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The effect of ageing and gender on calf vascular conductance and fatigue during plantar flexion exercise

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

2012

Heather Reilly

MSc. (Neuropsychopharmacology, Exercise physiology)

I Declaration

I declare that this thesis is entirely my own work with the exception of measurements of leg vascular conductance kinetic responses in 4 men during 60% MVC exercise tests, which were carried out by Ms Louise Lane. 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.

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Heather Reilly, BSc. (Physiology), MSc. (Neuropsychopharmacology), MSc. (Exercise physiology)

II Abstract

Background: Ageing is a complex multifaceted process, which ultimately results in a reduction in neuromuscular function and exercise tolerance. A reduced vasodilatory function and a slower rate of increase in blood flow (blood flow kinetics) to active skeletal muscles has been implicated as a possible underlying cause of the age-associated reduction in exercise tolerance. On the contrary, a vast number of studies indicate an age-associated enhanced fatigue resistance and muscular endurance. However, to date, no studies have looked at blood flow kinetics and fatigue responses simultaneously using the same exercise protocol in aged participants. Furthermore, the majority of previous research examining age-related vasodilatory function and fatigue parameters have involved cycling, knee extension, elbow or leg dorsiflexion exercise. Although all these exercise modalities are functionally relevant, the plantar flexion exercise modality, which is fundamental for walking and for the maintenance of postural stability has not been examined.

Methods: Three studies were performed. Our first study examined peak and exercising haemodynamic (blood flow) and vasodilatory functioning (vascular conductance = blood flow/MAP) using the technique of venous occlusion plethysmography during a graded intermittent (duty cycle; 2 s contraction /4 s relaxation) isometric plantar flexion exercise to maximal exertion using a custom built calf ergometer in 15 young men (YM), 13 older men (OM), 8 young women (YW) and 10 older women (OW). Leg vascular conductance (VC) responses were calculated by simultaneous measurements of leg blood flow (BF) and beat-to-beat mean arterial pressure (MAP). In our second study, VC kinetic responses were determined in 12 YM, 10 OM, 12 YW and 10 OW during constant-force isometric calf plantar flexion exercise performed at relative exercise intensities of 30%, 45%, 60% and 70% maximum voluntary contraction (MVC) forces (three 6 min bouts at each intensity). VC kinetic responses were fitted using a bi-exponential function. The third study examined muscle fatigue responses using the same exercise model (constant-load plantar flexion exercise) in 8 YM, 8 OM, 8 YW, and 10 OW performed again at relative intensities of 30%, 45%, 60% and 70% MVC. During these fatigue tests, an MVC was performed every 30 s (during the 70% MVC test) or every 60 s (during the 30%, 45% and 60% MVC tests). The 70% MVC fatigue test was brought to failure whereas the other three fatigue tests were limited to 20 min. The rate of muscle fatigue was determined by the degree to which maximal force was reduced over time using a linear regression.

Results: In the first study, older and young individuals exhibited similar peak BF and VC responses (blood flow: YM; 34.4±8.8, OM; 34.5±16.7, YW; 32.3±12.1, OW; 35.9±10.1 $ml.min^{-1}.100ml^{-1}$, peak vascular conductance: YM; 2.1±0.5, OM; 1.9±1.0, YW; 2.0±0.7, OW; 2.1±0.6 $ml.min^{-1}.100ml^{-1}.mmHg^{-1}$). Also, exercising BF and VC responses during the sub-maximal stages of the graded test were similar between older and young, despite an age-related decline in peak force (YM; 927±198N, OM; 700±212N, YW; 681±169N, OW; 610±110N, $P>0.05$). Consistent with the observation in the initial study, in our second study, older adults displayed a

preserved vasodilatory capacity at the end of the constant load exercises. However, there was an age-associated slower adjustment in the VC kinetic responses at the onset of exercise for all relative exercise intensities, evidenced by an overall lower mean response time (MRT) (time to reach ~63% of the overall steady state response) due primarily to a greater time constant of phase 2 (τ2) (time to reach ~63% of the steady state response within phase 2) of the bi-exponential response (Table 1).

Table 1. MRT and τ2 values performed at 30%, 45%, 60% and 70% MVC for young and older men and women.

% MVC	Young men		Older men		Young women		Older women	
	MRT (s)	**τ2 (s)**	**MRT (s)**	**τ2 (s)**	**MRT (s)**	**τ2 (s)**	**MRT (s)**	**τ2 (s)**
30%	13.8±5.6	14.1±9.4	33.9±26.9*	40.8±31.6*	18.2±12.9	13.7±7.2	23.3±20.6*	29.6±15.8*
45%	13.1±8.0	13.8±8.8	26.7±15.8*	31.8±17.1*	16.8±13.4	17.0±10.2	30.3±18.1*	39.9±15.7*
60%	16.2±5.9	21.1±10.6	24.5±9.5*	37.4±10.7*	15.8±7.5	20.4±6.6	29.9±22.6*	48.9±20.4*
70%	19.3±7.6	26.6±10.6	28.5±11.2*	48.5±11.6*	14.7±4.1	22.8±3.5	36.1±20.5*	59.2±23.5*

Despite, the age-associated slower VC kinetic responses, in our third study, older adults displayed similar fatigue resistance responses at 30% and 45% MVC, while they showed enhanced fatigue resistance responses at 60% and 70% MVC compared with young adults. In addition, older male adults displayed greater muscular endurance (time to failure) at 70% MVC (OM; 15.5±5.7 min) compared with young individuals (YM; 10.6±2.4, YW; 13.8±4.9 min).

Conclusion: In conclusion, despite deleterious haemodynamic alterations, ageing appears to confer positive benefits during fatiguing exercise, primarily in order to maintain the required level of functional capacity in the face of declining skeletal muscle strength. This may incorporate a greater reliance on oxygen extraction, as well as age-associated morphological alterations to a more fatigue resistant type fibres, a greater a reliance on oxidative metabolism and an overall greater age-related metabolic economy.

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V Abbreviations

A	amplitude
ACh	acetylcholine
AEMG	averaged electromyogram
ANOVA	analysis of variance
AP	action potential
ARH	arm reactive hyperaemia
ATP	adenosine triphosphate
AUC	area under the curve
BF	blood flow
BMI	body mass index
BP	blood pressure
Ca^{2+}	calcium ion
cAMP	cyclic adenosine monophosphate
CAR	central activation ratio method
cm	centimetres
cMAP	compound muscle action potential
CNS	central nervous system
CO_2	carbon dioxide
CO_{max}	maximum cardiac output
CP	creatine phosphate
CSA	cross sectional area
DEXA	dual energy x-ray absorptiometry
DNA	deoxyribonucleic acid
E	exponential
EDD	endothelial dependent dilation
EMG	electromyogram
eNOS	endothelial nitric oxide synthase
FBF	forearm blood flow
FT	fast-twitch

H^+	proton
h	hour
$H_2PO_4^-$	diprotonated inorganic phosphate
Hb	haemoglobin
Hct	haematocrit
HDL	High Density Lipoprotein
HHb	deoxyhaemoglobin
HP	hydrostatic pressure
HR	heart rate
HRT	hormone replacement therapy
Hz	hertz
iEMG	integrated electromyogram
IGF-1	insulin-like growth factor
IL	interleukin
K^+	potassium ion
$[K^+]$	concentration of K^+ ion
kg	kilogram
L	litres
LDL	low density lipoproteins
LED	light emitting diode
L-NMMA	N-methylarginine
LOPAR	low-level physical activity recall
LRH	leg reactive hyperaemia
LVC	leg vascular conductance
MAP	mean arterial pressure
MET	metabolic equivalent
$MET.h^{-1}.wk^{-1}$	metabolic equivalent hours per week
$mg.d^{-1}$	milligram per decilitre
min	minute
mL	millilitres

mmHg	millimetres of mercury
mM	millimoles
mm	millimetres
MRS	magnetic resonance spectroscopy
MRT	mean response time
MSNA	muscle sympathetic nerve activity
MUDR	motor unit discharge rate
MVC	maximum voluntary contraction
N	newton
n	number
NA	nor-adrenaline
NF-κB	nuclear factor kappa-light chain enhancer of activated B cells
NMJ	neuromuscular junction
NO	nitric oxide
O_2	oxygen
P2	phase 2
pCO_2	partial pressure of carbon dioxide
PCr	phospocreatine
PG	prostaglandins
Pi	inorganic phosphate
%	percentage
pO_2	partial pressure of oxygen
r	correlation
RBC	red blood cell
RNA	ribonucleic acid
ROS	reactive oxygen species
RTD	rate of torque development
s	second
SD	standard deviation

SEM	standard error of the mean
SNS	sympathetic nervous system
SR	sarcoplasmic reticulum
ST	slow-twitch
τ	time-constant
TBP	tonometric blood pressure
TD	time delay
TMS	transcranial magnetic stimulation
$t_{1/2}$	half time
V	volt
VC	vascular conductance
$\dot{V}O_2$	rate of pulmonary oxygen uptake
W	watt
WBC	white blood cell
WHO	World Health Organisation
yr	year

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Chapter 1: General Introduction

According to the World Health Organisation, the world's population is growing older, with the number of people over the age of 65 years projected to double in the next 20 years (WHO, 2008). Similarly, Ireland will experience rapid population ageing in the coming decades and it is predicted that the percentage of older (>65 years) males and females will increase by the year 2021 from 9.7% and 12.5% to between 13.9%-14.1% and 15.8%-16.4% respectively. The number of older males over the age of 65 years will rise from 189,555 recorded in 2001 to between 322,651 and 339,505 over the next 10 years. Also, the number of females will rise from 246,846 recorded in 2002 to between 375,835 and 389,101 over the next decade. There will also be a substantial increase in the number of male adults over the age of 75 years rising from 72,146 (recorded in 2002) to between 114,528 and 120,399 in 2021. The number of female adults over the age of 75 years will also rise from 118,252 documented in 2002, to between 158,761 and 164,014 in 2021 (National Council on Ageing and Older People, 2004). The shift in ageing demographics of the world's population presents challenges to society and health care providers in order to meet the needs of ageing individuals. Thus, as our population ages, it is imperative that we develop a greater understanding of the underlying mechanisms involved in the ageing process in order to develop strategies to prevent disability and optimise independence.

Ageing is a multifaceted process associated with a progressive loss in neuromuscular function and performance (Doherty, 2003). This usually results in a gradual decline in functional capacity and an ultimate loss of independence. Sarcopenia, which literally means "poverty of flesh" describes the degenerative loss of skeletal muscle mass and strength with biological ageing (Larsson *et al.*, 1979; Porter *et al.*, 1995; Doherty, 2003). Healthy older adults undergo a number of haemodynamic changes including a decline in resting (basal) blood flow (Leithe *et al.*, 1984) and vascular conductance (blood flow/mean arterial pressure (MAP)) (Dinenno *et al.*, 1999, 2001; Anton *et al.*, 2006). A number of structural changes to the vasculature of ageing individuals are also evident including increased stiffness of the central and peripheral arteries (Najjar

et al., 2005) and an impairment in arterial distensibility (Ferrari *et al.*, 2003). In addition, ageing is associated with endothelial dysfunction (DeSouza *et al.*, 2002) and a marked increase in sympathetic nerve activity (Sundlof & Wallin, 1978a; Ng *et al.*, 1994). Coupled with the haemodynamic alterations, healthy older adults display alterations in both central and peripheral nervous system innervations, changes in hormonal status (Janssen *et al.*, 2000) as well as changes in caloric and protein intake (Rosenberg & Mendez, 1989). Furthermore, age-related intramuscular changes have been implicated in fatigue patterns observed during various exercise protocols (Allman & Rice, 2001; Kent-Braun *et al.*, 2002; Lanza *et al.*, 2007). Thus, multiple inter-related factors contribute to the decline and inevitable loss of functional mobility and independence observed in many older adults, which ultimately has major health implications. This introduction aims to review the physiological adaptations related to ageing, while also encompassing gender specific differences. But first, the general control and regulation of blood flow will be discussed below.

1.1 Control and regulation of blood flow

The flow of blood through any vessel or network of vessels is dependent on the pressure gradient (ΔP) and the resistance (R) of the vessel, therefore flow is $\Delta P/R$. The overall pressure gradient driving flow through the systemic circuit is the mean arterial pressure, while the main influence on vascular resistance is vessel radius. The combined resistance of all blood vessels in the systemic circuit is the total peripheral resistance (TPR). When describing blood flow across the systemic circuit; CO = MAP/TPR.

Thus, two factors influence blood flow to an organ: mean arterial pressure (MAP) and resistance. MAP influences blood flow to all organs in the systemic circuit. Any decline in MAP tends to compromise blood flow to all the systemic organs. Blood flow is regulated both extrinsically and intrinsically and both will be discussed below.

