rest and after the first contraction were 15 and 0 mmHg respectively.

On average, the leg volume throughout exercise in the present study was lower in the inclined compared to supine posture both at 30 % F_{max} and 70 % F_{max} (Table 5.2, see results). This suggests that blood was more effectively expelled (or larger venous emptying effect) by the contracting muscles in an inclined position, despite the fact that arterial flow into the muscle was higher (Folkow *et al.*, 1971). Therefore, our findings suggest that both muscle pump action and vasodilation contribute to the rapid hyperaemic response observed in the present study.

The central cardiovascular responses at the onset of exercise appeared to be similar in each of the upright and supine positions. Heart rate was significantly lower in the inclined compared to supine posture at rest and during exercise at 30 % F_{max} but, the change in HR during exercise was not different among the postures. During high-intensity exercise heart rate increased very rapidly from rest to the first contraction (Table 5.2), probably due to vagal withdrawal, but the increase was similar in both postures. Mean arterial pressure was unaffected by tilt angle or intensity (Table 5.2).

Resting blood flow in the present study was not affected by posture or tilt angle (Table 5.2). Macdonald *et al.,* (1998) also found similar blood flows at rest in the upright and supine postures. However, Leyk *et al.,* (1994) observed significantly higher resting blood flows in the supine compared to upright position. In the study conducted by Leyk *et al.,* (1994), subjects performed calf plantar flexion exercise in a seated upright and supine position. In the upright position, the legs of the subjects were kept below the heart level, whereas in the supine position, the legs were above heart level. Similarly, subjects in the study by Macdonald *et al.,* (1998) performed knee extension-flexion exercise seated upright and supine; but during supine exercise subjects kept their tight muscles at the level of the heart and the calf muscles below the heart.

In contrast with our findings of greater blood flows in the incline posture during exercise, Leyk *et al.,* (1994) and Macdonald *et al.,* (1998) found no differences in blood flows during exercise in the upright and supine postures. In our study subjects kept their body completely extended whereas in the studies by Leyk *et al.,* (1994) and Macdonald *et al.,* (1998) subjects were seated. Therefore, in our study the larger effect of gravity on perfusion pressure in the incline posture may have induced larger differences in blood flows between the two postures. In addition, the intensity of the present study (~ 70% of peak graded workload, or 70% F_{max}) was higher than the intensity of the study by Macdonald *et al.,* (1998, 1/3 of peak graded workload) and the study by Leyk *et al.,* (1994, ~ 30% MVC).

The leg blood flow values recorded in the present study, both, at rest and during exercise, were lower than the ones observed in the studies conducted by Leyk *et al.,* (1994) and Macdonald *et al.,* (1998) using Doppler ultrasound equipment. However, these differences may be because:

a) Both, Leyk *et al.,* (1994) and Macdonald *et al.,* (1998) measured blood flow at the femoral artery of the thigh, whereas we calculated blood flow at the calf muscle.
b) In the present study, to prevent venous outflow during relaxation, a cuff around the thigh was inflated at 55 mmHg (see methods) and was maintained during the course of the exercise tests. It has been shown that a congestion cuff as used in our study impedes arterial inflow as a result of venous congestion (not arterial compression) by the second cardiac cycle following inflation (Tschakovsky *et al.*, 1995).

Venous occlusion plethysmography has not been compared with other more established methods of blood flow measurement such as Doppler ultrasound. However, there is evidence to suggest that venous occlusion plethysmography can be used to measure blood flow during exercise. On one hand, during the relaxation phases the leg was relaxed and fixed in order to minimize any movement artefact and allow accurate measurement of blood flow. On the other hand, the amplitude of the blood flow response increased by 2-fold (see Table 5.1) when the intensity was increased by 2.3-fold (i.e. from 30 to 70% Fmax), which is in agreement with the linear relationship between exercise intensity and blood flow (Andersen & Saltin, 1985, Radegran & Saltin, 1998).

However, one of the limitations of venous occlusion plethysmography is that by measuring the change in blood volume (usually across 2-3 cardiac cycles), there is a possibility that the veins may fill completely, enabling the venous pressure to increase to a level that may cause venous outflow. This was most likely to happen at the higher intensity (70% Fmax) and in the incline posture (67°) where the blood inflow was highest, and if this happened, it would have contributed to an underestimation of blood flow. In an attempt to eliminate this

possibility, a thigh cuff was inflated to 55 mmHg which prevented any decline of the strain gauge trace. In fact, none of the strain-gauge responses in the six subjects declined at any point during relaxation, indicating that venous outflow probably did not occur.

5.5. Conclusion

In conclusion this study showed that the initial or fast phase of the leg blood flow is increased in a tilted position, and that this effect contributes entirely to the positive effect of body tilting on leg blood flow observed during several minutes of exercise. This suggests that the tilt-induced increase in the fatigue-resistance and endurance of the plantar flexors is mediated by a larger response of blood flow to the first contraction. This mediation, however, is only evident at high intensities (i.e. 70 % F_{max}), because at low intensities (i.e. 30 % F_{max}) a similar postural response in blood flow does not increase fatigue-resistance.

Chapter 6: **General discussion**

Chapter 6: GENERAL DISCUSSION

The major novel findings of the present thesis are as follows:

1. Endurance time to failure during a high-intensity constant-load cycling exercise increased by 160 % in the upright compared to supine posture.
2. Endurance time to failure during plantar-flexion exercise increased by 17 % during a graded test and by 100 % during a high-intensity constant-load test when the body was tilted from the supine to the upright posture
3. The rate of fatigue during constant-load plantar flexion exercise was reduced by ~40 % when the body was tilted from the supine posture at moderate to high intensities (>40 % F_{max}) but this effect was not observed at low intensities (<40 % F_{max}).
4. The postural effect on endurance and fatigue during constant-load plantar flexion exercise was linked to a greater blood flow response in the inclined position.

6.1 Postural effect on endurance

6.1.1 Whole-body exercise

The literature shows that during incremental cycling exercise endurance time to failure is ~10-15 % higher in the upright compared to supine posture (Eiken, 1988, Leyk *et al.*, 1994a, Terkelsen *et al.*, 1999). This effect has been suggested to be mediated by a faster O_2 uptake ($\dot{V}O_2$) adjustment in the upright posture observed during the early phase of a high-intensity constant load cycling exercise (Convertino *et al.*, 1984, Koga *et al.*, 1999, Leyk *et al.*, 1994a). However, this assumption is indirect because experiments which have investigated $\dot{V}O_2$ alterations were done so during short duration constant-load exercise bouts, whereas the effect of posture on endurance cycling time has only been shown during incremental graded exercise tests. Therefore, during the first experimental study, we measured the performance time to failure during

a constant-load cycling exercise in the upright and supine postures in men and women, and the change in $\dot{V}O_2$ at the beginning of exercise was calculated simultaneously. Since the intensity for the constant-load test was set as a percentage of a maximal workload, prior to the constant-tests, incremental tests were also performed.

Consistent with previous findings, during graded tests, endurance time in the upright posture was ~10 % higher in the upright compare to supine posture both in men and women. The improvements in endurance in the upright posture during the ABS constant-load test were 10-fold higher than during graded tests, and were of a significantly greater magnitude for men compared to women. During the constant-load test the change in oxygen uptake in the upright posture was significantly faster than in the supine during the first 15 s of exercise, and the magnitude of this effect entirely explained the higher $\dot{V}O_2$ that was sustained during the first two minutes of exercise. Therefore the results of this study support the idea that a faster response in $\dot{V}O_2$ at the onset of exercise and, probably, blood flow to the working muscles mediates the postural effect on performance. The larger postural effect on performance observed in men during the constant-load test, might have been attributed to a relatively greater effect on muscle perfusion and oxygen uptake. Men were significantly taller than women, and had a longer 'hydrostatic' column between the heart and the proximal muscles engaged in cycling. It was observed that the height or the estimated distance of the hydrostatic column were positively correlated with the absolute and relative improvements in cycling time in the upright position (Fig 2.6A, 2.6B and 2.6C). Therefore, the results of this study suggest the possibility that the magnitude of the postural effect on cycle performance is linked to the magnitude of the increase in perfusion pressure induced by a change in posture.

Caution needs to be applied when analysing the relationships between exercise variables and anthropometric values. Even though these correlations show high r-values, it is possible they were strongly affected by the two different genders. In other words, it could have been that the highest changes in time to failure between the upright and supine postures were achieved by the fittest men due

to a higher temporal resolution as a result of significantly higher times to failure compared to women. We explored this possibility using Bland-Altmann plots, and performing a linear regression between the individual postural changes in time to failure (upright minus supine) and the mean individual times to failure (i.e. mean times between upright and supine). The test showed a significant relationship (r=0.9). Therefore, the correlations between exercise variables and anthropometric values are strongly influenced by the two different genders, counteracting, in part, their significance.

Limitations:

One of the limitations of the present study was that the phases of the menstrual cycle of the women were not controlled. This may have influenced the results since phases of the menstrual cycle produce different substrate, endocrine and ventilatory responses during continuous exercise (Bonen *et al.*, 1983, Lavoie *et al.*, 1987, Regensteiner *et al.*, 1989). For instance, when following a 24 hour carbohydrate-poor diet seven young women performed prolonged cycling exercise at 63% VO_{2max} in the upright posture both in the mid follicular and luteal phases, blood glucose concentration was significantly decreased after 70 and 90 min of exercise in the luteal but not in the follicular phase. In addition, cortisol and progesterone levels at the end of 90 min of exercise were higher in the luteal but not in the follicular phase, but in contrast, blood lactate was highest during the follicular phase (Lavoie *et al.*, 1987).

6.1.2 Individual muscle group exercise

The next step was to investigate the postural effect on muscle endurance in an isolated muscle group of the lower body (i.e. calf muscle). In this second study, isometric plantar flexion was applied while the body was positioned at various tilt angles from the supine to 90°. Prior to this second study there was no data regarding the postural effect on the performance either during a graded or constant-load test using an isolated muscle group; and thus, the results of our second study could only be compared with the ones obtained during whole-body exercise. To facilitate this comparison, the protocol of the plantar-flexion

graded test was similar from the graded cycling test, and the intensity chosen for the constant-load test (70 % F_{max}) was similar from the one during the constant-load cycling test (80 % Wload max).

During the graded plantar flexion test the time to failure increased by 17 % when the body was tilted from the supine posture, and during the constant-load test the improvement in time induced by a change in posture was of 100%. Therefore, these data suggest that the postural effect on performance time during high-intensity constant-load exercise is an order of magnitude greater than the postural effect seen for graded tests for both, whole-body exercise and exercise involving isolated muscle groups.

6.2 Postural effect on muscle fatigue

6.2.1 At 70 % F_{max}

During the performance to the point of failure during high-intensity constant-load plantar flexion test subjects performed maximal efforts every 30 s to calculate the rate of fatigue. The rate of fatigue was reduced by ~40 % when the body was tilted from the horizontal posture. Prior to this study there was no data on the effect of postural change on muscle fatigue during voluntary constant-load exercise involving lower limb muscles. Studies involving electrically-induced submaximal contractions of the adductor pollicis muscle in the human hand demonstrated that changes in perfusion pressure exert immediate changes in force production (Fitzpatrick *et al.*, 1996, Wright *et al.*, 1999). For instance, lowering the arm below heart level (and thus, elevating perfusion pressure) significantly increased muscle force production within seconds after the change in limb position (Fitzpatrick *et al.*, 1996), suggesting that muscle force production during repeated contractions is very sensitive to changes in blood flow.

Then, we reasoned that if blood flow plays an important role in mediating the postural effect on fatigue and muscle endurance, preventing arterial blood flow

into the leg (i.e. ischaemia) should reduce the postural effects in endurance times we observed during the graded and constant-load plantar flexion tests. Consistent with this, when the plantar flexion tests were repeated with the blood flow to the legs completely occluded, no differences were found in endurance times to failure or rate of fatigue between inclined and supine postures both during graded and constant-load tests, suggesting that ischaemia abolished the postural effect on calf performance.

Muscle fatigue has been assessed by measuring the rate of decline in maximal voluntary contraction (MVC) during repetitive maximal contractions in absence of any sub-maximal constant-load contractions (Hepple, 2002). This means that in addition to the submaximal contractions, the maximal contractions performed in the different experiments during chapters 3 and 4 may have affected (reduced) the endurance times to failure achieved by the participants by accelerating metabolite accumulation. However, in this instance, combining maximal and submaximal contractions was an appropriate approach because: a) it allowed quantification of the rate of fatigue, and b) even though performance times to failure were probably reduced, in these experiments, a repeated measures design was applied (each subject performed the same protocol at different tilt angles and force intensities).

6.2.2 Low to moderate (30-60 % F_{max}) vs. very high intensities (80-90 % F_{max})

The next question to resolve was whether the postural effect on muscle fatigue and/or endurance time seen during plantar flexion exercise at 70 % F_{max} is still observed at low to moderate intensities (below 70 % F_{max}) and very high intensities (above 70 % F_{max}). The literature suggests that fatigue during electrically-induced repeated contractions depends on the frequency of stimulation at least on the adductor pollicis muscle in humans (Fitzpatrick *et al.*, 1996). These authors demonstrated that raising the arm above the level of the heart decreased muscle force production during electrically-induced tetanic contractions at frequencies of 3-9 stimuli at 25 Hz each second, but when

contractions were induced less frequently (i.e. single twitches) elevating the arm above the heart level had no effect on muscle force production

The third experimental study found that fatigue from moderate to very high intensities (40-90 % F_{max}) was significantly diminished when the body was tilted from the supine posture, but was not different between postures at 30 % F_{max}. Therefore, these data demonstrate that the effect of posture on muscle performance during voluntary exercise is intensity-dependent. In addition, endurance was significantly prolonged during high intensity (80 and 90% F_{max}) exercise suggesting that the postural effect on endurance and fatigue is still present at near maximal exercise.

However, there was a considerable variation among subjects in the postural responses of mainly low to moderate intensities (see Appendix 19 for individual responses). Although the present study is unable to explain this variation, the composition of the muscle groups involved (fibre-type) and the training status of the participants may have been important factors. It is well establish that endurance trained individuals have a higher proportion of slow-twitch fibers compared to untrained individuals, and for a given level of force production fast-twitch fibers produce greater quantity of metabolites than slow-twitch fibres (Saltin & Gollnick, 1983).

Importantly, during isometric plantar flexion exercise when the knee is fully extended to 180° (like in the present study), the mono-articular (ankle joint) soleus muscle (majority of slow muscle fibers) of the triceps surae and the bi-articular (ankle and knee joints) gastrocnemius muscles (~50% slow fibers and ~50% fast fibers) show a gradual EMG activation during augmenting efforts at a parallel time course (Kennedy & Cresswell, 2001, Nardone & Schieppati, 1988). Therefore, the substantial contribution of the "slow" soleus muscle during isometric plantar-flexion exercise together with the presumably higher percentage of slow fibers in the gastrocnemius muscles in the more trained compared to untrained subjects suggest a delayed metabolite accumulation and subsequently muscle fatigue among the "fitter" individuals, primarily at low to moderate intensities (i.e. 30 to 50% F_{max}). This may have resulted in a minimal

differences on the rates of fatigue mainly at these low intensities among the fitter individuals (Appendix 17).

In addition, even if we found an intensity threshold (40% F_{max}) below which the effect of body tilt angle on calf muscle fatigue does not occur; the relationship between the intensity of contraction (%F_{max}) and the absolute change in the rate of fatigue (upright minus supine) showed a positive curvilinear response. Therefore, this may imply that the absolute change in the rate of fatigue across different intensities shows a continuum rather than a threshold (see Figure 4.5).

At this point it was observed that at high intensities (70 % F_{max}) muscle fatigue and endurance were dependent on the intact peripheral circulation, but the blood flow response was unknown. In addition, the causes why a change in posture did not affect fatigue at low intensities (30 % F_{max}) were unknown. To explore whether the postural effect on high intensity exercise performance is mediated by a faster blood flow and to find the causes of the lack of postural effect on fatigue at low intensities (i.e. possibly due to a lack of faster blood flow response or insensitivity to a faster blood flow response), the last experimental study measured the blood flow responses at 30 % and 70 %F_{max} in the supine and incline postures.

6.3 Postural effect on muscle blood flow

Blood flow to the active limb has been reported to be faster during the first minute of submaximal exercise when the exercising limb is placed below compared with above the heart level (Leyk *et al.*, 1994b, MacDonald *et al.*, 1998, Tschakovsky *et al.*, 2004a). Since muscle fatigue is very sensitive to the changes in perfusion pressure (Fitzpatrick *et al.*, 1996, Wright *et al.*, 1999), it has been suggested that the faster rate of increase in blood flow may mediate the postural effect on fatigue and endurance. However, this is hypothetical because muscle endurance and/or fatigue and blood flow in response to a postural change have not been assessed within the same study. Therefore, the present thesis explored for the first time the postural effect on endurance time to

failure, the rate of fatigue and muscle blood flow using the same exercise protocol.

The fourth experimental study showed in agreement with previous studies a higher increase in leg blood flow in the inclined position over the first few minutes of calf exercise, difference that was established after the first contraction. The blood flow response was similar for both 30% and 70% F_{max} intensities. Therefore, it was reasoned that the lack of postural effect observed at 30 $\%F_{max}$ was due to an insensitivity to changes in blood flow and O_2 delivery to low intensities.

Although the present study cannot explain the specific mechanisms that caused the immediate increase in blood flow, the mechanisms that most likely contribute to the immediate exercise hyperaemia are the following:

a) Metabolic vasodilation (in muscle activation)

It has been demonstrated that vasodilation increases during steady state exercise in proportion to the tension developed by a given number of active motor units, and in proportion to the number of active motor units at a given tension (VanTeeffelen & Segal, 2000). This is consistent with the spatial distribution of microvascular units relative to motor unit fibers (Fuglevand & Segal, 1997). Therefore, the rapid vasodilatory mechanism(s) responsible for immediate vasodilation may be related to muscle activation. The two main candidates are potassium (K^+) and acetylcholine.

The present evidence regarding K^+ is mixed. Bunger *et al.*, (1976) observed 20% dilation within 4s (the first measurement taken) after application of K^+ in the coronary vasculature. In contrast, Wunsch *et al.*, (2000) observed a 4-to 6-s delay in the onset of vasodilation after direct application of K^+ to secondary resistance vessels. With regard to acetylcholine, although it has been observed that a motor nerve source of acetylcholine evokes vasodilation in hamster cremaster muscle (Welsh & Segal, 1997), blockage of muscarinic receptors with

atropine does not alter the immediate exercise-induced hyperaemia in exercising humans (Brock *et al.*, 1998, Buckwalter *et al.*, 1998).

b) Mechanical compression/distortion of resistance vessels.