1.1.1 Extrinsic control of blood flow

Control of MAP is achieved by extrinsic regulatory mechanisms; mechanisms involving the control of organs and tissues by the nervous and endocrine systems.

1.1.1.1 Neural control of MAP

Neural control of MAP is co-ordinated primarily by the medulla oblongata. It possesses a diverse set of neural networks encompassing several nuclei, called the cardiovascular control center, that regulate different aspects of cardiovascular function. The cardiovascular control center is able to evaluate various indicators of cardiovascular performance (such as arterial pressure) and decide whether their performance is sufficient to meet the body's current requirements. If it is not the cardiovascular control center instructs the cardiovascular system to make the necessary adjustments by sending output to effectors via autonomic nerves. Information from a variety of sensory receptors projects to the cardiovascular control center. Foremost among these are the arterial baroreceptors.

Arterial baroreceptors, located in the aortic arch and carotid sinus of the carotid arteries, are sensors that monitor MAP. They respond specifically to the stretching that occurs during pressure changes in the arteries. Their sensory endings are embedded within arterial walls. When the arterial pressure rises, the arteries expand, stretching the walls of the arteries and the sensory endings of the baroreceptors within them, and inducing depolarization, triggering action potentials which are then conducted to the central nervous system by the baroreceptors axons.

The cardiovascular control centre integrates information received and determines what adjustments in the cardiovascular system are needed. The centre communicates to the autonomic nervous system, affecting the level of activity in sympathetic and parasympathetic nerves to the heart and blood vessels. Major autonomic innervations of the cardiovascular system include; sympathetic and parasympathetic nerves to the sinoatrial node, which controls heart rate; sympathetic nerves to the ventricular myocardium, which control ventricular contractility; sympathetic nerves to arterioles

and other resistance vessels, which control vascular resistance, and sympathetic nerves to veins, which control vasomotor tone

1.1.1.2 Hormonal control of MAP

Arterial baroreceptors exert control over cardiovascular function not only via the baroreceptor reflex but also by regulating the secretion of hormones epinephrine, vasopressin, and angiotensin II, which work together with the autonomic nervous system to monitor MAP. Epinephrine is released in response to sympathetic nerve activity to the adrenal medulla. Low arterial pressure is a stimulus for epinephrine secretion. Epinephrine affects both cardiac output and total peripheral resistance as follows; At the SA node, epinephrine increases the action potential frequency of the pacemaker cell, which increases heart rate. In the myocardium, epinephrine increases cardiac contractility, which increases stroke volume. In both cases, these effects are brought about due to epinephrine binding to β_1 receptor in cardiac tissue. Under most circumstances, epinephrine causes an increase in total peripheral resistance and increases in blood pressure. Thus, epinephrine tends to increase MAP by increasing heart rate, stroke volume, and total peripheral resistance.

During periods of increased sympathetic nerve activity, norepinephrine binds to α adrenergic receptors on arteriolar smooth muscle and activates the phosphatidylinositol biphosphate second messenger system. The end result is vasoconstriction, which increases TPR and thus increases MAP.

Vasopressin and angiotensin II both cause vasoconstriction, thereby increasing total peripheral resistance and mean arterial pressure. Vasopressin secretion is controlled by factors including the level of activity in arterial baroreceptors. When arterial pressure falls, vasopressin release is enhanced, which promotes an increase in mean arterial pressure. Vasopressin also reduces urine output by the kidneys to maintain plasma volume.

Angiotensin II is produced in response to renin secretion from the kidneys. When arterial pressure falls, the release of renin is stimulated both directly by reduced

arterial pressure and via activity of sympathetic nerves to the kidneys. As a result of the increase in renin secretion, the plasma concentration of angiotensin rises, and this is accompanied by an increase in the concentration of angiotensin II. Angiotensin II increases MAP in several different ways including enhancing vasoconstriction, reducing urine output by the kidneys and stimulating thirst.

1.1.2 Intrinsic/local regulation of blood flow

The varying ways in which tissues and organs within the body are able to intrinsically regulate their own blood supply in order to meet their metabolic and functional needs is known as local regulation of blood flow. Several mechanisms are responsible for local blood flow regulation and include myogenic mechanisms, secretion of chemical messengers, active hyperemia and reactive hyperemia and each will be discussed below.

1.1.2.1 Regulation in response to stretch of arteriolar smooth muscle: myogenic mechanism.

Arteriolar smooth muscle is responsive to stretch, and this occurs when the pressure of the blood within the arterioles increases. When these fibres are stretched, they respond by contracting. Alteration in vascular resistance that occur in response to stretch of blood vessels, and that does not require the action of sympathetic nerves, hormones, or other chemical agents is known as a myogenic response. When perfusion pressure increases in an organ or tissue, blood flow increases and arteriolar pressure rises, which increases the degree of stretch of arteriolar walls because it increases the distending pressure across them. In arterioles containing stretch-sensitive smooth muscle, the muscle fibres then contract, which increases the arteriole's resistance and decreases the flow of blood through it (Figure 1.1). A decrease in perfusion pressure (which causes blood flow to decrease) brings about the opposite response; vasodilation and an increase in blood flow.

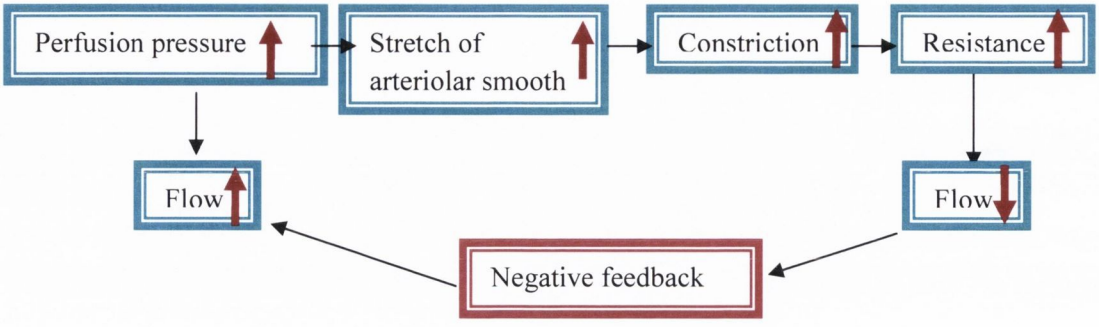


Figure 1.1 The myogenic response to changes in perfusion pressure

1.1.2.2 Regulation of locally secreted chemical messengers

Contractile activity of vascular smooth muscle is also influenced by a variety of chemical substances, the majority of which are secreted by blood vessel endothelial cells or by cells surrounding the tissues. One such substance is nitric oxide, which is released by endothelial cells in arterioles and acts on smooth muscle to enhance vasodilation. The role that nitric oxide plays in blood flow regulation will be discussed in a later section. Other chemical substances and their actions on vascular smooth muscle can be seen in Table 1.1.

Table 1.1 Local vasoactive substances and their actions on vascular smooth muscle.

Substance	Source	Effect on vascular smooth muscle
Oxygen	Delivered by blood	Vasoconstriction
Carbon dioxide	Generated by aerobic metabolism	Vasodilation
Potassium ions	Released from cells (muscle cells particularly)	Vasodilation
Acids (hydrogen ions)	Generated during anaerobic metabolism (lactic acid)	Vasodilation
Adenosine	Released by cells in certain tissues in response to hypoxia	Vasodilation
Nitric oxide	Released by endothelial cells in response to chemical signals	Vasodilation
Bradykinin	Generated from kininogen by action of kallikrein secreted by cells in certain tissue in response to various chemical signals	Vasodilation
Endothelin-1	Released by endothelial cells in response to various chemical signals and mechanical stimuli	Vasoconstriction
Prostacyclin	Released by endothelial cells in response to various chemical signals and mechanical stimuli	Vasodilation

1.1.2.3 Regulation in response to changes in metabolic activity: active hyperemia

Vascular smooth muscle cells in arterioles respond to alterations in the concentration of a wide variety of chemical substances including oxygen, carbon dioxide, potassium ions and hydrogen ions. The alterations in concentration occur as a result of metabolic activity. Arteriolar smooth muscle either relaxes or contracts depending on whether the concentrations of particular substances rise or fall due to changes in metabolism. For example if changes in oxygen and carbon dioxide concentration occur, due to increased metabolic rate then oxygen consumption and carbon dioxide production increase. This causes a decrease in tissue oxygen (hypoxia) and an increase in tissue carbon dioxide. Initially blood flow is inadequate to maintain the metabolic needs. The decrease in oxygen and the increase in carbon dioxide both act on the arteriolar smooth muscle, causing it to relax. When the muscle relaxes,

vascular resistance in the tissue drops, and blood flow in that region increases. The increase in blood flow following an increase in metabolic activity is known as active hyperemia. As a consequence of this increased blood flow, the oxygen delivery to the tissue and the carbon dioxide removal from the tissue increases and eventually a new steady-state is reached.

1.1.2.4 Regulation in response to changes in blood flow: reactive hyperemia

If blood flow is obstructed/reduced below sufficient levels, the oxygen concentration falls and the carbon dioxide level rises because rates of oxygen consumption and carbon dioxide production exceeds rates of delivery and removal. Both of these alterations induce vasodilation and a reduction in vascular resistance, which tend to increase blood flow. Once the obstruction is removed, the rate of flow will be higher than normal and remain elevated until excess metabolites are removed and tissue oxygen concentration is restored to normal.

1.2 Age-associated haemodynamic alterations

1.2.1 Basal (resting) blood flow and vascular conductance

Original investigations using venous occlusion plethysmography reported a reduction (Leithe *et al.*, 1984), no difference (Allwood, 1958) or an increase (Hellon & Clarke, 1959) in resting forearm or calf blood flow responses in older compared with young adults. These discrepancies, however, may have been due to poor matching of subject groups in terms of their activity levels, with some studies including subjects with cardiovascular disease.

More recent data using the technique of Doppler ultrasound indicates that resting blood flow responses are significantly reduced in older compared with young adults and that these reductions in blood flow are accompanied by reductions in vascular conductance (Dinenno *et al.*, 1999; Miyachi *et al.*, 2005; Anton *et al.*, 2006; Donato *et al.*, 2006; Parker *et al.*, 2008).

Dinenno *et al.*, (1999) examined the effects of age on absolute resting limb blood flow using duplex ultrasound in healthy humans and showed that older men (n=15, 63 ± 1 years) displayed a 26% decline ($P<0.005$) in basal femoral arterial blood flow compared with young men (n=16, 28 ± 1 years). The lower basal femoral artery blood flow in the older men was related to a 32% reduction in vascular conductance ($P<0.001$) and a 45% higher vascular resistance ($P<0.005$).

In order to better establish the relationship between ageing, blood flow and vascular conductance, Dinenno *et al.*, (2001) studied a larger group of 142 men (18-79 years) and concluded that basal femoral arterial blood flow and vascular conductance declined progressively with increasing age in healthy men and this related to a concomitant decline in both limb fat free mass (r=-0.48) and estimated limb oxygen demand (r=-0.49) (both $P<0.001$).

Furthermore, they also investigated whether age-associated reductions in basal whole leg blood flow and vascular conductance were modulated by habitual physical activity. The researchers studied a population (n=89) of older (55-75 years) and young men (20-35 years), who were either sedentary (n=26, did not perform regular exercise), physically active (n=31, performed light to moderate physical activity three or more times/week) or endurance trained (n=32, performed vigorous aerobic exercise 5 or more times/week and active in local road running races). They observed that basal femoral artery blood flow was reduced by 18-22% ($P<0.05$) in the older men compared with the young men irrespective of fitness levels. Femoral vascular conductance was reduced by 20-30% ($P<0.05$) and vascular resistance was 25-38% higher in the older compared with the young group of men ($P<0.05$). In addition, basal femoral artery blood flow (r=-0.4), vascular conductance (r=-0.51) and vascular resistance (r=0.47) were significantly correlated with age.

Most of the studies, which examine the age-related declines in basal limb blood flow, have included only male adults (Proctor *et al.*, 1998; Dinenno *et al.*, 2001). However, Moreau *et al.*, (2003) revealed that similar to men, post-menopausal healthy women show a 23% decline ($P<0.001$) in basal limb blood flow compared with young

females (Moreau *et al.*, 2003). Further, this study showed that the age-related decline in leg blood flow in women was associated with a 33% decline in leg vascular conductance and a 42% increase in vascular resistance (Moreau *et al.*, 2003).