Early speculation about the cause(s) of the rapid dilation following the onset of exercise suggested that the compression/distortion of arterioles during a brief tetanic contraction of skeletal muscle resulted in a myogenic dilation (Mohrman & Sparks, 1974). Subsequent studies in humans suggest that myogenic dilation may not account for such rapid changes in blood flow at the onset of contractions. When the forearm was elevated above the heart in an attempt to empty the veins and a cuff was inflated and then deflated repeatedly over 1 min, there was no hyperaemia (Tschakovsky *et al.*, 1996). However, simple muscle compression may not represent the true mechanical distortion/compression presented during muscle contraction.

Hamann *et al.,* (2004) recently proposed that this mechanical distortion affects the endothelial cells lining the vasculature in a similar manner to pressure and shear stress (Lamontagne *et al.*, 1992). However, Tschakovsky et al (2004) proposed that mechanical distortion my directly effect smooth muscle. Nitric oxide, prostaglandins, and endothelial-derived hyperpolarizing factor are known mediators of endothelial-dependent vasodilation (Bolz *et al.*, 1999, Pohl & Busse, 1990). To date, experiments that employed prostaglandin or nitric oxide blockade in isolation have not affected the change in blood flow in the first 0-5 s of exercise in humans (Brock *et al.*, 1998). Recently, combined nitric oxide and prostaglandin blockade has been demonstrated to substantially reduce steady state blood flow (Boushel, 2003). In contrast, the first investigation of combined nitric oxide and prostaglandin blockade on the rapid vasodilation in an mild to moderate exercise transition found no effect on the immediate percent increase in blood flow with increased contraction intensity (Saunders *et al.*, 2005).

Recent evidence from Hamann et al., (2004) demonstrated that clamping smooth muscle membrane potential virtually eliminates immediate vasodilation. This was evidenced by elimination of a blood flow response to a single 1 s

stimulation of isolated dog muscle under these conditions. These data indicate that, if muscle compression/distortion can rapidly reduce arteriolar tone in isometric contractions, it must do so by evoking changes in smooth muscle membrane potential. It is also possible that shortening/lengthening contractions are required to disrupt latched smooth muscle cross bridges. Clearly, this area warrants further investigation through the application of different in vivo, in situ, and in vitro experimental approaches.

c) Neural mechanisms

Neural regulation has also been speculated to facilitate an early exercise hyperaemia. Human skeletal muscle is under tonic sympathetic tone and removal of this constrictor influence can double flow at rest. Therefore, sympathetic withdrawal at the onset of contractions (Callister *et al.*, 1994) would enhance the flow response. Although brachial artery flow in the inactive arm was not changed during the transition from rest to exercise performed by the contralateral arm (Shoemaker *et al.*, 1997), lower limb vascular conductance increased significantly within 10 s of both voluntary and electrically evoked exercise of the contralateral limb (Fisher & White, 2003). However, it has recently been demonstrated that the rapid increase in vascular conductance of the contra-lateral limb is not mediated by sympathetic withdrawal (Fisher *et al.*, 2005).

It is important to note that venous occlusion plethysmography measures the change in calf volume which accounts for muscle blood flow in addition to other tissues such as skin and bone surrounded by the gauge. For instance, in the case of the forearm, under resting conditions, ~70% of total blood flow is through skeletal muscle, with skin blood flow accounting for most of the remainder (Wilkinson & Webb, 2001). However, during isometric plantar flexion exercise at moderate intensities, a very small changes in skin conductance at sites over the tibia and the gastrocnemius have been reported after 10 seconds of exercise (Fisher & White, 2003). Therefore, the rapid increase in lower limb

conductance observed in the present study is probably mostly due to the increase in muscle blood flow.

In conclusion, these data suggests that tilting the body from a supine to an inclined posture reduces the rate of fatigue and increases the endurance of the plantar flexors, and that this effect is mediated by a greater increase of blood flow that is produced immediately after the first contraction. This mediation, however, is only evident at high intensities (i.e. 70 % F_{max}), because at low intensities (i.e. 30 % F_{max}) a similar postural response in blood flow does not decrease the rate of fatigue.

IX Appendices

Appendix 1: Medical questionnaire

DEPARTMENT OF PHYSIOLOGY, TRINITY COLLEGE, DUBLIN.

Medical Questionnaire

The purpose of this survey is to keep a record of all subject/participant personal, medical and general health details for later comparison and data analysis. It is also essential to ensure any unnecessary risk or injury is avoided to all involved in the experimental series. Please complete all of the personal information at the top of this page and answer all of the questions accurately. All information will be kept as confidential as possible.

Subject Name:_____ Date:_____

Height:_____ Weight:_____

Sex:_____ Age & D.O.B.:_____

Contact Telephone Numbers:_____

Please circle the appropriate answer and provide details in all cases.

1. Are you a smoker? YES NO _____
2. Do you suffer from asthma? YES NO _____
3. Do you drink alcohol? YES NO _____
4. Do you drink tea/coffee? YES NO _____
5. Do you drink Coke/Pepsi etc? YES NO _____
6. Are you a diabetic? YES NO _____
7. Are you lactose intolerant? YES NO _____
8. Have you ever had any soft tissue injuries (ie: broken bones, ligament damage...)? YES NO _____
9. Does your family have a history of stroke and/or heart disease? YES NO____
10. Do you have any allergies? YES NO _____

11. Do you have any other medical/health related complaints that should be made aware to the investigators? YES NO_____

_____

12. Do you perform any regular physical activity? YES NO

If YES, please indicate type, duration and frequency._____

_____

_____

13. Are you currently taking any prescribed medication? YES NO

If YES, please indicate which drugs, and reasons for prescription._____

_____

_____

14. Have you ever knowingly or unknowingly taken any performance enhancing agents (e.g.: anabolics, steroids, β-blockers...)? YES NO

If YES, please indicate which agents, and why._____

_____

_____

15. Are you currently taking any other dietary supplements (e.g.: vitamins, iron, proteins...)? YES NO

If YES, please indicate which supplements, and why._____

_____

_____

Please sign and date this survey below if the answers you have given are, to the best of your knowledge, true and correct. If you are unsure of any questions or have any information you think may be important, but not specifically addressed by these questions, please make it known to the principal investigator of the study.

Signature of Subject:_____Date:_____

Title of Project:_____

Name of Principal Investigator:_____

Appendix 2: Subject information form

Project Title: EFFECT OF POSTURE ON CYCLING PERFORMANCE (I)

Investigator: MIKEL EGAÑA

Supervisor: DR SIMON GREEN

Background

As a subject in this study, you will be required to attend the Physiology department at Trinity College on **two** occasions:

- Session 1: You will have your height and weight recorded; you will be familiarised with the exercise device and then you will try and complete an incremental exercise test to failure either in the supine or upright position. During the exercise test you will be required to increase your level of effort in a stepwise manner until you reach and cannot sustain a maximum effort. Approx. time 45-60 minutes.

- Session 2: You will repeat the same procedures as in session one either in the supine or upright position. Approx. time: 45-60 minutes.

Exercise tests do create muscle pain, breathlessness and occasionally dizziness. If you require medical assistance at any time, the research personnel are trained in first aid and a medical practitioner is on-site to help, if needed.

Project Title: EFFECT OF POSTURE ON CYCLING PERFORMANCE (II)

Investigator: MIKEL EGAÑA

Supervisor: DR SIMON GREEN

Background

As a subject in this study, you will be required to attend the Physiology department at Trinity College on **five** occasions:

- Session 1: You will have your height and weight recorded; you will be familiarised with the exercise device and then you will try and complete an incremental exercise test to failure either in the supine or upright position. During the exercise test you will be required to increase your level of effort in a stepwise manner until you reach and cannot sustain a maximum effort. Approx. time 45-60 minutes.
- Session 2: You will repeat the same procedures as in session one either in the supine or upright position. Approx. time: 45-60 minutes.
- Session 3, 4 and 5: You will try and complete 3 constant load tests, one in the upright and two in the supine postures. During the upright and one of the supine constant load tests you will exercise at a fixed relative intensity equivalent to 80% of the maximal workload achieved during the upright incremental test whereas during the second supine test at 80% of the maximal workload achieved during the supine incremental test. Approx. time for each test: 30-60 minutes.

Exercise tests do create muscle pain, breathlessness and occasionally dizziness. If you require medical assistance at any time, the research personnel are trained in first aid and a medical practitioner is on-site to help, if needed.

Appendix 3: Written consent

DECLARATION

I have read this consent form and the accompanying Subject Information Sheet. I have had the opportunity to ask questions and all my questions have been answered to my satisfaction. I freely and voluntarily agree to be part of this research study, though without prejudice to my legal and ethical rights and recognising that I may leave the study at any time, without question. I have received a copy of this agreement and I understand that, if there is a sponsoring company, a signed copy will be sent to that sponsor.

Name of sponsor:

PARTICIPANT'S NAME:.....

PARTICIPANT'S SIGNATURE:.....

Date:.....

Statement of investigator's responsibility: I have explained the nature, purpose, procedures, benefits, risks of, or alternatives to this research study. I have offered to answer any questions and fully answered such questions. I believe that the participant understands my explanation and has freely given informed consent and I have witnessed the participant's signing of this agreement.

INVESTIGATOR'S NAME:.....

INVESTIGATOR'S SIGNATURE:.....