1.2.2 Associated between basal blood flow, limb skeletal muscle mass and oxygen demand

A decline in skeletal muscle mass and limb oxygen demand may be significant physiological determinants in the decline in absolute levels of basal limb blood flow observed with ageing (Dinenno *et al.*, 1999; Donato *et al.*, 2006). Whole limb blood flow takes into account blood flow to the skin, skeletal muscle, subcutaneous tissue and bone. Since skeletal muscle accounts for the largest mass of total tissue in the human leg, it has the greatest requirement for oxygen delivery and blood flow (Dinenno *et al.*, 1999). Indeed, Dinenno *et al.*, (1999) estimated that leg oxygen consumption was 15% lower in older (n=15) men compared with young (n=15) men (254 ± 8 versus 217 ± 9 $ml.min^{-1}$, $P<0.01$). This was accompanied by a 26% reduction in basal femoral blood flow (determined using Doppler ultrasound), which was significantly correlated with estimated leg oxygen consumption ($r=0.78$, $P<0.001$). Since oxygen consumption is directly associated with tissue mass, Dinneno *et al.*, (1999) initially assumed that a reduction in limb blood flow and oxygen consumption in older adults may have occurred as a result of a smaller limb tissue mass. However, the authors did not find any age-associated differences in tissue mass between older and young individuals, which indicated that the decline in resting limb blood flow was due to a reduced oxygen demand independent of tissue mass. This also suggested that with increasing age, limb perfusion at rest is reduced per unit tissue mass. The low subject numbers in the study may have been the reason for the authors failing to find a relationship between blood flow and muscle mass.

Hence, using a larger sample size (n=142), Dinenno *et al.*, (2001) further investigated the relationship between leg blood flow and leg muscle mass in aged versus young men. Basal femoral blood flow was lower in the older men and this corresponded to an 18-22% significant reduction in leg fat-free mass and estimated leg oxygen

consumption ($P<0.001$), irrespective of each individual's physical activity level. Basal femoral blood flow per unit leg fat free mass was 12% lower in the older group compared with the young group of men (28.1 ± 0.7 vs. 32 ± 1.1 ml $kg^{-1}min^{-1}$, $P<0.01$). In addition, the age-associated differences in estimated leg oxygen consumption were reduced by 54% after taking leg fat free mass differences into account, although a significant difference in blood flow between both groups remained.

On the other hand, Donato *et al.*, (2006) found that seated resting femoral blood flow was approximately 26% lower in older subjects compared with young subjects but this difference was abolished when expressed as blood flow per kilogram of quadriceps muscle mass (young, 115 ± 28; older: 114 ± 39; $ml.kg^{-1}.min^{-1}$). The contrasting findings between the study of Dinenno *et al.*, (2001) and Donato *et al.*, (2006) suggests that posture might affect resting haemodynamic differences between young and older people, due to changes in the perfusion pressure acting across the skeletal muscle (Egana & Green, 2005).

Among women, the age-related reduction in muscle mass doesn't seem to be the key determinant in the decline in basal blood flow. This was highlighted in a study of 103 healthy females, 41 of whom were post-menopausal taking hormone replacement therapy (HRT) (61 ± 2 yr), 32 were post menopausal not taking HRT (63 ± 2 yr) and 30 were pre-menopausal (29 ± 1 yr). Compared with pre-menopausal women, absolute femoral blood flow (duplex ultrasound) was 23% lower ($P<0.001$) in the post-menopausal group not taking HRT, but only 13% lower ($P<0.01$) in the post-menopausal HRT group. The lower absolute femoral artery blood flow in the post-menopausal group not taking HRT compared with the pre-menopausal women was associated with a 33% lower femoral artery vascular conductance and a 42% higher vascular resistance. Within the post-menopausal women, the greater absolute femoral blood flow in the HRT group was related to a 25% greater femoral vascular conductance and a 25% lower vascular resistance. Femoral blood flow normalised to leg fat free mass was 17% lower ($P<0.05$) in post-menopausal women not taking HRT compared with pre-menopausal women but was similar between the post-

menopausal taking HRT and pre-menopausal groups. Normalizing femoral blood flow to leg fat free mass reduced the age-related difference (from 23% to 17%) between post-menopausal not taking HRT and post-menopausal taking HRT, but did not eliminate it ($P<0.05$). Thus, leg blood flow per kg of leg fat free mass, assumed to be skeletal muscle mass, is lower in oestrogen deficient post-menopausal women compared with young females. The greater absolute femoral blood flow in the post-menopausal HRT group compared with the post-menopausal non-HRT group may be due to the independent vasodilatory effects of circulating oestrogen, which influence locally produced vasoactive factors such as nitric oxide, reactive oxygen species and/or endothelin-1 (Moreau *et al.*, 2003). Indeed, tonic declines in nitric oxide and elevations in endothelin-1 production strongly correlated with oestrogen deficiency in ageing individuals (Amrani *et al.*, 1996; Taddei *et al.*, 2001).

Overall, these results support the notion that an age-related reduction in limb fat-free mass may have a significant role in the initial decline in blood flow at least in part by lowering limb oxygen demand. However, other physiological factors that are altered with ageing may also contribute to the decline in blood flow and will be discussed in later sections.

1.2.3 Structural vasculature alterations

Data from cross-sectional and post mortem studies demonstrate that elastic arteries, such as the central aorta dilate with age, resulting in an increase in lumen size. The thickness of the arterial wall, as determined by the intimal and medial layer, increases progressively by almost 3 fold between the ages of 20 and 90 years, even in the absence of atherosclerosis. This is coupled with an increase in central arterial stiffness and is associated with repeated cycles of distensions and elastic recoils, which may accelerate the breakdown and loss of elastin as well as increasing deposits of collagen. This augmented central arterial stiffness results in increased central pressure. Thus, not only does peripheral blood pressure and pulse pressure increase with age, central blood pressure is also significantly higher (Najjar *et al.*, 2005).

Structural adaptations in the vascular system are thought to contribute to the age-associated reduction in muscle blood flow and muscle performance and these will be discussed in the remainder of the discussion (Proctor *et al.*, 2004).

1.3 Human ageing and the sympathetic nervous system.

1.3.1 Association between basal blood flow and muscle sympathetic nerve activity (MSNA)

The sympathetic nervous system (SNS) plays a vital role in the maintenance of arterial blood pressure under both resting and exercising conditions.

Under habitual resting conditions, sympathetic outflow to skeletal muscle vascular beds, or MSNA is under tonic control by the arterial and cardiopulmonary baroreflexes. Upon discharge or "firing" of sympathetic nerves, norepinephrine is released from sympathetic nerve varcosities. Once released, norepinephrine diffuses and binds to postjunctional α_1 and α_2-adrenergic receptors, evoking vascular smooth muscle contraction and thus, vasoconstriction (Seals and Murray, 2000). During exercise, although sympathetic vasoconstriction persists in the active skeletal muscle, the vasoconstriction responses to sympathetic stimulation are blunted in the vascular beds of contracting muscles, a phenomenon known as functional sympatholysis, thus allowing for major increases in blood flow via enhanced vasodilation. However, with ageing there is evidence for reduced functional sympatholysis. This may have a role to play in the reduction in blood flow in older adults at rest and during some exercise protocols compared with young adults and this will be discussed below (Dinneno *et al.*, 2005).

The increases in sympathetic nervous system (SNS) activity have been investigated using direct (intra-neural) recordings of post-ganglionic sympathetic nerve activity to skeletal muscle using the technique of microneurography. Indeed a number of studies have indicated a significant increase in muscle sympathetic vasoconstrictor nerve activity with advancing age in both sedentary and physically active men (Sundlof & Wallin, 1978b; Ng *et al.*, 1994; Davy *et al.*, 1998). Age-associated reductions in basal

whole leg blood flow and vascular conductance in men are mediated, at least in part, by tonically augmented sympathetic α-adrenergic vasoconstriction as muscle sympathetic nerve activity (MSNA) has been shown to be inversely proportional to basal whole limb vascular conductance, (Dinenno *et al.*, 1999), the pathway which can be seen in Figure 1.2.

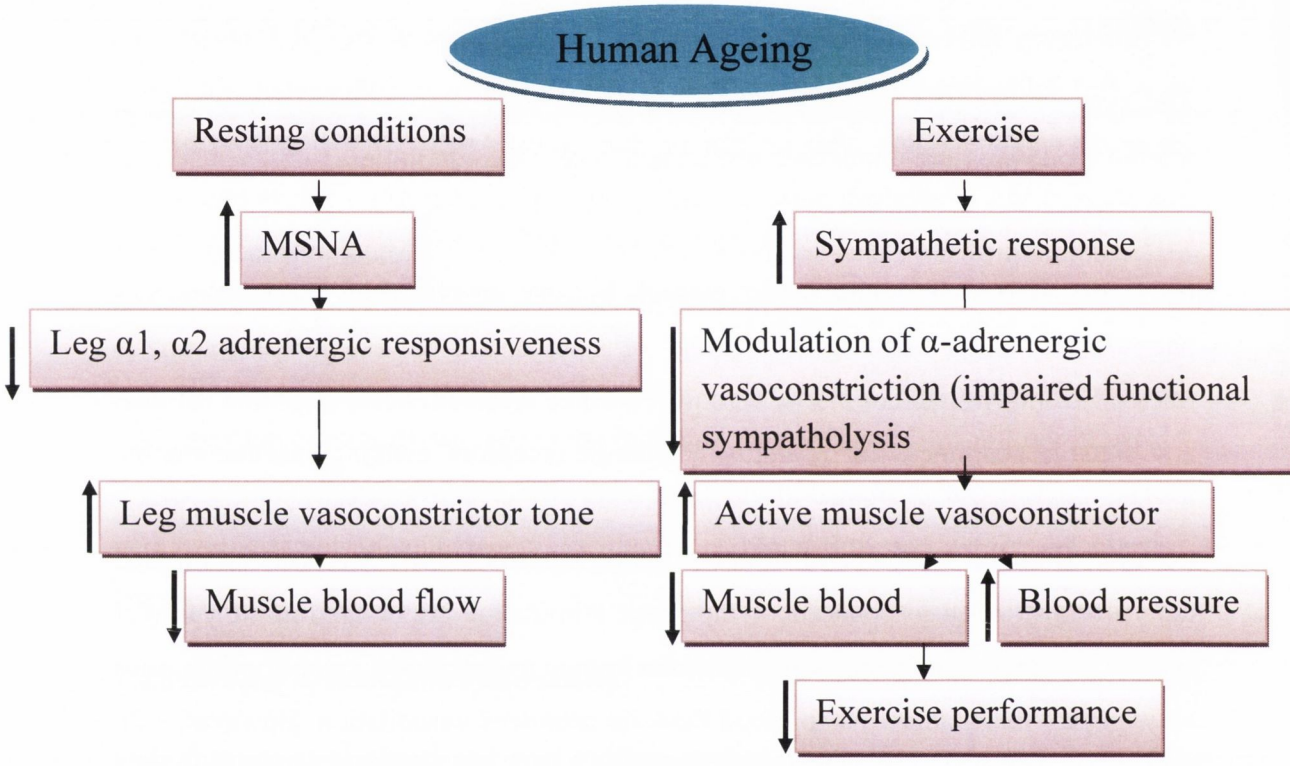


Figure 1.2 Alterations in sympathetic α-adrenergic control of skeletal muscle circulation at rest and during exercise in ageing humans. MSNA is muscle sympathetic nerve activity.

Older men (n=15; age 63 ± 1 year) displayed a 32% decline in femoral artery vascular conductance, a 45% increase in vascular resistance and an accompanying 75% higher leg MSNA compared to young men (n=15; age 28 ± 1 year) (MSNA older: 38 ± 1.4 vs. young: 22.1 ± 1.8 bursts/min, $P<0.001$). The augmented MSNA determined by peroneal neurography, correlated strongly with femoral blood flow (r=-0.55, $P<0.005$), vascular conductance (r=-0.65, $P<0.001$) and vascular resistance

(r=0.61, $P<0.001$). When corrected for MSNA, age group differences in haemodynamics were abolished. Furthermore, similar to the reduction in vascular conductance with ageing, MSNA activity was found to increase linearly with advancing age (Sundlof & Wallin, 1978b; Iwase *et al.*, 1991). Thus, these data suggest that heightened MSNA may have a role to play in age-associated haemodynamic alterations at rest in men.

In contrast to men, differences in leg MSNA do not explain the differences in basal leg blood flow and vascular conductance in older compared with young women. Despite, leg MSNA during supine rest being 95-100% higher in post-menopausal compared with pre-menopausal women, basal MSNA was not related to either leg blood flow (absolute or normalised for leg fat free mass), vascular conductance or vascular resistance (Moreau *et al.*, 2003). The absence of a relationship between leg vascular conductance and MSNA may be due to the independent vasodilatory effects of circulating oestrogen, which influence locally produced vasoactive factors such as nitric oxide, reactive oxygen species and/or endothelin-1 (Moreau *et al.*, 2003). Indeed, tonic declines in nitric oxide and elevations in endothelin-1 production strongly correlated with oestrogen deficiency in ageing individuals (Amrani *et al.*, 1996; Taddei *et al.*, 2001). Therefore, in contrast to men, the reduction in basal limb blood flow is independent of MSNA and instead, may be influenced by a number of different factors including reductions in fat free mass, a decline in vascular conductance and oestrogen deficiency, which is known to be a potent acute vasodilator (see section 1.6.2) (Gilligan *et al.*, 1994a;1994c).