Date:.....

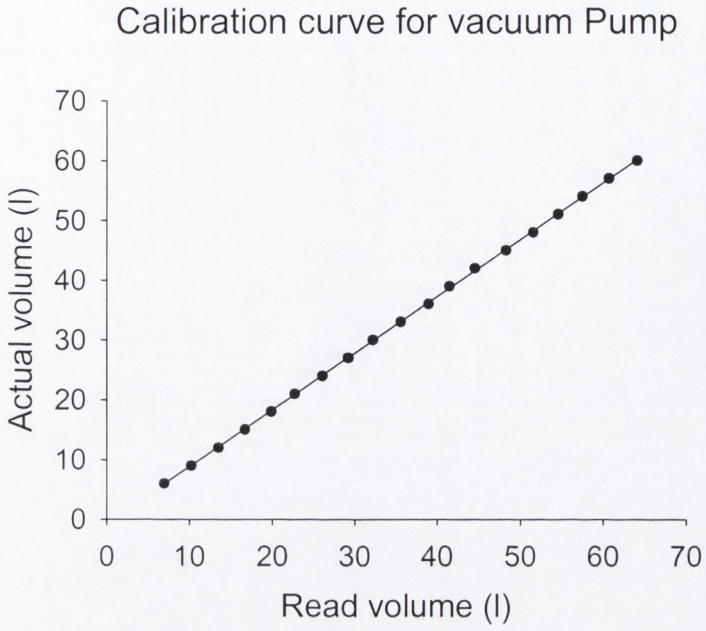
Appendix 4: Vacuum Pump Calibration (l)

Calibration of Vacuum Pump (U.G.I. Meters Ltd, England)

To ensure that vacuum pump was giving accurate readings it was calibrated over a variety of volumes to produce a calibration curve.

Actual Volume(l)	Read volume (l)			Average read volume (l)
	Day 1	Day 2	Day 3	
6	7.2	6.8	7	7
9	10.2	10.7	9.8	10.23
12	13.2	13.4	13.9	13.5
15	16.7	16.6	16.8	16.7
18	19.8	19.8	20.1	19.9
21	22.7	22.9	22.6	22.73
24	26	26	26.4	26.13
27	29.3	29.2	29.2	29.23
30	32.1	31.9	32.6	32.2
33	35.2	35.9	35.6	35.57
36	38.7	38.6	39.6	38.97
39	41.8	41.2	41.4	41.47
42	44.4	44.6	44.6	44.53
45	47.8	48.5	48.6	48.3
48	52	51	51.7	51.57
51	55.1	54.3	54.4	54.6
54	57.5	57.8	57.2	57.5
57	61.1	60.6	60.4	60.7
60	64.4	63.8	64.1	64.1

The equation of the line of best fit **y = 0.9498x – 0.74121** where **y** is the actual volume and **x** is the read volume, was used to translate the raw pump reading into a more accurate indication of ventilation.


Calibration curve for vacuum Pump
Actual volume (l)
Read volume (l)
0
10
20
30
40
50
60
70

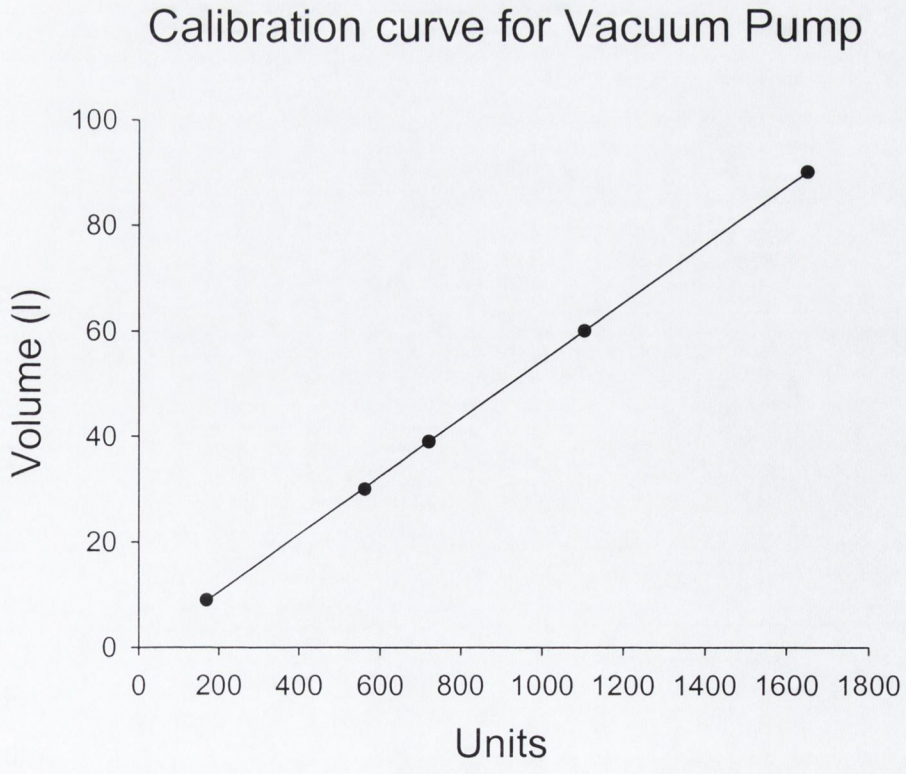
Appendix 5: Vacuum Pump Calibration (II)

Calibration of Vacuum Pump (Cranlea and company, Birmingham, England)

To ensure that vacuum pump was giving accurate readings it was calibrated over a variety of volumes to produce a calibration curve.

Actual Volume (l)	Units 1	Units 2	Units 3	Average units
9	170	167	168	168
30	555	559	569	561
39	715	727	723	722
60	1113	1100	1100	1104
90	1646	1650	1656	1651

The equation of the line of best fit: **y = 0,0547x – 0.4441** where **y** is volume and **x** is unit, was used to translate the raw pump reading into a more accurate indication of ventilation.



Appendix 6: Subject information form

Experiment 1

Project Title: EFFECT OF POSTURE ON CALF MUSCLE ENDURANCE DURING A GRADED EXERCISE

Investigator: MIKEL EGAÑA

Supervisor: DR SIMON GREEN

Background

As a subject in this study, you will be required to attend the Physiology department at Trinity College on **six (or eight)** occasions:

- Session 1 and 2 (familiarisation): You will have your height and weight recorded and you will be familiarised with the exercise device and exercise protocol. Approx. time for each session: 30 min.
- Session 3 (strength test): You will try and complete 3-4 maximum voluntary efforts in each of the following tilt angles: 0°, 47°, and 90°. Approx time: 15 min.
- Session 4, 5 and 6: You will try and complete an incremental exercise test to failure in each tilt angle (0°, 47°, and 90°). During the exercise test you will be required to increase your level of effort in a stepwise manner until you reach and cannot sustain a maximum effort. Approx. time for each session: 45-60 min.
- Session 7 and 8: In addition you might be ask to perform 2 further tests under ischaemic conditions during which you will repeat the same procedures as in sessions 4, 5 and 6 with the difference that the blood flow to your leg will be stopped by inflating a thigh cuff above 240 mmHg. Approx. time for each session: 30 min

Exercise tests do create muscle pain, breathlessness and occasionally dizziness. If you require medical assistance at any time, the research personnel are trained in first aid and a medical practitioner is on-site to help, if needed.

Experiment 2

Project Title: EFFECT OF POSTURE ON CALF MUSCLE FATIGUE AND ENDURANCE DURING A CONSTANT-FORCE EXERCISE

Investigator: MIKEL EGAÑA

Supervisor: DR SIMON GREEN

Background

As a subject in this study, you will be required to attend the Physiology department at Trinity College on **seven (or nine)** occasions:

- Session 1 and 2 (familiarisation): You will have your height and weight recorded and you will be familiarised with the exercise device and exercise protocol. Approx. time for each session: 30 min.
- Session 3 (strength test): You will try and complete 3-4 maximum voluntary efforts in each of the following tilt angles: 0°, 32°, 47°, and 67°. Approx time: 15 min.
- Session 4, 5, 6 and 7: You will try and complete 4 constant load tests to the point of failure in each tilt angle (0°, 32°, 47°, and 67°). You will exercise at a fixed relative intensity equivalent to 70% of the maximal voluntary effort (70 % F_{max}) and you will exercise until you reach and cannot sustain a maximum effort. Approx. time for each session: 45-60 min.
- Sessions 8 and 9: In addition you might be ask to perform 2 further tests under ischaemic conditions during which you will repeat the same procedures as in sessions 4, 5, 6 and 7 with the difference that the blood flow to your leg will be stopped by inflating a thigh cuff above 240 mmHg. Approx. time for each session: 30 min

Exercise tests do create muscle pain, breathlessness and occasionally dizziness. If you require medical assistance at any time, the research personnel are trained in first aid and a medical practitioner is on-site to help, if needed.

Appendix 7: Familiarisation sessions

Familiarisation sessions

In the two experiments, during the first familiarization session, subjects performed 4 maximal voluntary efforts in each tilt angle, each contraction separated by 1 min. Subjects also repeated the maximal voluntary efforts during the second familiarisation session.

In addition, in Experiment 1, during the first familiarization session, subjects performed one-leg isometric plantar flexions intermittently so that the calf was contracted for 3 s and relaxed for 3 s at an intensity equivalent to 40 % of the maximum voluntary effort (F_{max}) at the three tilt angles until subjects felt comfortable in each position. The rhythm of the contractions was helped by a digital metronome. During the second familiarisation session subjects performed the same type of contractions, but the force sustained during each contraction started at a fixed intensity of 100 N and was increased by 100 N every two minutes until subjects reached the maximum fixed intensity of 500N.

In Experiment 2, during the first familiarization session, one leg isometric plantar flexions were performed intermittently at 40 % F_{max} at the four tilt angles so that the calf contracted for 2 s and relaxed for 4 s until subjects felt comfortable in each position. During each fifth contraction (i.e. every 30 s) a maximum effort was sustained for 2 s. The second preliminary session differed from the first only in the intensity of the constant load efforts which were set at 70% F_{max}.

Appendix 8: Relative calf muscle activation during walking

Calf muscle activation as a percentage of the gait cycle

1 Aim: The main aim of the present pilot study was to identify the calf muscle activation as a percentage of the gait cycle during treadmill locomotion. In addition, we investigated the relative EMG activity of the calf muscle at different speeds and terrains.

2 Methods

2.1 Subjects: 5 subjects (4 male and 1 female).

2.2 Experimental design: Fine-wire electromyography (EMG) data of Gastrocnemius lateralis (GL) and Gastrocnemius medialis (GM) were collected from 5 subjects at three walking speeds and two gradients (6 conditions). Before this test, the maximal EMG signal was recorded during a strength test.

2.3 Strength test: In order to measure the maximal EMG of the calf muscles, we simulated a calf-rise exercise harnessing the body with a rock-climbing rope towards the floor creating the maximum resistance without preventing a complete calf rise. Subjects stood on one edge of the treadmill on their right leg toes, and they performed a maximum full calf rise contraction.

2.4 Exercise sessions: The study was conducted in a gait laboratory. Subjects were familiarised to all the speeds and gradients before the test. During the tests, they walked comfortably for at least 1 minute in each condition, and EMG was recorded during the last 15 seconds. In total 10 traces were analysed. The different speed and gradients are shown in Table 1.

Table 1: Speed and grades

Speed ($km.h^{-1}$)	Gradient (degrees)
3	0
6	0
3	7
6	7
3	14
6	14

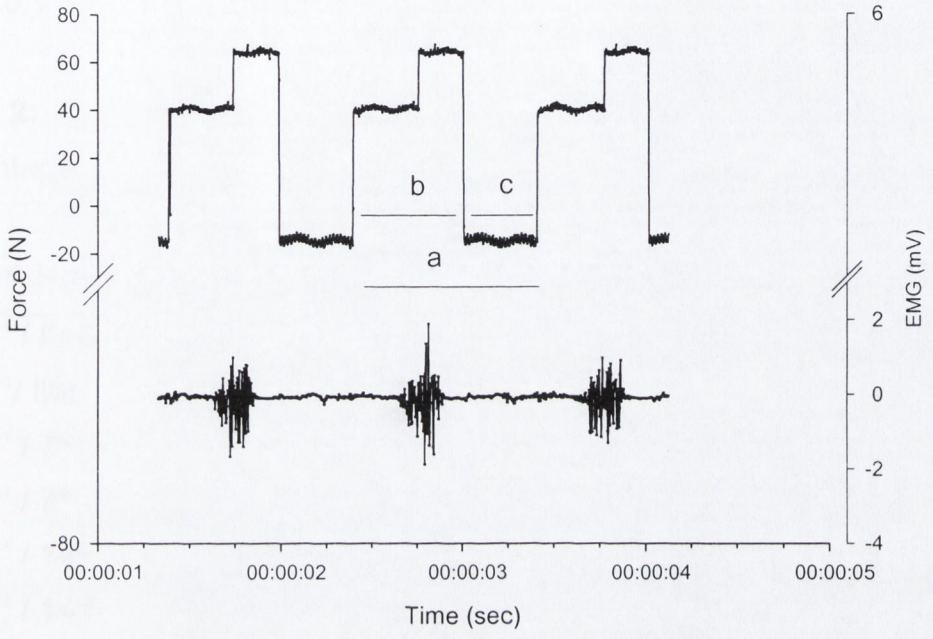
2.6 Gait cycle and EMG recording: Subjects walked barefoot on a motorised treadmill. Pressure sensitive switches were attached by Elastoplast to the sole of the right foot (toe and hell) and were used to record total gait cycles as well as stance and swing phases of the cycle.

Bipolar surface EMG were recorded from the GL and GM muscles of the right leg. The skin was shaved and cleaned with 95% ethanol prior to surface electrode application. Red dot monitoring electrode 3 m with micropore tape and solid gel were placed with an inter-electrode distance of 2.5 cm. Fine wire electrodes were used to record muscle activity.

2.7 Signal processing: Fifteen to twenty traces were recorded at each speed and the best 10 were analysed and averaged. Foot pressure (Force) and EMG data were collected on-line at 400 Hz before being processed by a PowerLab (ML 795, AD Instruments) analog-digital converter and displayed on the screen (Chart v4.12, AD Instruments). Once stored in the hard disc, data was analysed off-line (Figure 1)

Average Root Mean Square (RMS) was calculated for the period of time that each muscle was activated.

Figure 1: EMG signal (voltage) and foot pressure (force) of a subject walking at 6km.h^{-1} and at 7° ; where **a** is the total gait cycle, **b** the stance phase and **c** the swing phase of the gait cycle.



3. Results

3.1 Activation time of the calf muscles as a percentage of the total gait cycle: The average activation time between the gastroc. muscles as a percentage of the total gait cycle was 27.2 % (Table 2). However, it was noted that during recordings at flat and at 7° terrain, it was quite difficult to identify the point at which calf muscle contraction started (i.e. point where the EMG signal increased from baseline) because each contraction was preceded by a small increase in EMG signal which was not clear whether it was produced by an artefact or by the actual calf activation. To solve this problem, we did not consider the small initial EMG signals, but in doing so, we probably underestimated the calf activation time as a percentage of the total gait during flat and 7° terrain (average ~25%) (Table 2). On the other hand, EMG traces at 14° terrain were more clear and obvious to identify the point where the calf muscle was activated. The activation time of the calf as a percentage of the

total gait at the inclination of 14° was higher at both speeds, averaging ~30 % (Table 2)

Therefore, we reasoned that the relative period of activation of the calf muscle during walking on flat and inclined terrain was closer from 33 % or 1:2 ratio than from 25 % or 1:3 ratio.

Table 2: Activation times of GL, GM and the average activation of both as a percentage of the total gait duration.

Speed / degree	GL	GM	Mean	SD
3 km.h^{-1} / flat	27.10	27.72	27.41	0.44
6 km.h^{-1}/ flat	23.52	27.43	25.47	2.77
3 km.h^{-1} / 7°	23.20	24.62	23.91	1.00
6 km.h^{-1} / 7°	23.92	27.12	25.52	2.26
3 km.h^{-1} / 14°	29.19	28.34	28.77	0.60
6 km.h^{-1} / 14°	28.68	35.28	31.98	4.66
Average:			**27.18**	**1.96**

3.2 % EMG max of calf muscles at different walking speed and terrains:

Table 3 shows the relative EMG activity of the calf muscle at different speeds and terrains.

Table 3: Percentage of the maximum EMG (% EMG max) during walking in GL, GM and the average of both.

Speed / degree	GL	GM	Mean	SD
3 km.h^{-1} / flat	31.88	36.98	**34.43**	3.60
6 km.h^{-1} / flat	47.65	49.89	**48.77**	1.58
3 km.h^{-1} / 7°	39.96	49.58	**44.77**	6.80
6 km.h^{-1} / 7°	69.97	64.79	**67.38**	3.66
3 km.h^{-1} / 14°	52.92	56.18	**54.55**	2.31
6 km.h^{-1} / 14°	87.78	79.86	**83.82**	5.60

In a study carried out by Creswell *et al.*, (1995), normalised EMG RMS data and relative Maximum voluntary contraction data (% of MVC) were correlated for the same muscles (lateral and medial gastrocnemius) during isometric plantar flexion exercise of the right calf (Cresswell *et al.*, 1995). In the present study EMG RMS data for the same muscle group was calculated. When applying our findings into the correlation figure performed by Creswell et al., we found the following relationships between treadmill speeds and relative MVC values.

- Walking in a flat terrain at 4.5 $km.h^{-1}$ the relative calf contraction intensity corresponds to 40 % of the maximum voluntary contraction (MVC).
- Walking in a 7° inclined terrain at 4.5 $km.h^{-1}$ the relative calf contraction intensity corresponds to 50 % MVC.
- Walking in a 14° inclined terrain at 4.5 $km.h^{-1}$ the relative calf contraction intensity corresponds to 70 % of MVC.

Appendix 9: Effect of hell lifting on force

Effect of Hell Lifting on Force production

Aim: The aim of this pilot study was to investigate the effect of heel lifting from the leather boot on a maximal voluntary effort (F_{max}).

Subjects: 4 young male subjects.

Exercise type: One-leg (i.e. right leg) isometric plantar flexion exercise.

Protocol: Each subject performed 3 maximal voluntary efforts (F_{max}) with their right foot strapped and tight (test A) and another 3 F_{max} with their right foot strapped but loose (test B) so that their heel was substantially lifted from the perspex footplate. During each maximal effort the force output (N) and the distance between the footplate and the heel (cm, "heel lift") was measured

Results: In test B subjects lifted their heel on average 2.3 cm more than in test A (table 1), but the F_{max} was similar ($p=0.26$; paired t-test) in both tests (see table 2).