1.4 Blood flow, ageing and exercise

The age-related decline in leg blood flow to active skeletal muscle has been implicated as one of the underlying factors contributing to a decline in physical activity and exercise performance evident with ageing process. The relationship between blood flow, ageing and exercise performance has been investigated using a variety of exercise models (Martin *et al.*, 1991a; Proctor *et al.*, 1998; Proctor *et al.*, 2003a; Proctor *et al.*, 2004; Donato *et al.*, 2006; Parker *et al.*, 2008) but there is still

conflicting views regarding age and gender related differences in blood flow responses during exercise (Table 1.1).

1.4.1 Exercising blood flow responses in older men

In a recent study by Parker *et al.*, (2008), healthy normally active (neither extremely trained or extremely sedentary, aerobic exercise <3 days.wk^{-1}, resistance training < 2 days.wk^{-1}) older (n=13, 60-79 years) and young (n=15, 20-30 years) men performed a graded (8 W increases every 3 mins) single knee extension exercise to maximal exertion where blood flow was determined using the technique of Doppler ultrasound. The exercise was performed in an inclined position in order to minimise the influence of age-associated limitations imposed by cardiorespiratory baroreceptor mediated decreases in MSNA. Although older men displayed a reduction in resting leg blood flow, they exhibited a preserved exercising peak leg blood flow (young: 1886 ± 63 ml.min^{-1} vs. older: 2032 ± 152 ml.min^{-1}, P=0.30) and peak vascular conductance (young: 17.9 ± 0.7 ml.min^{-1}mmHg^{-1} vs. older: 19.0 ± 2.1 ml.min^{-1}mmHg^{-1}, P=0.55) compared with young male subjects (Figure 1.3) (Parker *et al.*, 2008). Furthermore, when comparing the slopes for the hyperaemic response from 0 to 24 W, older men demonstrated a significantly greater hyperaemic (slope of femoral blood flow vs. absolute work rate in young: 35 ± 2 ml.min^{-1}W^{-1} vs. older: 49 ± 3 ml.min^{-1}.W^{-1}; P<0.01) and vasodilatory response (slope of femoral vascular conductance vs. absolute work rate in young: 0.30 ± 0.03 ml.min^{-1}.mmHg^{-1}.W^{-1} vs. older: 0.44 ± 0.04 ml.min^{-1}.mmHg^{-1}.W^{-1}; P<0.01) compared with young men. Of interest was that at rest and during the initial stages of the exercise protocol (3 minutes of passive movement, followed by 3 minutes of active unloaded kicking), older men displayed lower vascular conductance compared with the young group, which may have been due to heightened baseline leg vasoconstriction evident in this group. This may also be explained by slower vasodilator kinetics in the microcirculation of the leg, which has been recently observed in aged male rat (Bearden *et al.*, 2007).

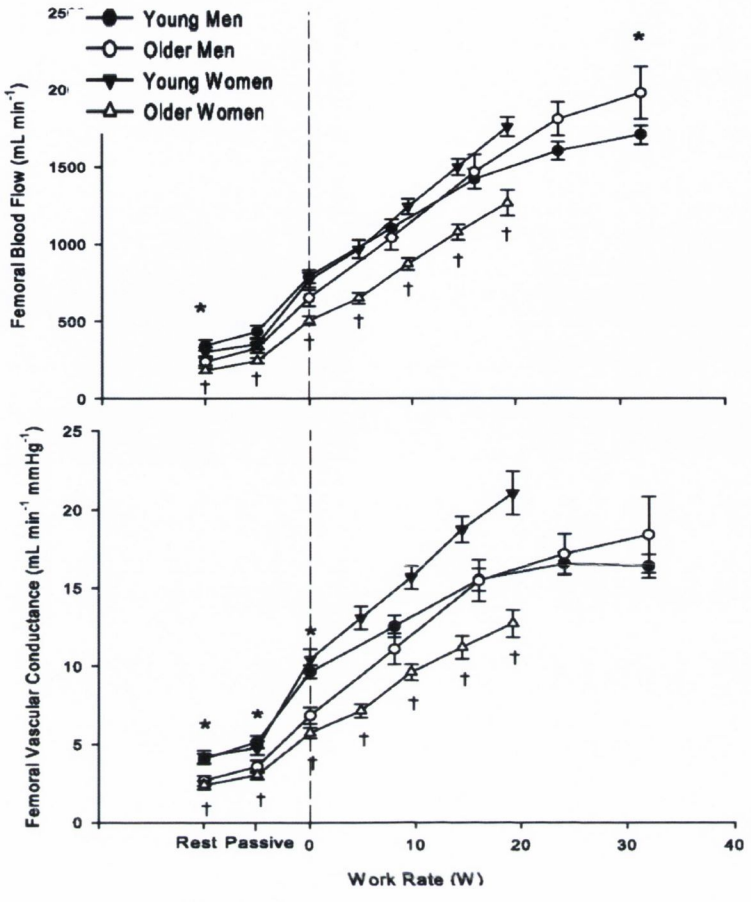


Figure 1.3 Femoral blood flow and femoral vascular conductance expressed as group means ± SE at absolute work rates in young and older men and women.

The age-associated preservation of leg blood flow and vascular conductance responses are supported by previous research involving older and young males during both sustained submaximal (7-80% workload max for 8 min) and graded (workload increments of 0.3 $J.min^{-1}$) dynamic handgrip exercise performed to exhaustion (Jasperse *et al.*, 1994). Submaximal and peak forearm blood flow were similar between young and older men (young: 53 ± 3 ml $(100\ ml)^{-1}min^{-1}$, older: 59 ± 4 ml $(100\ ml)^{-1}min^{-1}$), however the older group displayed a significantly greater peak forearm vascular conductance relative to pre-exercise resting control levels compared with the young group (young: 955 ± 144, older: 1495 ± 165 % of control, $P<0.05$).

Similarly, eight moderately active (aerobic exercise < 3 days.wk^{-1}, resistance exercise <1 day.wk^{-1}) older men (62-73 years) displayed a preservation of leg blood flow (assessed using the technique of femoral thermodilution) and vascular conductance (VC) during both graded and constant-load bouts of leg cycling at the same sub maximal absolute (20-100 W) and relative to percentage of peak oxygen consumption ($\dot{V}O_{2\,peak}$) exercise intensities compared with 11 young men (20-25 years) (Proctor *et al.*, 2003b). Older adults displayed a higher (8-12 mmHg) perfusion pressure compared with the young group and this was thought to be a factor in the maintenance of blood flow in older men. Exercise induced increase in leg arterio-venous oxygen difference [(a-v) O2] was well preserved with age (young: 13.8 ± 0.5, older: 13.8 ± 0.6 ml.dl^{-1}), in spite of reductions in arterial O_2 content. The fractional extraction of O_2 by the legs was higher in the older men for a given submaximal workload and this indicated a greater maintenance of muscle O_2 diffusion capacity relative to that of peak leg blood flow in healthy ageing men. Also, although absolute levels of cardiac output were reduced in older men, a significant higher percent distribution of cardiac output to the legs was apparent in the older group of adults. Due to the strong correlation between limb oxygen demand and limb blood flow (young: r^2=0.92, older: r^2=0.85), it is thought that the former has a major role to play in preservation of leg blood flow with ageing (Proctor *et al.*, 2003b).

However, in contrast, 8 normally active older men (71 ± 5 years) displayed an attenuation in leg blood flow (measured using Doppler ultrasound) when performing dynamic single knee extensor exercise (at 0, 3, 6 and 9 W and 20, 40, 60% of their maximum work rate) compared with 8 young men (22 ± 6 years) (Donato *et al.*, 2006). Both blood flow and vascular conductance were attenuated in the older group whether expressed in absolute terms for a given absolute workload or whether normalised per unit muscle mass at a given relative exercise intensity (young: 1532 ± 329; older: 1340 ± 157 ml.kg^{-1}.min^{-1} at 40% maximum workrate) (Figure 1.4). Although the mean quadriceps mass of the older adults (1.6 ± 0.1 kg) was significantly smaller compared with the young adults (2.1 ± 0.2 kg), this did not account for the attenuation in leg blood flow. These findings are similar to results

from previous studies showing an attenuated absolute blood flow response in older compared with young adults during both cycling (Wahren *et al.*, 1974; Beere *et al.*, 1999; Poole *et al.*, 2003; Proctor *et al.*, 2003a) and during isolated knee extensor exercise (Magnusson *et al.*, 1994).

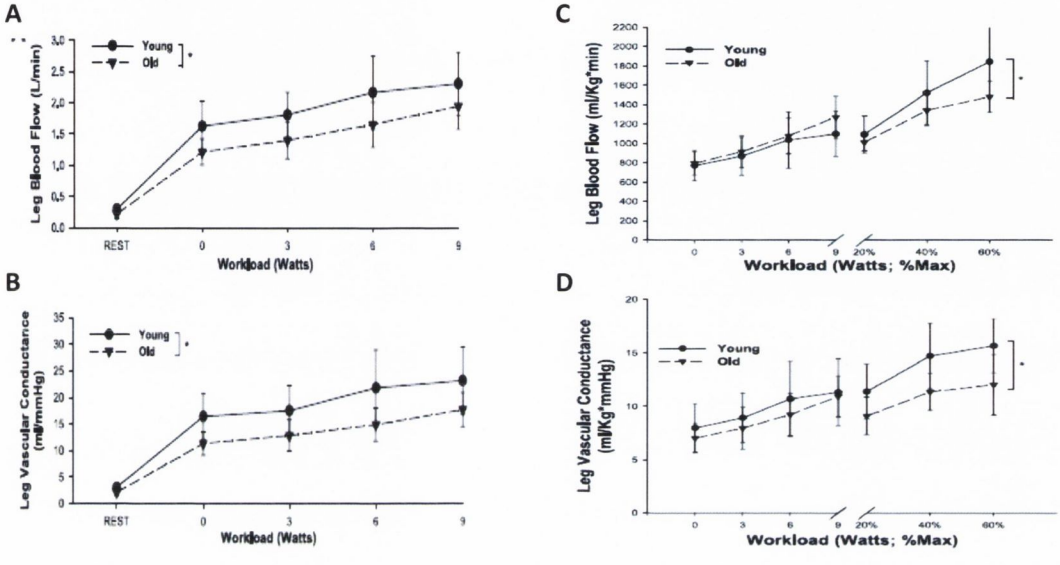


Figure 1.4. Absolute blood flow (A) and vascular conductance (B) and blood flow (C) and vascular conductance (D) per kilogram of quadriceps muscle mass during leg exercise across a series of submaximal absolute and relative workloads in young and older subjects.* significant difference ($P<0.05$).

During one such study, Proctor *et al.*, (1998) measured femoral blood flow using thermodilution technique, in a group of 6 older (55-68 years) and 6 young (22-30 years) chronically endurance trained men during submaximal two-leg cycling exercise at power outputs of 70, 140 and 210 W. For each of the given power outputs, absolute femoral blood flow (20-30%) and vascular conductance (26-30%) were significantly reduced in the older group of men compared with young men. Furthermore, the lower absolute levels of leg blood flow were not due to an age-related decline in total limb muscle mass or cardiac output. However, leg (a-v) O_2 differences averaged 2 $ml.dL^{-1}$ higher in the older group compared with the young group. The augmented O_2 extraction may be indicative of the extensive capillary supply and/or greater oxidative enzyme activation of their trained leg muscles. In

addition, measurements of SNS activity revealed an increase in nor-epinephrine spillover in the older subjects at 210 W. Thus, it is possible that sympathetic limitations to active muscle blood flow contributed to the reduced vasodilation of the older subjects at higher workloads. Also, other factors including a blunted skin blood flow response to exercise, reduced shunting of blood from visceral organs and/or increased blood flow to respiratory muscles may have been involved (Proctor *et al.*, 1998).

The discrepancies within the literature may be due to differences in fitness or activity levels of the subjects or perhaps the exercise modalities employed in studies (Table 1.2). Parker *et al.*, (2008) pointed out that older men who had the greatest fitness levels (assessed using incremental exercise test to maximal exertion) displayed the least reduction in vascular conductance compared with young individuals. Furthermore, Miyachi *et al.*, (2005) revealed that age-related reductions in basal whole leg blood flow and vascular conductance were absent in middle-aged men who habitually performed resistance training. This was in part due to larger muscle mass as a result of resistance training. In addition, an increase in skeletal muscle turnover and basal metabolic demand in older resistance trained subjects may also contribute to the preservation of leg blood flow. Therefore, it seems the greater fitness level of the individual, the smaller the reduction in vasodilation with age. Further studies are required in order to elucidate the relationship between fitness levels and leg haemodynamics during exercise in older men.