Table 1: The distance of the hell from the footplate (hell lift) and maximal force duting test A (right foot tight) and test B (right foot loose).

	Subject 1		Subject 2		Subject 3		Subject 4	
Test	A	B	A	B	A	B	A	B
Heel lift (cm)	0.2	2	2	4.5	1.5	3.5	1	4
F_{max} (N)	788	793	1110	1112	1056	1002	1151	1120

Table 2: Average maximal forces achieved in test A and test B.

	Test A	Test B
Subject 1	788	793
Subject 2	1110	1112
Subject 3	1056	1002
Subject 4	1151	1120
Mean:	1026	1007
SD:	164	152

Appendix 10: Force at different foot positions

Effect of the position of the foot on force production

Background: During constant load plantar flexion preliminary tests, it was noticed that towards the end of the tests some subjects changed the position of their feet from the centre of the footplate to increase their performance time to failure.

Aim: The aim of the present pilot study was to investigate the effect of foot position on the footplate on force production.

Protocol: Several precisely measured weights were placed initially on specific centre-points of the right footplate corresponding to the centre-point of the right strain gauge ("centre" on the table below). Then, the same weights were placed 2 cm to the right, 2 cm to the left, 2 cm back and 2 cm forward from the initial specific center-point.

Statistical analysis: The effects of the position of the weights on the percentage change in force from the center point values were identified using repeated-measures ANOVA and differences were then located using Tukey's HSD test.

Results: Table 1 (below) shows the absolute forces (N) recorded in all the different positions and the percentage changes in force in relation to the initial recordings made at the center-point. When moving the position of the weights 2 cm to the left or right from the center point, the mean force was not different. However, moving the position of the weights 2 cm back or forward from the center point, the mean forces were significantly lower ($p<0.05$) and higher ($p<0.001$) respectively compared to the center-point.

Table 1: Absolute forces and percentage changes in force, in relation to the forces recorded at the center-point when different weights were placed 2 cm left, right, back or forward from the center.

	Right footplate				
	Force (N) at different positions				
Weight (g)	Center	Left	Right	Back	Forward
0	7	7	7	7	7
1013	31	31	32	29	35
3151	83	81	84	74	92
36360	843	845	841	736	961
72551	1537	1547	1605	1479	1899
	Percentage change in force (%) at different positions				
Weight (g)	Center	Left	Right	Back	Forward
0	100.0	100.0	100.0	100.0	100.0
1013	100.0	100.0	103.2	93.5	112.9
3151	100.0	97.6	101.2	89.2	110.8
36360	100.0	100.2	99.8	87.3	114.0
72551	100.0	100.7	104.4	96.2	123.6
Mean:	100.0	99.6	102.2	91.6 *	115.3 *
SD:	0	1.4	2.0	4.0	5.7

* significantly different ($p<0.05$) from center.

Conclusion: This data shows that during plantar flexion exercise subjects must keep the position of their feet constant during the entire exercise session on the place in the footplate.

Appendix 11: Calibration and reliability of the ergometer

Calibration procedure for the calf ergometer

A spreadsheet template was set up in Microsoft Excel for calibration. The ergometer was repositioned vertically to start. A small screwdriver was used to reset the residual voltage reading as close to zero as possible. The baseline voltage was recorded on the excel worksheet. A base for the weights was positioned centrally on the footplate corresponding to the centre-point of the strain gauge. Precisely measured weights ranging from 1 to 11kg were placed onto the base and on top of each other and the voltage readings were recorded (in V) in sequence. When all weights were placed, the total weight on the footplate was 80,034 kg, which when converted to Newtons represented around 70 % of the maximal voluntary force for most of the subjects. Once all weights were on the ergometer, each weight was removed and the corresponding voltage reading was also recorded on the excel worksheet. Therefore, two voltage recordings for each weight were obtained; the first one when the ergometer was loaded and the second when it was unloaded (Figure 1). All measurements were changed from V to mV. Two graphs (one while loading and the other while unloading the erg) showing the linear function ($y : a + bx$) between weight and voltage were produced, where y is voltage, x is weight, parameter *a* represents voltage at weight = 0 and parameter *b* represents the rate of increase in voltage. Using this equation voltage values for two weights, 20 and 80 Kg, were calculated, and averaged. This values were then converted from mV readings to force (N) readings in Chart for Windows.

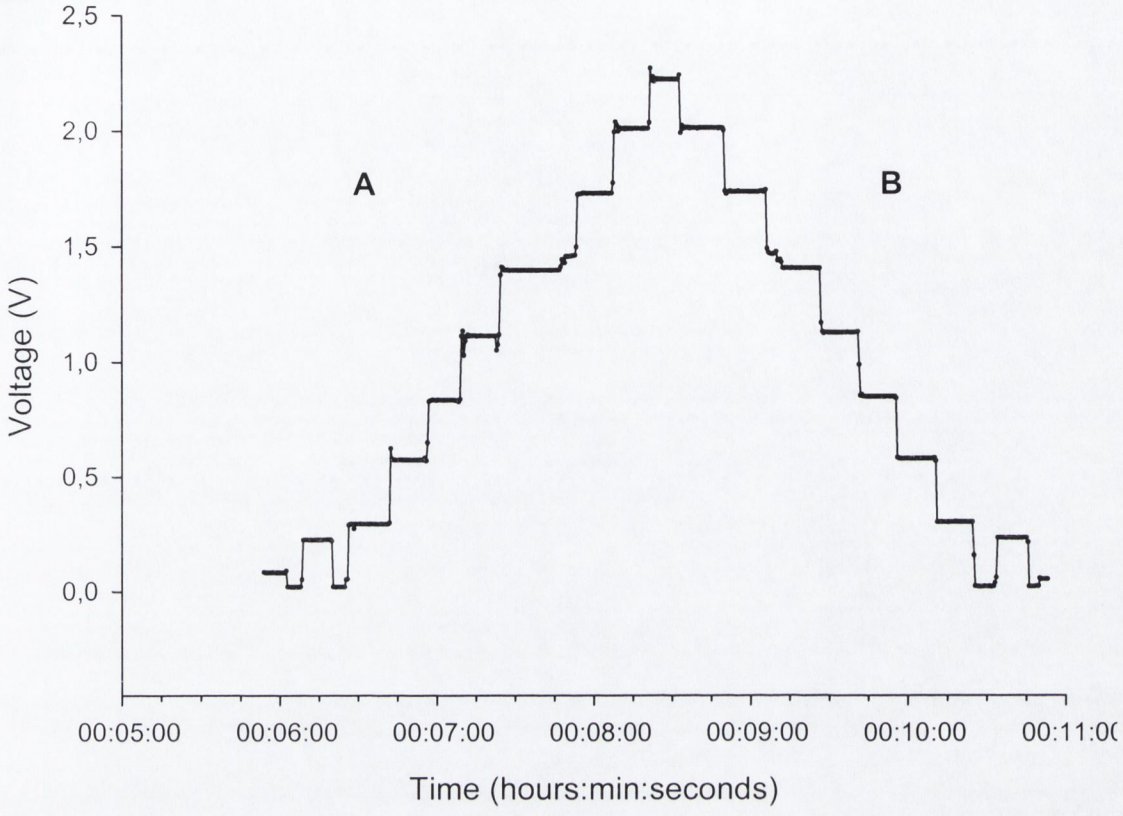


Figure 1: Voltage recording when the Ergometer was loading (A) and unloading (B).

Reliability of the calf ergometer

Table 1 shows the average of the two voltage recordings (one while the erg was loading and the other one while unloading) for 20 and 80 kg during different testing days. The reliability of the ergometer was assessed calculating the percentage coefficient of variation using the following equation:

% CV = (standard deviation/mean) x 100

Percentage CV for 20 kg was 3.22 whereas for 80 kg 3.71.

Table 1: Voltage recordings and percentage coefficient of variation for 20 and 80 kg during 21 testing days.

dates	x=20kg (in mV)	x=80kg (in mV)
22.07.03	742.3	2830.3
24.07.03	735.2	2847.2
25.07.03	772.7	2938.7
28.07.03	730.4	2767.4
31.07.03	743.8	2885.8
01.08.03	731.7	2774.7
06.08.03	719.5	2795.5
07.08.03	736.3	2827.3
08.08.03	766.2	2884.2
11.08.03	720.8	2787.8
12.08.03	745.2	2887.2
13.08.03	726.9	2850.9
15.08.03	739.3	2836.3
18.08.03	742.2	2842.2
20.08.03	705.4	2607.4
22.08.03	753.9	2886.9
25.08.03	742.0	2863.0
26.08.03	779.1	3017.1
27.08.03	686.6	2624.6
29.08.03	722.8	2726.8
01.09.03	687.5	2607.5
MEAN	734.8	2813.8
SD	23.7	104.3
CV (%)	**3.22**	**3.71**

Appendix 12: Effect of the left foot dorsiflexion on force

Effect of dorsiflexion of the non-exercising foot on force production of the exercising foot.

Background: Initially during preliminary tests, subjects performed one-leg (i.e. right leg) plantar flexion exercise with both feet strapped to the footplates of the ergometer. It was observed that some of the subjects used to dorsiflex with the non-exercising left leg mainly in the horizontal position producing a "liver effect" that resulted in higher forces.

Aim: The aim of the present pilot study was to investigate the effect of dorsiflexion of the non-exercising left foot on the force production of the exercising right foot.