1.4.2 Exercising blood flow responses in older women

Blood flow responses in older women during exercise are more consistent, with studies finding an attenuation in leg blood flow and vascular conductance (Proctor *et al.*, 2003a; Proctor *et al.*, 2004; Parker *et al.*, 2008). Compared with young women, older women demonstrated an attenuated peak hyperaemic (young: 1913 ± 72 $ml.min^{-1}$ vs. older: 1349 ± 92 $ml.min^{-1}$, $P<0.01$) and peak vascular conductance response (young: 22.6 ± 1.4 $ml.min^{-1}$ vs. older: 13.6 ± 1.0 $ml.min^{-1}$, $P<0.01$) following a single leg graded knee extension exercise and this was still evident when

both leg blood flow and vascular conductance were normalised to quadriceps muscle mass (Figure 1.3) (Parker *et al.*, 2008). Furthermore, when comparing the slopes of the hyperaemic response from 0-19.2 W, older women demonstrated a significantly blunted blood flow (young: 52 ± 3 $ml.min^{-1}W^{-1}$ vs. older: 40 ± 4 $ml.min^{-1}.W^{-1}$, $P<0.01$) and vasodilatory (young: 0.56 ± 0.06 $ml.min^{-1}.mmHg^{-1}.W^{-1}$ vs. older: 0.37 ± 0.04 $ml.min^{-1}.mmHg^{-1}.W^{-1}$, $P<0.01$) response compared with young women. Age-related alterations in vasoregulatory mechanisms, structural changes and a loss of oestrogen were cited as possible reasons for this reduction. Also, older women were capable of exercising at the same absolute workrates for the same amount of time as the young women, with no difference in peak workrate achieved, but with reduced blood flow. Thus, since the leg oxygen uptake to workrate relationship is maintained with age, then the older women may compensate for the reduced leg blood flow with augmented leg oxygen extraction. Moreover, age-associated changes in muscle metabolism and fibre type size distribution may also contribute to the attenuated hyperaemic and vasodilatory responses among aged women.

In a different study Proctor *et al.*, (2003a) examined blood flow responses in groups of young (n=13, age: 20-27 yr) and older post-menopausal (n=13, age: 61-71 yr) non endurance trained females during both graded and constant-load dynamic leg cycling exercises performed at the same absolute exercise intensities. Limb blood flow in older subjects was significantly lower compared with young subjects at moderate workloads (50-60 W), but leg blood flow was similar between the groups at light workloads (20-40 W) (Proctor *et al.*, 2003a). Furthermore, the age-related attenuation in leg blood flow during exercise was only partly attributed to differences in total leg muscle mass observed between the groups. Thus, in response to dynamic exercise performed at a moderate intensity, older women are limited in their ability to augment leg blood flow and vascular conductance. Reduced release of endothelial derived vasodilator substances including nitric oxide and prostacyclin coupled with an increase in the release of vasoconstrictor substances such as endothelin-1 or thromboxane A_2 may be involved.

In a similar study conducted by the same group, older post-menopausal oestrogen deficient females (n=12, 60-71 yr) demonstrated a 29% lower ($P<0.05$) peak leg blood flow (young: 89 ± 6, older: 71 ± 3 mL.min-1.kg-1 leg muscle) during cycle ergometry compared with young female (n=12, age: 20-27 yr) subjects (Proctor et al., 2004). This was accompanied by a 32% decline ($P<0.001$) in $\dot{V}O2$ and a 38% decline ($P<0.001$) in vascular conductance (young: 86 ± 7, older: 54 ± 2 mL.mmHg.min-1). The age group difference in estimated leg vascular conductance denotes a markedly impaired leg vasodilator response in the older women. When the leg blood flow response of each subject was normalised to estimated leg muscle mass, the mean age group difference in peak flow was reduced by 9% but the difference remained significant ($P<0.01$). Thus, leg muscle mass accounted for only a small percentage of the variance in leg O2 demand observed in these subjects. Therefore, it appears that blood flow at rest and during exercise is coupled with oxygen demand, but it is not clear if it related to muscle mass. Again structural limitations as well as reduced CO and higher perfusion pressure were cited as other possible mechanisms (Proctor et al., 2004).

There are a number of factors that may be responsible for differences in exercise blood flow responses between genders. The dramatic loss of leg vasodilatory functioning in older women may involve the loss of oestrogen due to the menopause. In men, it could involve the influence of chronic fitness levels such that reduced leg blood flow is observed in sedentary men, while trained older men have similar responses compared with young subjects. However, further studies are required to elucidate the exact mechanisms involved.

Table 1.2: A summary of studies in which blood flow and/or vascular conductance responses were measured during exercise in both older and younger men and/or women. Y=young, O=older, m=men, w=women, n=number taking part in study.

Investigating Team	Participant number	Age (yr)	Gender	Activity/fitness Status	Type of exercise	Exercise Modality	Blow flow measurement technique	Outcome
Parker *et al.*, (2008)	n=31 (Y) n=31 (O)	20-30 60-79	M /W	Normally active	Incremental single knee extensor kicking	Knee extensor ergometer	Doppler ultrasound	Preservation
Jasperse *et al.*, (1994)	n=11 (Y) n=11 (O)	19-29 60-74	M	Chronic physically active	Incremental to failure and submaximal forearm dynamic handgrip exercise	Hand dynamometer	Venous occlusion plethysmography	Preservation
Lawrenson *et al.*, (2003)	n=6 (Y) n=6 (O)	18-27 61-77	M	Sedentary	Incremental single knee extension exercise	Knee extensor ergometer	Thermodilution	Preservation
Magnusson *et al.*, (1994)	n=8 (Y, O)	44-69	M	Regularly physical activity	Dynamic knee extension (1 - 2 legged) exercise to failure	Knee extensor ergometer	Doppler ultrasound	Preservation
Donato *et al.*, (2006)	n=8 (Y) n=6 (O)	20-29 65-80	M	Normally active	Submaximal dynamic knee extensor exercise Submaximal handgrip exercise	Knee extensor ergometer Hand dynamometer	Doppler ultrasound	Attenuation Preservation
Beere *et al.*, (1999)	n=13 (Y) n=10 (O)	20-40 60-80	M	Healthy (activity status undefined)	Upright 2 legged cycling	Cycle Ergometer	Thermodilution	Attenuation
Lawrenson *et al.*, (2004)	n=6 (Y) n=6 (O)	18-27 61-77	M	Sedentary	Incremental knee extension exercise	Knee extensor ergometer	Thermodilution	Attenuation
Poole *et al.*, (2003)	n=9 (Y) n=9 (O)	19-21 68-72	M	Sedentary	Incremental 2 legged cycling exercise	Cycle ergometer	Thermodilution	Attenuation
Proctor *et al.*, (2003a)	n=6 (Y) n=6 (O)	22-30 55-68	M	Chronically endurance trained	Submaximal 2 legged cycling	Cycle ergometer	Thermodilution	Attenuation
Proctor *et al.*, (2003b)	n=11(Y) n=8 (O)	20-25 62-73	M	Normally active	Submaximal constant load and incremental 2 legged cycling	Cycle ergometer	Thermodilution	Preservation
Proctor *et al* (2003c)	n=13(Y) n=13(O)	20-27 61-71	W	Non endurance Normally active	Incremental and constant load 2 legged cycling	Cycle ergometer	Thermodilution	Attenuation
Proctor *et al* (2004)	n=13 (Y) n=13 (O)	20-27 60-71	W	Normally active	Incremental and constant load 2 legged cycling	Cycle ergometer	Thermodilution	Attenuation

1.5 Ageing and the rate of increase in blood flow (blood flow kinetics)

It remains vital for physical performance that any increase in muscle work is rapidly matched by adequate increases in the rate of muscle blood flow and thus muscle oxygen requirements.

The rate of increase of blood flow (i.e. blood flow kinetics) has normally been characterised by a biphasic response (Figure 1.5), although at very high intensities, the response has shown to be triphasic (Saunders *et al.*, 2005).

Phase 1 is characterised by an immediate and rapid increase in blood flow, which plateaus between 5 and 7 s (Radegran & Saltin, 1998; Shoemaker *et al.*, 1998). Phase 2 occurs approximately 20 s after exercise onset and is characterised by a further increase in flow that slowly reaches a steady state plateau. The third phase is sometimes apparent at very high intensity efforts (> 80% MVC), and occurs approximately ~ 90 s after the onset of exercise, reaching a plateau at a much slower rate. Each of these phases are represented by a time delay (TD), time constant (τ) and an amplitude (A). The time delay for each phase represents the time taken for the phase to commence. Each time constant represents the rate at which blood flow increases, while each amplitude represents the size of the blood flow response. The mechanisms responsible for phase 3 have not been well established and given that the blood flow responses analysed in the present thesis are biphasic, only those factors contributing to this biphasic response will be discussed in the following section.

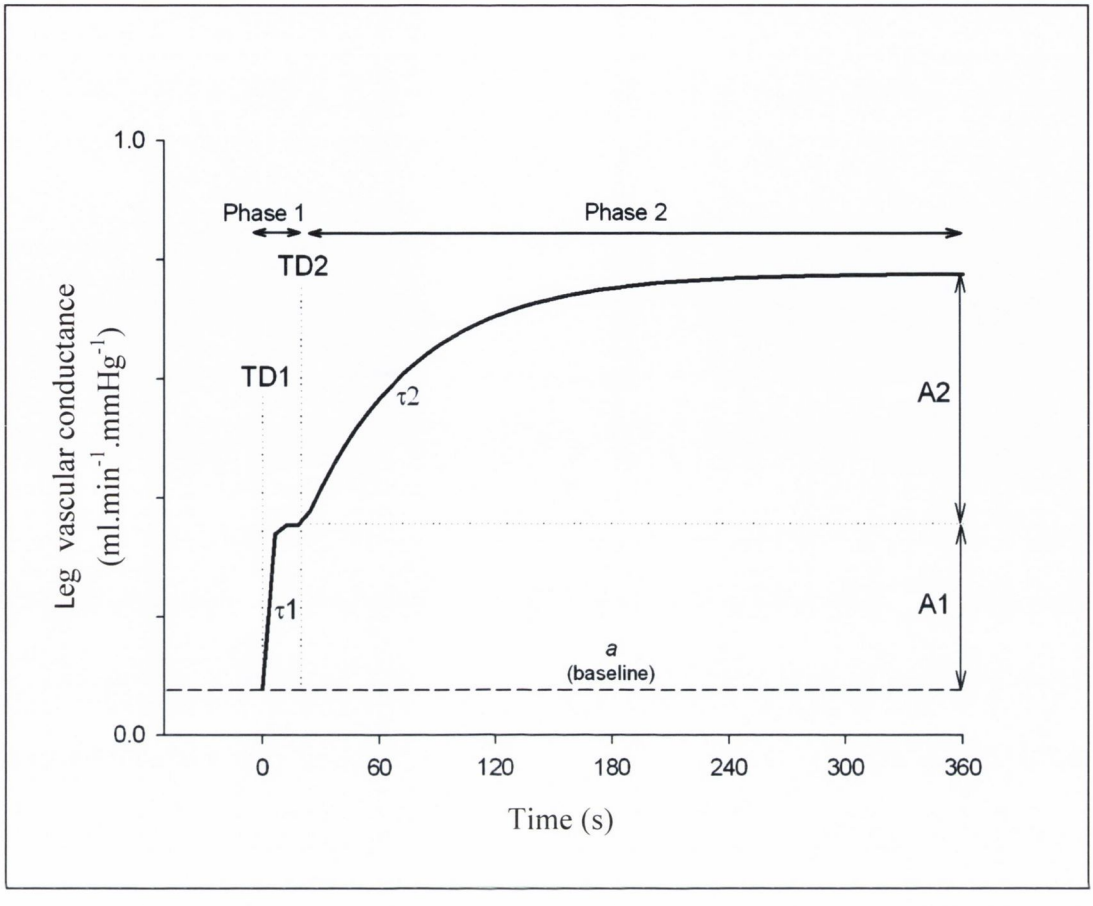


Figure 1.5: Representative diagram of leg vascular conductance (LVC) responses during dynamic plantar flexion exercise showing Phase 1 with time delay (TD_1), time constant (τ_1), amplitude (A_1) and Phase 2 with time delay (TD_2), time constant (τ_2) amplitude (A_2) (MacAnaney *et al.*, 2010).

1.5.1 Mechanisms responsible for Phase 1

The initial increase in blood flow is thought to involve the "muscle pump" action and/or a rapid vasodilatory response (Saltin *et al.*, 1998; Tschakovsky & Sheriff, 2004). A number of studies have indicated that the instantaneous rise in blood flow following the initial muscle contraction may be attributed to muscle mechanical factors (Laughlin, 1987; Sheriff *et al.*, 1993; Tschakovsky *et al.*, 1996). Mechanical interactions between the contracting and relaxing muscles and the blood vessels create a "muscle pump" mechanism, that can increase blood flow independently of vasodilation, by augmenting perfusion pressure in the exercising muscles (Sheriff *et*

al., 1993). The initial muscle contraction results in a block of inflow of blood and a rise in intramuscular pressure. Following the release of the contraction and simultaneous decline in intramuscular pressure and thus, mechanical hindrance to blood flow, there is an immediate pronounced rise in blood velocity, which reaches a peak just prior to the next contraction. In addition, mechanical interactions may also result in vasodilation via myogenic mechanisms (Joyner & Proctor, 1999).

The contribution of vasodilator substances to the instantaneous rise in blood flow has also been investigated (Welsh & Segal, 1997; Brock *et al.*, 1998; Dyke *et al.*, 1998). After 5-10 s, substances released by the active muscles cause vasodilation, which may contribute to the continued increase in flow until a steady state is reached (Sheriff *et al.*, 1993; Tschakovsky *et al.*, 1996). A number of vasodilator substances have been implicated including ions, metabolites, gases (CO_2, O_2) and nitric oxide. When nitric oxide binds to haemoglobin and when Hb is in the oxygenated state, there is substantial "offloading" of NO from Hb when oxygen is extracted by active muscles from the arterial blood, amplifying the total vasodilatory response (Saltin *et al.*, 1998).

In addition, ACh spillover from motor nerves may cause vasodilation via muscarinic receptor-linked release of NO from the vascular endothelium. ACh released by activation of the motor nerve, spills over into muscarinic receptors in the skeletal muscle vasculature and this may diffuse to nearby arterioles initiating a rapid vasodilatory signal (Welsh & Segal, 1997; Brock *et al.*, 1998; Dyke *et al.*, 1998).

Furthermore, one of the many vasoactive agents that may evoke a response following a single contraction is the cation potassium. It is well established that potassium ions cause vasodilation. Following a brief muscle contraction, there is an increase in K^+ in the venous blood from the active muscle, which would implicate it in the initiation of vasodilation at the onset of exercise (Joyner & Proctor, 1999). However, micro-electrode measurements of peri-arteriolar concentrations of potassium following twitch and tetanic stimulation demonstrated that very small alterations in interstitial levels of potassium are apparent during the initial 10-20 s of exercise, despite

substantial hyperaemia (Hnik *et al.*, 1976). Thus, there is evidence for and against the contribution of potassium in the initial hyperaemic response.

1.5.2 Mechanisms responsible for Phase 2

The steady-state phase occurring approximately 20-30 s following the initial phase is thought to be under feedback regulation (Shoemaker & Hughson, 1999). Metabolic factors may play a role in the regulation of flow at this point. Also, the increase in the blood velocity during the initial few seconds of exercise may initiate the production and release of endothelial derived dilating factors that would contribute to the blood flow adaptation between rest and steady state exercise. These include nitric oxide (Rubanyi *et al.*, 1986, Pyke *et al.*, 2010) and prostaglandins (PG) (Koller *et al.*, 1993). However, there are conflicting reports regarding the contribution of both substances.

Endothelial derived prostaglandins are vasodilator agents that are released promptly following the initial increase in blood velocity (Koller *et al.*, 1993). However, upon further investigation, using human subjects who consumed 1200 $mg.d^{-1}$ of ibuprofen to halt prostaglandin (PG) synthesis, blood flow responses were unaltered over the time course of the exercise. In contrast, a different study Wilson & Kapoor, (1993) demonstrated that inhibition of PG synthesis resulted in small alterations in limb blood flow under steady state conditions.

Both the endothelium and the skeletal muscle cells are potential sources of nitric oxide in skeletal muscle tissue during contraction. The enzyme nitric oxide synthase (NOS), which catalyses the formation of NO from L-arginine, exists in two isoforms: endothelial NOS (eNOS) and neuronal NOS (nNOS). In human skeletal muscle eNOS and nNOS are localised in the vascular endothelium and in skeletal muscle cells. The generation of NO from the endothelium may be increased in response to receptor binding of compounds such as bradykinin, acetylcholine, and ATP as well as mechanical factors such as sheer stress (Clifford *et al.*, 20004). Indeed, experimental evidence has revealed that the shear stress resulting from the initial rise in blood flow causes vasodilatory substances to be released including the above mentioned, nitric

oxide (Furchgott & Zawadzki, 1980; Wilson & Brackett, 1983; Pohl & Lamontagne, 1991, Pyke *et al.*, 2010). Administration of sodium nitroprusside, which resulted in an increase in NO in the muscle vasculature, caused blood flow to be augmented at rest and during exercise (Radegran, 1997; Radegran & Saltin, 1998). In contrast, NO synthesis blockade using L-NMMA resulted in a significant reduction in blood flow by 50% at rest and during the recovery phase of knee extensor exercise. However, there was no effect on blood flow during either the voluntary or passive exercise phase during knee extensor exercise in humans (Radegran & Saltin, 1998). Thus, further research to clarify the role of NO in the haemodynamic response is warranted.

Adenosine is a metabolite and when given exogenously to skeletal muscles causes rapid vasodilation (Joyner & Proctor, 1999). Furthermore, adenosine is elevated in venous blood draining into the contracting skeletal muscle and when injected into vascular beds resulted in vasodilation (Sollevi, 1986). Also, during knee extensor and knee flexion exercise, which caused blood flow to increase to between 5-6 $L.min^{-1}$, infusion of adenosine further enhanced the blood flow response by 2 -3 $L.min^{-1}$ (Radegran, 1997). In addition, when adenosine was infused at a rate of 2 $mg.min^{-1}.L^{-1}$ into the femoral artery, an increase in blood flow of up to 8-10 L min^{-1} occurred and this increase persisted as long as the same dose of adenosine was infused (Radegran, 1997). Furthermore, when infusion of adenosine was halted, the time of return of blood flow to resting levels is similar to the onset of vasodilation i.e. 10-15 s. Also, the elevation in blood flow is proportional to the adenosine dose (Radegran, 1997).

Overall, muscle pump action and/or vasodilatory factors may be responsible for the initial hyperaemic phase I response. Phase II involves many factors including the accumulation of interstitial potassium (K^+), adenosine, and a release of nitric oxide (NO) from the endothelium due to shear stress. In addition, a decreased pH, decreased pO_2, increased temperature and/or increased pCO_2 may also be involved (Saltin *et al.*, 1998; Kingwell, 2000). However, more research regarding the exact mechanisms is warranted.

To our knowledge, there is only one study, which has investigated leg blood flow (LBF) in older and young adults. DuManoir *et al.*, (2010) examined the steady state and kinetic responses of femoral (conduit) artery blood flow (measured using Doppler ultrasound) as well as quantifying $\dot{V}O_{2p,}$ and local muscle deoxygenation (ΔHHb). The three parameters were evaluated in young (n=7, age: 26 ± 3 yr) and older (n=7, 71 ± 7 yr) adults during the same absolute (24 W) and relative (~80% lactate threshold) intensities during alternating leg knee-extension exercise. At the same absolute WR, the amplitude and the end-exercise responses for $\dot{V}O_{2p}$, LBF, HR and LVC were similar in older and young adults. However, $\dot{V}O_{2p}$ (young: 31 ± 9 s, older: 58 ± 21 s) and conduit artery blood flow kinetics were slower in older adults compared with young adults at both absolute (24 W: young: 18 ± 7 s, older: 44 ± 19 s) exercise intensities as evidenced by a larger $\tau\dot{V}O_{2p}$ and τLBF. The adjustment of deoxygenation (ΔHHb) was not different between the two groups. This implied that while the amplitude of bulk O_2 delivery is maintained, the adjustment (kinetics) of microvascular O_2 delivery is attenuated. Thus, as well as the slower O_2 delivery, microvascular blood flow may be attenuated in older adults and thus they may require a greater reliance on oxygen extraction at a faster rate or for a longer period of time during the transition to exercise (DuManoir *et al.*, 2010).

1.6 Ageing and oxygen uptake kinetics

Muscle contractions can evoke major increases in limb blood flow that are closely matched to the oxygen uptake of the active tissue (Anderson *et al.*, 1985; Rowell, 1988). Recently, the effects of age on the adaptation of pulmonary oxygen uptake ($\dot{V}O_2$ kinetics) and muscle deoxygenation (HHb), reflective of O_2 extraction, at the onset of heavy-intensity cycling exercise were investigated (DeLorey *et al.*, 2005). It was shown that older adults displayed slower pulmonary O_2 uptake kinetics compared with young adults.

During heavy-intensity exercise, apart from the cardiopulmonary phase (phase 0) and the primary phase (phase 1) observed in moderate intensity exercise, an additional component of the oxygen uptake response, referred to as the slow component or

phase 2 (P2) of $\dot{V}O_2$ kinetics exists. The pulmonary $\dot{V}O_2$ kinetics of the primary phase largely reflects the kinetics of O_2 consumption in the exercising muscles. The time constant (τ) of the primary phase of $\dot{V}O_2$ kinetics was slower in older (49 ± 8 s) adults compared with their young (29 ± 4 s) counterparts at the onset of heavy-intensity cycling exercise (DeLorey *et al.*, 2005). In addition, heavy-intensity exercise had a greater muscle oxygen demand and required a greater fraction of maximum cardiac output (CO_{max}) in the older adults.

DeLorey *et al.*, (2005) also demonstrated that muscle deoxygenation (HHb) during heavy- intensity cycling exercise adapted at a faster rate in older adults (8 ± 2s) compared with young adults (14 ± 2s). This implied that muscle blood flow increases at a slower rate throughout the exercise in older adults compared with young subjects. This also signified that there was a greater mismatch between muscle oxygen delivery and muscle oxygen utilisation during the on-transient of heavy-intensity exercise in older compared with young adults. In contrast, during the steady state phase ($\Delta HHb/\Delta\dot{V}O_2$), muscle deoxygenation was similar ($P>0.05$) between young and older adults. Thus, following a slow increase in O_2 delivery during the on-transient of exercise, muscle perfusion to metabolism matching was similar in young and older adults (DeLorey *et al.*, 2005). These findings suggests that local muscle perfusion may be altered at a slower rate in older compared with young adults during the onset of heavy-intensity exercise and $\dot{V}O_2$ may be limited by the convective delivery of O_2 in older adults (DeLorey *et al.*, 2005). This in fact is consistent with findings by DuManoir *et al.*, (2010) who showed a significant reduction in both $\dot{V}O_2$ and blood flow kinetics responses in the aged at the onset of moderate intensity knee extension exercise.

1.7 Muscle fatigue

Muscle fatigue can be defined as "a decrease in or the inability to sustain the maximum force generating capacity of the muscle and assumes the voluntary activation of all motoneurons" (Gandevia, 2001). While, muscular endurance, a related characteristic of fatigue, is the ability to resist fatigue and is measured using

the time that a given target force can be maintained (Allman & Rice, 2001). The fatigability and endurance of a muscle is determined by either a) the endurance time (the time that the required force or power output can be maintained) or b) the degree to which force or power is reduced over time (rate of fatigue). The kinetics of muscle blood flow and thus, the delivery and consumption of O_2 by the exercising muscle may have an important potential role in the underlying mechanisms involved in the development of muscle fatigue, and this will be discussed below.

1.7.1 Factors contributing to development of muscle fatigue

Muscle fatigue can originate from both peripheral and central factors (Table 1.3). This can occur because of failure in force production at numerous sites along the pathway from the central nervous system to the muscle itself (Kent-Braun *et al.*, 2002). The peripheral sites of the origin of fatigue involve the peripheral nervous system and the skeletal muscle and incorporate the neuromuscular junction, the sarcolemma T-tubule-sarcoplasmic reticulum and the myofilaments (Table 1.2). The mechanisms involved in the development of peripheral fatigue may be excitation-contraction coupling, accumulation of metabolites and/or a depletion of energy sources (Kent-Braun *et al.*, 2002).