Subjects: Two young subjects (1 female and 1 male).

Protocol: Each subject performed 4 sets of 3 maximal voluntary efforts (F_{max}): 2 sets in the horizontal and 2 sets in the vertical posture. In each posture subjects performed a set of 3 F_{max} with both feet strapped and dorsiflexing the non-exercising leg, and another set of 3 F_{max} placing the left foot on a padded platform that lay ~30 cm anterior to the heel of the exercising foot.

Results: The highest F_{max} of each set is shown in table 1. When subjects dorsiflexed their non-exercising left foot the force was ~200 N higher than when the left foot was placed on the platform, both on the horizontal and vertical positions. A repeated-measures ANOVA showed that when the non-exercising foot was dorsiflexed the force achieved was significantly higher than when the non exercising foot was placed on the platform on the horizontal position.

Table 1: Highest F_{max} of each set.

	Both legs strapped (dorsiflex with the left)		Left foot on the platform	
	Horizontal	Vertical	Horizontal	Vertical
Subject 1	817 N	822 N	537 N	625 N
Subject 2	1201 N	1235 N	1057 N	1105 N
Mean	1009 N	1029 N	797 N *	865 N
SD	272 N	292 N	368 N	339 N

* Significantly different ($p<0.05$) from "both legs strapped" condition both in the horizontal and vertical postures.

Appendix 13: Effect of harness tension on force

Effect of the harness and its tension on force production

Background: Initially during preliminary tests, when subjects performed one-leg (i.e. right leg) plantar flexion exercise, it was noted that in the horizontal posture most subjects tended to displace backwards, and we suspected that this was effecting the maximal voluntary efforts (F_{max}) on the horizontal postures, which were lower compared to the other incline positions.

Aim: The aim of the present pilot study was to investigate which was the most adequate harness tension in order to eliminate the differences observed on F_{max} production among different postures.

Protocol: During this pilot study we first investigated the effect of different harness tensions on force production on 2 subjects at 3 tilt angles (0°, 47° and 90°, test A). When the ideal harness tension ("comfortably tight") was identified, we then validated our finding on 5 subjects at 3 tilt angles (0°, 47° and 90°, test B).

Test A: Each of the 2 young subjects (1 male and 1 female) performed 9 sets of 3 F_{max}: 3 sets harnessing the body to the ergometer with a loose tension, 3 sets with tight but comfortable tension and 3 sets with very tight and uncomfortable tension. Under those 3 different tensions, subjects performed 1 set of 3 F_{max} at each tilt angle (i.e. 1 set at 0°, 1 set at 47° and 1 set at 90°).

Within each harness tension, the effects of body tilt angle on F_{max} were identified using repeated-measures ANOVA and differences were then located using Tukey's HSD test. The results showed that wearing the harness at a "tight comfortable" tension eliminated the differences in F_{max} previously observed with loose harness tension between the horizontal and more inclined postures (Table A).

Table A: Highest maximum forces in each tilt angles within each harness tension conditions.

Subjects	"Tight comfortable" tension			"Loose tension"			"Tight uncomfortable" tension		
	0°	47°	90°	0°	47°	90°	0°	47°	90°
Sub. 1	1020 N	1088 N	1004 N	614 N	877 N	781 N	1198 N	1183 N	1049 N
Sub. 2	1065 N	1095 N	1080 N	610 N	814 N	822 N	1088 N	1117 N	1137 N
Mean	1043 N	1092 N	1042 N	612 N	846 N *	802 N	1143 N	1150 N	1093 N
SD	32 N	5 N	54 N	3 N	45 N	29 N	78 N	47 N	62 N

* significantly different from 0° at same harness tension.

Test B: Each of the 5 young subjects (4 male and 1 female) performed 5 sets of 3 F_{max} harnessing the body to the ergometer at a "comfortably tight" tension on the following order: set 1 at 0° , set 2 at 90° , set 3 at 0° , set 4 at 47° and set 5 at 0° . Therefore, out of 5 sets 3 were performed at 0° one at 90° and one at 47°.

A one-way repeated-measures ANOVA showed that tilt angle had no effect on maximal force ($p = 0.365$).

Table B: Highest F_{max} achieved in each of the tilt angles.

Subjects	Tilt angles				
	0° (1)	90°	0° (2)	90°	0° (3)
Subject 1	1052 N	1204 N	1140 N	1220 N	1174 N
Subject 2	1093 N	1118 N	1119 N	1220 N	1082 N
Subject 3	1644 N	1561 N	1684 N	1704 N	1590 N
Subject 4	803 N	810 N	822 N	752 N	782 N
Subject 5	1566 N	1262 N	1617 N	1539 N	1584 N
MEAN:	1232 N	1191 N	1276 N	1287 N	1242 N
SD:	360 N	270 N	365 N	365 N	346 N

Conclusions: These results confirmed that a "comfortably tight" harness tension eliminated the lower F_{max} values observed during horizontal exercise compared to more inclined plantar flexion exercises.

Appendix 14: Effect of time in the upright posture on force.

Effect of time remained in the incline posture on force production

Aim: The aim of the present pilot study was to investigate the effect that had the amount of time remained in the incline posture on maximal force production

Subjects: Two young subjects (1 female and 1 male).

Protocol: Each subject performed 6 maximal voluntary efforts (F_{max}) in the vertical posture. 3 F_{max} were performed without moving the subjects from the vertical position at the following times: the first contraction at min 1, the second one at min 10 and the third one at min 20. Before the production of each of the remaining 3 F_{max} the subject was tilted back to the horizontal posture, and then, he/she performed each maximum effort immediately after tilting the body from the horizontal to the vertical posture.

Results: When subjects performed maximal forces while remaining in the incline posture for 20 min or when the F_{max} were performed immediately after tilting the body to the incline posture, the maximal force was not different among the 3 contractions (Table 1, p=0.60, one-way repeated measures ANOVA)

Table 1:F_{max} data in Newtons.

	Body kept in the incline posture			Body tilted from the horizontal before each F_{max}		
	min 1	min 10	min 20	n.1	n.2	n.3
Subject 1	1072	1062	980	1065	1100	1090
Subject 2	708	695	715	717	698	722
Mean	890	879	848	891	899	906
SD	257	260	187	246	284	260

Conclusion: Keeping the body inclined for several minutes do not decrease the force output.

Appendix 15: Subjects information form

Experiment 1

Project Title: EFFECT OF POSTURE ON CALF MUSCLE FATIGUE DURING A CONSTANT-FORCE EXERCISE AT LOW TO MODERATE INTENSITIES

Investigator: MIKEL EGAÑA

Supervisor: DR SIMON GREEN

Background

As a subject in this study, you will be required to attend the Physiology department at Trinity College on **eleven** occasions:

- Session 1 and 2 (familiarisation): You will have your height and weight recorded and you will be familiarised with the exercise device and exercise protocol. Approx. time for each session: 30 min.
- Session 3 (strength test): You will try and complete 3-4 maximum voluntary efforts in each of the following tilt angles: 0°, and 67°. Approx time: 15 min.
- Sessions 4 to 11: You will try and complete 8 constant load tests in 2 tilt angles (0° and 67°) and at 4 different intensities equivalent to 30, 40, 50 and 60 % of the maximal voluntary effort (F_{max}) and you will exercise for a maximum period of time of 20 min. Approx. time for each session: 30 min.

Exercise tests do create muscle pain, breathlessness and occasionally dizziness. If you require medical assistance at any time, the research personnel are trained in first aid and a medical practitioner is on-site to help, if needed.

Experiment 2

Project Title: EFFECT OF POSTURE ON CALF MUSCLE FATIGUE AND ENDURANCE DURING A CONSTANT-FORCE EXERCISE AT HIGH INTENSITIES

Investigator: MIKEL EGAÑA
Supervisor: DR SIMON GREEN

Background

As a subject in this study, you will be required to attend the Physiology department at Trinity College on **seven** occasions:

- Session 1 and 2 (familiarisation): You will have your height and weight recorded and you will be familiarised with the exercise device and exercise protocol. Approx. time for each session: 30 min.
- Session 3 (strength test): You will try and complete 3-4 maximum voluntary efforts in each of the following tilt angles: 0° and 67°. Approx time: 15 min.
- Sessions 4, 5, 6 and 7: You will try and complete 4 constant load tests to the point of failure in 2 tilt angles (0° and 67°) and at 2 different intensities equivalent to 80 and 90 % of the maximal voluntary effort (F_{max}). Approx. time for each session: 30 min.

Exercise tests do create muscle pain, breathlessness and occasionally dizziness. If you require medical assistance at any time, the research personnel are trained in first aid and a medical practitioner is on-site to help, if needed.

Appendix 16: Familiarisation sessions

Familiarisation sessions

In the two experiments, during the first familiarization session subjects performed 4 maximal voluntary efforts in each tilt angle, each contraction separated by 1 min. Subjects also repeated the maximal voluntary efforts during the second familiarisation session.

In addition, during the first familiarization session, subjects allocated to Experiment 1 performed one-leg isometric plantar flexions intermittently (2 s on, 4 s off) at intensities equivalent to 30 and 40 % of the maximum voluntary effort (F_{max}) at the two tilt angles until they felt comfortable in each position. The rhythm of the contractions was helped by a digital metronome. During each tenth contraction (i.e. every minute) a maximum effort was sustained for 2 s. The second familiarisation session differed from the first only in the intensity of the constant load efforts which were set at 50 and 60 % F_{max}.