As well as peripheral factors, a lack of drive from the central nervous system may also be responsible for the loss of force generated during exercise. The inability to voluntarily recruit all motor units, or a decrease in discharge rates indicates that some failure in central activation has occurred. This progressive decline in voluntary activation is called "central fatigue" and is due to failure at a site within the central nervous system. Central fatigue may originate from all structures above the neuromuscular junction. Indeed, it may arise from a number of factors including intrinsic motoneuronal properties, reflex inhibition and disfacilitation, renshaw cell inhibition and insufficient drive from supraspinal sites, all working in part or in combination (Gandevia, 2001). On average, central factors are thought to account for up to 20% of muscle fatigue in healthy young adult (Kent-Braun *et al.*, 2002). A number of techniques have been developed to assess the failure of central drive and

thus assess central fatigue, including the central activation ratio method (CAR), examination the ratio of maximum voluntary contraction (MVC) to tetanic force, examination of changes in integrated electromyography (iEMG) signal during exercise, twitch interpolation and assessment of low frequency fatigue (Latash *et al.*, 1994). These techniques will be referred throughout this section.

Table 1.3. Peripheral and central factors involved in the development of muscle fatigue.

Central factors	**Peripheral factors**		
	Peripheral nervous system and skeletal muscle		
Malfunction of neurons • reduced motor unit firing rate • reduced motor unit firing frequency Voluntary effort inhibition • motor cortex Psychological factors	• neuromuscular junction (NMJ) -inhibition of axon terminals -depletion of neurotransmitters: Ach, acetyl coenzyme A, choline -receptors on motor end plate	•Sarcolemma –T tubules sarcoplasmic reticulum -failure of electrical signal to cross sarcolemma/T tubule -problem with Ca^{2+} release from sarcoplasmic reticulum -inability of Ca^{2+} and troponin to bind -Na^{+}/k^{+} pump not working optimally -AP alters the concentration on both sides of the cell membrane	•Depletion of ATP, PC and/or glycogen • Myofilaments -substrate depletion (ATP, CP)

1.7.2 Ageing and fatigue

Although, over the last decade numerous studies have focused on the effect of ageing on muscle fatigue, there is still a lack of consensus in the literature. Some studies have found that older adults fatigue less compared with young individuals under numerous exercise conditions using various muscle groups (Narici *et al.*, 1991; Ditor & Hicks, 2000; Kent-Braun *et al.*, 2002; Lanza *et al.*, 2004; Rubinstein & Kamen, 2005; Chung *et al.*, 2007; Russ *et al.*, 2008). Other studies (Lindstrom *et al.*, 1997; McNeil & Rice, 2007) suggest that fatigue development is similar between young and older individuals, while yet others (Baudry *et al.*, 2007) demonstrate that older adults fatigue more compared with young individuals. The contrasting results across the different studies may be attributable to differences in contraction mode, exercise

protocol, muscle groups used and subject characteristics employed during different studies.

1.7.2.1 Association between muscle fatigue and changes in the neuromuscular system with ageing

There are a number of prominent age-associated changes that occur within the neuromuscular system that may affect the degree to which fatigue develops. These factors include alterations in motor unit discharge rate (MUDR) (Kamen *et al.*, 1995; Connelly *et al.*, 1999), loss of muscle mass (sarcopenia) (Kent-Braun & Ng, 1999), fibre type alterations (Jakobsson *et al.*, 1990) and therefore changes in metabolic capacity (Kent-Braun *et al.*, 2002; Lanza *et al.*, 2004; Lanza *et al.*, 2005) which support an age-related metabolic economy (Tevald *et al.*, 2010). Also, muscle activation patterns and alterations in the pressor response differ between older and young individuals (Russ *et al.*, 2008; Wust *et al.*, 2008). Most studies use an integrated approach in order to study muscle fatigue. Measurements of central and peripheral activation, contractile functioning as well as intracellular energy metabolism are made *in vivo* and usually non-invasively.

The motor unit remodelling theory illustrates the age-associated progressive loss of motor units. Initially there is a loss of motor neurons innervating fast-twitch (FT) muscle fibres. Then FT fibres are either lost or reinnervate a motor neuron responsible for slow-twitch (ST) fibres, thus adopting a more fatigue resistant characteristic (Doherty *et al.*, 1993). Overall, the greater percentage of the remaining muscle fibres possess slow twitch properties. Furthermore, histochemical studies have indicated a decline in the number of large sized lumbar motoneuron cells in older adults. With an age-related loss of motoneurons and fast twitch muscle fibres, slow twitch fibres determine the degree of muscle contraction (Doherty *et al.*, 1993). Muscles from older adults are weaker and slower in contracting. For instance, declines of up to 40% in maximal voluntary isometric contraction for the tibialis muscle have been observed in men over the age of 80 years (Vandervoort & McComas, 1986). Also, the duration of the contraction (determined using stimulated

twitch) is prolonged by as much as 50% in older individuals. Furthermore, moderate reductions in firing rates (up to 33%) at forces less than 20% MVC have been observed in older adults (67-89 years) compared with young adults (Hunter *et al.*, 2005b).

A number of studies have used electromyography (EMG) to study motor units and recruitment patterns in aged and young participants. One such study examined endurance capacity in older and young strength matched men (Hunter *et al.*, 2005a). Eight young (18-34 yr) and older (67-76 yr) men sustained an isometric contraction using the elbow flexors at 20% MVC torque until the target torque could no longer be maintained for at least 5 s. Both groups sustained similar maximum torque prior to and following the fatiguing task. The time to task failure was longer for the older men (22.6 ± 7.4 min) compared with strength matched young men (13.0 ± 5.2 min, $P<0.05$). An increase in torque fluctuations, EMG bursting activity, and heart rate were greater for the young men compared with the older group and each parameter was also lower at task failure for the older men. Furthermore, the averaged EMG (AEMG) activity and ratings of perceived exertion were similar at task failure for both groups. However, the rates of increase in AEMG were less for the older men for all arm extensor muscles compared with young men ($P<0.05$), which correlated with a longer time to failure. The increase in EMG activity during this low force fatiguing contractions was largely due to the recruitment of larger motor units as the muscle fatigued, without alterations in discharge rates. The difference in bursting activity of EMG indicated that the young men relied more on the transient recruitment of motor units to maintain the task. The mechanisms responsible for the longer time to failure for the older men involved a reduced need for the transient recruitment of motor units (Hunter *et al.*, 2005a).

When using the same exercise protocol and comparing older men (n=10, 65-80 yr) and women (n=8, 65-80 yr) with young men (n=14, 18-35 yr) and women (n=13, 18-35 yr), the rate of EMG activity was greater for young men compared with either of the other three groups, while the time to task failure was longer for older adults (older

men: 24.1 ± 8.0 min, older women: 21.3 ± 10.7 min) and the young girls (18.3 ± 8.0 min) compared with the young men (10.8 ± 5.2 min) (Hunter *et al.*, 2004). EMG bursts were less frequent for older adults (older: 0.0 ± 0.2, young: 0.2 ± 0.5 bursts.min^{-1}) at the end of the fatiguing contractions and this was accompanied by a decline in torque fluctuations (young: 160 ± 97%, older: 122 ± 68%). The increase in EMG activity represented an increase in motor unit recruitment, and implied that young men displayed a greater rate of recruitment as active muscle fibres gradually failed. Furthermore, the EMG burst analysis increased more quickly for young men. The alterations in average EMG activity, EMG bursts and torque fluctuations implied that increase in motor unit activity, which specifically influenced endurance capacity, was more rapid for young and more gradual for older adults. Overall, the results indicated that the time to task failure for submaximal contractions with the elbow flexor muscles was longer for the older compared with the young adults and that changes in MAP, heart rate, EMG activity and fluctuations in torque between the groups were responsible for the differences in fatigue (Hunter *et al.*, 2004).

1.7.2.2 Association between muscle fatigue, muscle mass, strength and blood flow in ageing

The influence of muscle mass and strength on the development of muscle fatigue has been investigated in a number of studies (Kent-Braun *et al.*, 2002; Lanza *et al.*, 2004; Russ *et al.*, 2008). Before examining the association between muscle fatigue, muscle mass, strength and blood flow in ageing, sarcopenia and ageing will be discussed.

1.7.2.2.1 Sarcopenia

Sarcopenia was reported to range from 13-24% in a pool of 883 adults aged 65-70 years, increasing to over 50% for those over the age of 80 years (Baumgartner *et al.*, 1998). In this study, sarcopenia was indirectly determined using dual energy x-ray absorptiometry (DEXA) by applying previously validated predictive equations for determining muscle mass. Sarcopenia was defined as the appendicular skeletal muscle mass being less than 2 standard deviations below the mean of a young reference group. Similarly, further investigations have revealed that the prevalence of

sarcopenia based on skeletal muscle mass determined again by DEXA was 10% for men and 8% for women aged between 60 and 69 years, increasing to 40% for men and 18% for women over the age of 80 years (Melton *et al.*, 2000). Using the same technique to measure muscle mass, Iannuzzi-Sucich *et al.*, (2002) reported that the overall severity of sarcopenia in 64-92 year olds was 22.6% in women and 26.8% in men and this increased to 31% and 45% for those in their eight decade.

The age-related loss of skeletal muscle mass is undoubtedly one of the major contributing factors to a reduction in strength and decline in functional ability observed in older adults (Doherty, 2003). For example, Janssen *et al.*, (2000) highlighted the fact that a decline in muscle mass with ageing was more apparent in the lower limbs and this may be the reason for the major age-related decline in functional lower limb mobility. Furthermore, there is a strong correlation between loss of skeletal muscle mass, strength and a decline in functional capacity with ageing (Doherty, 2003).

1.7.2.2.1 (a) Age associated decline in strength

It is well established that older adults experience a loss of skeletal muscle strength due to the ageing process (Larsson *et al.*, 1979; Murray *et al.*, 1980; McDonagh *et al.*, 1984; Young *et al.*, 1984; Murray *et al.*, 1985; Young *et al.*, 1985; Davies *et al.*, 1986; Vandervoort & McComas, 1986; Cunningham *et al.*, 1987; Kallman *et al.*, 1990; Bassey & Harries, 1993; Doherty *et al.*, 1993). This has been highlighted in a number of cross-sectional studies comparing the limb muscles of older, middle aged, and young adults tested under both isometric and dynamic conditions.

Studies examining the knee extensor muscles have reported that older men and women in their seventh and eight decades display strength reductions in the range of 20 - 40% compared with young men and women (Larsson *et al.*, 1979; Murray *et al.*, 1980; Young *et al.*, 1984, 1985). This can increase up to 50% for older adults in their ninth decade, in comparison to young adults (Murray *et al.*, 1980; Murray *et al.*, 1985). Similar strength reductions have been observed for the elbow flexor and extensor muscles, handgrip strength and ankle plantar and dorsiflexors (McDonagh *et*

al., 1984; Davies *et al.*, 1986; Vandervoort & McComas, 1986; Cunningham *et al.*, 1987; Kallman *et al.*, 1990; Bassey & Harries, 1993; Doherty *et al.*, 1993).

Different strength testing procedures have reported different findings on strength alterations in ageing adults. Vandervoort & McComas, (1986) examined strength changes using a group (n=111, aged 20-100 yr) of older, middle aged and young men and women. Maximal voluntary and electrically evoked maximal twitch forces were assessed for the ankle plantar flexors and dorsiflexor muscles. Men displayed greater strength than women at all ages. Ageing was associated with a reduction in force production for the ankle plantar flexor and dorsiflexor muscles (approximately 49%: 80-90 year olds; 29%; 60-80 year olds). Reductions in strength were similar for both men and women and during evoked and voluntary contractions (Vandervoort & McComas, 1986). In addition, the loss in plantar and dorsiflexor strength was only apparent in the sixth decade and occurred at a rate of approximately 1.3% per year thereafter (Vandervoort & McComas, 1986).

Longitudinal studies provide an understanding of the rate of strength declines with ageing. A 12-year follow up study (Winegard *et al.*, 1996) involving 22 of the original 69 subjects (73-79 yr) from the initial study of Vandervoort & McComas, (1986). Over the 12-year period, a significant reduction in ankle plantar flexor strength was evident for men (30%) and for women (25%). Similarly, both male and female subjects displayed a significant reduction in dorsiflexor strength (9.5%, 3.3% respectively), but to a lesser extent when compared with that observed for the plantar flexor muscles (Winegard *et al.*, 1996).

Furthermore, Lindle *et al.*, (1997) assessed the rate of decline in muscle strength, isometric, concentric and eccentric peak torques at slow (0.52 rad/s) and fast (3.14 rad/s) velocities for the knee extensors in 654 men and women aged 20-93 years. Both men and women displayed age-related reductions in knee extensor isometric and concentric strength beginning in the fourth decade at a rate of 8-10% per decade in both genders. The age-associated decline for eccentric strength was similar for men and women, but the decline for women began a decade earlier (Lindle *et al.*, 1997).