During the first familiarization session subjects allocated to Experiment 2 performed one leg isometric plantar flexions intermittently (2 s on , 4 s off) at 60 and 70 % F_{max} at the two tilt angles until subjects felt comfortable in each position. During each fifth contraction (i.e. every 30 s) a maximum effort was sustained for 2 s. The second preliminary session differed from the first only in the intensity of the constant load efforts which were set at 80 and 90 % F_{max}, the intensities to be used in the Experiment 2.

Appendix 17: Individual responses for rate of fatigue ($N.s^{-1}$, Exp 1 and 2)

A) Low to moderate intensities (30 to 60% F_{max}, Experiment 1)

	30% F_{max}		40%F_{max}		50%F_{max}		60%F_{max}	
	0°	67°	0°	67°	0°	67°	0°	67°
Subject 1	-0,001	0,000	-0,132	-0,063	-0,130	-0,059	-0,210	-0,129
Subject 2	-0,110	-0,126	-0,294	-0,144	-0,176	-0,141	-0,942	-0,574
Subject 3	-0,194	-0,183	-0,162	-0,090	-0,343	-0,186	-0,669	-0,415
Subject 4	-0,064	-0,058	-0,242	-0,167	-0,113	-0,115	-0,082	-0,063
Subject 5	-0,010	-0,017	-0,110	-0,006	-0,187	-0,050	-0,130	-0,010
MEAN	-0,076	-0,077	-0,188	-0,094	-0,190	-0,110	-0,406	-0,238
SD	0,079	0,077	0,077	0,064	0,091	0,057	0,379	0,244

B) High intensities (80-90 F_{max}, Experiment 2)

	80% F_{max}		90% F_{max}	
	0°	67°	0°	67°
Subject 1	-0,85	-0,51	-1,61	-0,62
Subject 2	-1,25	-1,19	-1,51	-1,60
Subject 3	-0,79	-0,39	-1,37	-0,36
Subject 4	-2,87	-0,75	-3,33	-1,85
Subject 5	-0,89	-0,86	-1,24	-0,97
Subject 6	-2,62	-1,89	-2,25	-1,47
Subject 7	-0,63	-0,50	-1,56	-1,16
MEAN	-1,41	-0,87	-1,84	-1,15
SD	0,93	0,53	0,73	0,54

Appendix 18: Subjects information form

Project Title EFFECT OF POSTURE ON CALF MUSCLE BLOOD FLOW DURING A CONSTANT-FORCE EXERCISE

Investigator: MIKEL EGAÑA
Supervisor: DR SIMON GREEN

Background

As a subject in this study, you will be required to attend the Physiology department at Trinity College on **seven** occasions:

- Session 1 and 2 (familiarisation): You will have your height and weight recorded and you will be familiarised with the exercise device and exercise protocol. Approx. time for each session: 30 min.

- Session 3 (strength test): You will try and complete 3-4 maximum voluntary efforts in each of the following tilt angles: 0° and 67°. Approx time: 15 min.

- Session 4, 5, 6 and 7: You will try and complete 4 constant load tests in the two tilt angles (0° and 67°) and 2 different intensities (30 and 70% F_{max}). You will try and complete the constant load tests with a thigh cuff inflated at 55 mmHg. During each test, you will perform 3 bouts of a maximum duration of 6 min. Approx. time for each session: 60-90 min.

Exercise tests do create muscle pain, breathlessness and occasionally dizziness. If you require medical assistance at any time, the research personnel are trained in first aid and a medical practitioner is on-site to help, if needed.

Appendix 19: Familiarisation sessions

Familiarisation sessions

During the first familiarization session subjects performed 4 maximal voluntary efforts in each tilt angle, each contraction separated by 1 min. Subjects also repeated the maximal voluntary efforts during the second familiarisation session.

During the first familiarization session, one leg isometric plantar flexions were performed intermittently so that the calf contracted for 2 s and relaxed for 4 s at 30 % F_{max} at the two tilt angles (0° and 67°) until subjects felt comfortable in each position. During the session subjects had a thigh cuff inflated at 55 mmHg. The second preliminary session differed from the first only in the intensity of the constant load efforts which were set at 70% F_{max}.

Appendix 20: Force at which blood starts to flow.

FORCE AT WHICH BLOOD STARTS FLOWING INTO THE LEG

Aim: The aim of this pilot study was to identify the force at which blood starts flowing into the leg.

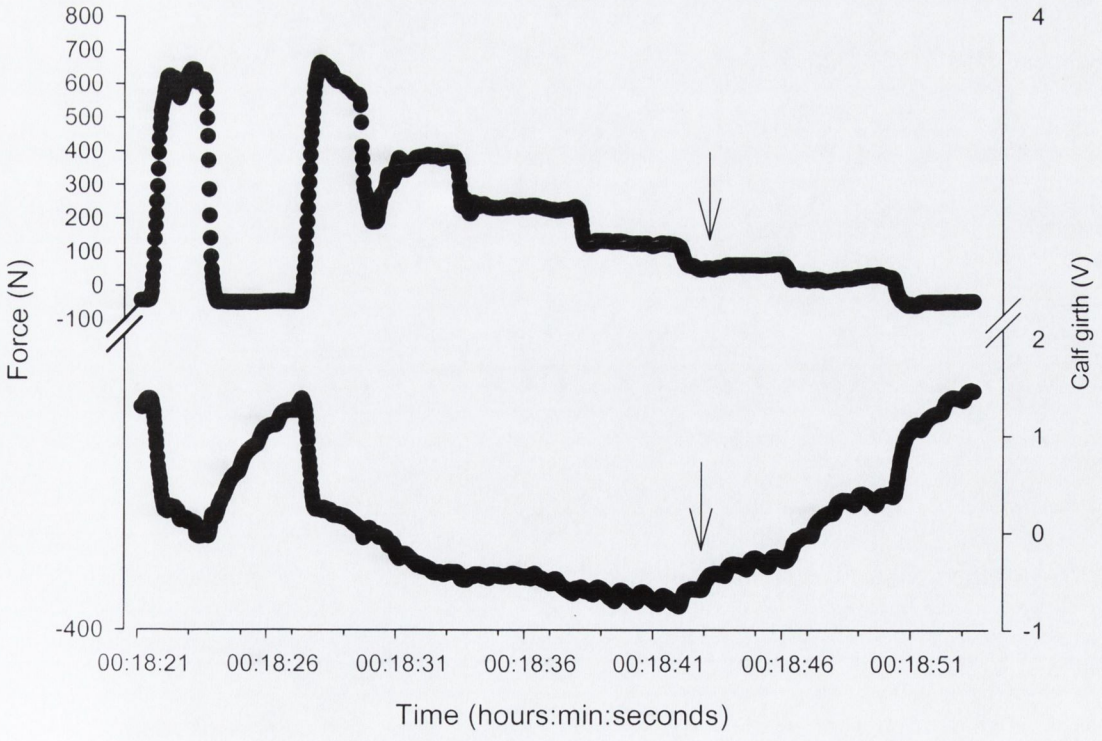
Subjects: 6 young male subjects.

Exercise type: One-leg (i.e. right leg) isometric plantar flexion exercise (2 s on, 4 s off)

Protocol: Each subject performed isometric plantar flexions at 70 % F_{max} for 2 minutes. Immediately after the last contraction the force was stepped down to zero in 50 and 100 N decrements, each of which lasted 5 s.

Results: A pulsatile rise in the calf girth recording indicative of arterial flow into the leg, was only observed when force reached an absolute value of 100-150 N (~ 10 % F_{max}). An example for one subject is shown in Figure 1.

Figure 1: The top arrow indicates the force at which blood starts flowing into the leg (arrow below).



X Publications

X PUBLICATIONS

Journal articles

Egaña, M. and Green, S. (2005) Effect of body tilt on calf muscle performance and blood flow in humans. *Journal of Applied Physiology* 98: 2249-2258

Egaña, M. and Green, S. (in review) Intensity-dependent effect of body tilt angle on calf muscle performance in humans. *Journal of Applied Physiology*

Egaña, M., Green, S., Garrigan, E. and Warmington, S.A. (in review) Effect of posture on high-intensity constant-load cycling performance in men and women. *European Journal of Applied Physiology*

Conference papers

Egaña, M. and Green, S. (2004) Effect of body tilt angle on calf muscle performance in humans. Journal of Physiology, 555P C130.

Egaña, M., Garrigan, E., Warmington, S.A. and Green, S. Effect of posture on exercise performance in men and women. Royal Academy of Medicine in Ireland, Winter Meeting. Dublin, January 2004 (in press)

Egaña, M. and Green S. Effect of body tilt angle on calf muscle performance at different intensities in humans. The Australian Association of Exercise and Sport Science Inaugural Conference. Brisbane, Australia. April 2004

Egaña, M., Warmington, S.A. and Green, S. Effect of posture on constant load cycling performance in men and women. The Australian Association of Exercise and Sport Science Inaugural Conference. Brisbane, Australia, April 2004.

Green, S. and **Egaña, M**. Venous occlusion plethysmography and the assessment of leg blood flow during calf exercise. Freycinet Symposium on "Diabetes and Exercise: Impact of Muscle Blood Flow". Coles Bay, Tasmania, August 2004.

Green, S. and **Egaña, M**. Effect of body tilt angle on leg blood flow during low- and high-intensity calf exercise. The Australian Health and Medical Research Congress. Sydney, Australia, November 2004.

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