Additional longitudinal studies have revealed significant age-related losses in strength for both men and women. A reduction in grip strength was evident for both men (12%) and women (19%) per year over 4 years (Bassey & Harries, 1993). In addition, there was a significant age-related reduction in knee extensor strength, with a 7 year follow up study revealing a decline of 3.2% per year in a group of 23 men aged between 73 and 86 years (Aniansson *et al.*, 1986). Furthermore, a 12 year follow up study revealed a significant decline in isokinetic strength for both the elbow (9%) and knee extensor (30%) muscles in 9 men (Frontera *et al.*, 2000). In this study, computerised tomography scans revealed a significant reduction in muscle cross-sectional area for the same group of men (Frontera *et al.*, 2000). Thus, as a consequence of reduced muscle mass, reduction in strength occurring with ageing may have important functional (Hyatt *et al.*, 1990) as well as haemodynamic implications.

1.7.2.2.2 Factors contributing to the development of sarcopenia

1.7.2.2.2 (a) Muscle activation and ageing

The development of sarcopenia may in part be due to older adults experiencing a loss in muscle activation during maximum voluntary contractions. Thus has been investigated in a number of studies but results are inconclusive. Several studies have reported no change in the ability of older adults to fully activate their muscles (Vandervoort & McComas, 1986; Kent-Braun *et al.*, 1993; Roos *et al.*, 1999; Scaglioni *et al.*, 2002). Other studies have found that older adults display a reduced muscle activation compared with young adults (Jakobi & Rice, 2002; Stevens *et al.*, 2003). Methodological differences in relation to muscle groups tested and protocols employed may account for some of the conflicting results. For instance, twitch interpolation technique has been used to examine the level of voluntary drive during an effort (voluntary activation) and involves the delivery of one or more electrical stimuli (single, double or tetanic pulse) to the nerve (Gandevia, 2001). This technique has been employed in studies using the quadriceps (Roos *et al.*, 1999; Stackhouse *et al.*, 2001), tibialis anterior (Vandervoort & McComas, 1986; Connelly *et al.*, 1999;

Kent-Braun & Ng, 1999) and elbow flexor muscles (Allman & Rice, 2001; Bilodeau *et al.*, 2001). However, the results are inconclusive as to whether older subjects can activate their muscles to the same extent as young subjects. The discrepancies within the results may be due to methodological differences employed in the studies. For example, studies utilizing single or double pulse for interpolation did not display any age-related impairment (Connelly *et al.*, 1999), while studies which have interpolated tetanic trains resulted in a decline (2- 6%) in voluntary activation in older adults (Lanza *et al.*, 2003). It is controversial as to whether these small age-associated differences in voluntary activation are functionally meaningful or not.

Moreover, Jakobi & Rice, (2002) reported that older adults have the ability to match young adults and achieve similar voluntary activation during isometric contraction. In this study the older men were fully familiarised and were allowed several attempts to fully activate their muscles. It was suggested that lack of practice as opposed to an inability of the nervous system to maximally activate muscles may account for the decrease in maximal voluntary activation observed in other findings (Jakobi & Rice, 2002).

1.7.2.2.2 (b) Reduction in type II fibre size, number and motorneuron number

The age-associated decline in strength can be attributed to a reduction in the number of active motorneurons (Campbell *et al.*, 1973), in particular type II motorneurons (Tomonaga, 1977; Larsson *et al.*, 1978; Aniansson *et al.*, 1986). It is well reported that the percentage of type I fibres increase with age. Furthermore, the remaining fast twitch fibres are reinnervated by type I neuronal axon branches (Kugelberg, 1976), and thus fast twitch fibres take on the properties usually exhibited by slower type I motor units (Buller *et al.*, 1987).

Furthermore, biopsy studies using the vastus lateralis muscle have demonstrated age-related reductions (20-50%) in the size of type II fibres. A reduction in size is also evident in type I fibres, although not as great as that seen in type II, with losses varying from 1 to 25% (Larsson *et al.*, 1978; Grimby & Saltin, 1983; Lexell & Downham, 1992; Doherty *et al.*, 1993; Roos *et al.*, 1997; Vandervoort, 2002).

Reductions in muscle fibre number are also evident with ageing (Lexell *et al.*, 1988). Post-mortem studies from 43 men (15-83 yr) (using whole muscle cross sections from the vastus lateralis muscle) demonstrate up to a 50% reduction in fibre type I and II number by the ninth decade compared to muscles of 20 year olds (Lexell *et al.*, 1988). Using the techniques of macro-electromyography (Stalberg & Antoni, 1980) and motor unit estimation (Doherty *et al.*, 1995; Doherty *et al.*, 2002), a substantial decline in the number of whole functioning motor units in both proximal and distal muscles from upper and lower regions of older men and women have been reported (de Koning *et al.*, 1988; Stalberg *et al.*, 1989; Doherty *et al.*, 2002).

The decline in the total number of muscle fibres leads to a loss in muscle cross sectional area (Booth *et al.*, 1994), which is a contributing factor in force production. Loss of motoneurons is also evident with ageing and this may be a contributing factor in the decreased fibre number and reduced muscle mass observed in older men and women. The age-associated loss in motoneurons has been highlighted in a number of studies, which demonstrate losses up to 50% for the thenar, hypothenar and biceps-brachialis muscle groups (Brown, 1972; Sica *et al.*, 1974; Brown *et al.*, 1988; Doherty *et al.*, 1993). Some studies have indicated that motorneuron numbers are well maintained until the seventh decade and then begin to progressively decline thereafter (Campbell *et al.*, 1973; McComas, 1991, 1998). These findings are also consistent with studies demonstrating an age-associated loss in ventral root fibres and anterior horn cells (Kawamura & Dych, 1977; Kawamura *et al.*, 1977; Tomlinson & Irving, 1977; Mittal & Logmani, 1987; Doherty *et al.*, 1993).

There is also evidence that the conduction velocities of surviving motoneurons are slowed with increasing age (Gutmann & Hanzlikova, 1976). For instance, adults aged between 60 and 96 years demonstrated a reduction in the conduction velocities of the fastest conducting nerve fibres innervating the extensor digitorum brevis muscle fibres when compared with adults under the age of 60 years, due to a loss of functioning motor units, and in particular loss of axons of fibres (Campbell *et al.*, 1973).

Furthermore, it has been reported that the size of human motor units increase with advancing age (Campbell *et al.*, 1973). The mean amplitude of the extensor digitorum brevis muscle single motor unit action potentials recorded from older adults (aged 60-96 years) was significantly greater compared to those recorded from humans aged between 3 and 58 years. The increase in the size of motor units may occur as a result of surviving axons adopting denervated muscle fibres thus forming large or "giant" motor units (Campbell *et al.*, 1973; Vandervoort & McComas, 1986). This may lead to older individuals experiencing a loss of muscle control (Campbell *et al.*, 1973) and/or decline in muscle force generating capacity (Vandervoort & McComas, 1986).

It is well established that older adults display a significant reduction in muscle mass and strength compared with young individuals (Kent-Braun *et al.*, 2002). Therefore, it is assumed that weaker older adults may be able to sustain a relative submaximal force for a greater duration compared with young adults, as they experience less intramuscular and blood flow occlusion. This results in a smaller accumulation of metabolites due to a greater delivery of oxygen to the muscle resulting in an enhanced ability to sustain a task for a longer period of time.

The influence of muscle mass and strength on endurance capacity was examined in young and older men and women during an ankle dorsiflexion fatiguing exercise (4 s contraction, 6 s relaxation), with workloads beginning at 10% of MVC and increasing by 10% every 2 minutes for a duration of 16 minutes (Kent-Braun *et al.*, 2002). To determine the time course of fatigue, an MVC was performed at the beginning of every 2 min stage. Older subjects fatigued less, (determined by ratio of pre-exercise strength to post-exercise strength) compared with young subjects ($P<0.01$). Men were stronger than women, with no significant age effect. The relationship between muscle mass, strength and fatigue was determined using univariate linear regression and was associated with approximately 24% of fatigue evident during the exercise. It was suggested that subjects who were stronger (i.e. young men) might have displayed greater blood flow occlusion to the muscle due to greater intramuscular pressure during each exercise contraction. Furthermore, greater blood flow occlusion was evident at the higher contraction intensities. Interestingly, the significant association

observed between pre-exercise strength and end-exercise $H_2PO_4^-$ concentrations also implied that oxygen delivery may have been somewhat better in the weaker subjects, which allowed them to use the oxidative pathways for a longer period of time during the exercise protocol (Kent-Braun *et al.*, 2002).

Similarly, Russ *et al.*, (2008) did not find a major relationship between baseline MVC and fatigue when assessing healthy young (men, n=8; female n=8, 20-35 yr) and older (men n=7, female n=9, 67-78 yr) participants during maximal dorsiflexor isometric contractions. A duty cycle of 70% (7 s contraction, 3 s relaxation) was used in order to minimise the metabolic advantage of women and older adults by limiting muscle reperfusion between contractions. But still, greater fatigue resistance (pre- versus post-fatigue MVC forces) was apparent in the older group of adults compared with the young group. Thus, other mechanisms independent of absolute force were cited as important contributors to the fatigue resistance observed, including those originating within the muscle itself (age-related fibre type alterations and changes in muscle metabolism) (Russ *et al.*, 2008).

In order to further examine the role of blood flow in the development of dorsiflexor muscle fatigue, young (n=12, 26 ± 4 yr) and older men (n=12, 72 ± 4 yr) performed intermittent (50% duty cycle, 5s on/ 5s off) maximal contractions for 6 minutes under both free-flow and ischaemic conditions (Chung *et al.*, 2007). The older group demonstrated 9% less fatigue (% decline on peak force) at minute 3 and 12% less at minute 6, under both conditions. When subgroups of older (n=6) and young adults (n=6) were matched for strength (young: 283 ± 33N, older: 281 ± 32 N), fatigue differences still remained (P=0.001 at min 3, P<0.001 at min 6). The greater fatigue in young individuals evident under ischaemic conditions was explained by central and peripheral activation failure. However, this was not the case for older individuals (Chung *et al.*, 2007). The smaller metabolic changes in the older muscle may have been responsible for the differences and will be discussed in the next section.

1.7.2.3 Association between muscle fatigue, oxidative metabolism and metabolic economy with ageing

Age-related differences in muscle bioenergetics may account for the enhanced fatigue resistance displayed by older adults during various exercise protocols. Quantification of skeletal muscle energetics can be made both non-invasively and continuously during various fatiguing conditions using phosphorus magnetic resonance spectroscopy (MRS). During such studies, a number of key metabolites including inorganic phosphate (Pi), proton (H^+) and diprotonated inorganic phosphate ($H_2PO_4^-$) are measured. MRS is also used to determine the synthesis rate of intracellular adenosine triphosphate (ATP) by the creatine kinase pathway, glycolysis and oxidative phosphorylation. The concentration of ATP ([ATP]) is measured experimentally by examining changes in PCr and H^+ (Kent-Braun, 2009).

Age-associated differences in intramuscular energy metabolism and the effect on muscle fatigue were quantified in a group of older (65-85 yr, n=11 men; n=11 women) and young (25-45 yr, n=10 men; n=10 women) individuals of similar activity levels during a progressive intermittent isometric dorsiflexion exercise (Kent-Braun *et al.*, 2002). Exercise began at 10% MVC and was increased by a further 10% MVC every 2 minutes. Similar to previous findings, older subjects fatigued less than young subjects ($P<0.01$). Furthermore, strength was associated with a greater production of metabolic by-products including H^+, $H_2PO_4^-$ and Pi as well as lower pH. In both groups, the decline in MVC was significantly related to increased levels of $[H_2PO_4^-]$. The smaller increase in H^+ concentration in the older group reflected their reduced capacity for glycolytic metabolism compared with young adults, mainly thought to be due to a loss of fast twitch fibres associated with ageing. Also, metabolic data demonstrated significant age and gender related differences in response to exercise. During the early part of the steady-state exercise, where Pi/PCr represents the capacity of the muscle to respond oxidatively to the need for ATP, steady state Pi/PCr was maintained in a similar manner for all groups. However, beyond the 8th minute (where intensity was > 50% MVC) the ratio of Pi/PCr increased at a greater rate in young men, while both older men and women and young women continued to derive

their energy requirements via oxidative phosphorylation for the duration of the exercise. This resulted in less accumulation of metabolites and allowed for more efficient maintenance of force production in the older group.

This is consistent with previous findings demonstrating the preservation of oxidative capacity in the tibialis anterior (Kent-Braun *et al.*, 2002; Lanza *et al.*, 2005) and plantar flexor muscles of older adults (Chilibeck *et al.*, 1998). Chilibeck *et al.*, (1998) demonstrated that moderately active older (n=12, mean age: 66.7 yr) and young men (n=13, mean age: 26.2 yr) had greater oxidative capacity, (quantified by 31P-MRS) during a progressive unilateral ankle plantar flexor exercise.

In order to further examine the relationship between muscle fatigue in ageing and the degree to which older adults use either oxidative phosphorylation or anaerobic glycolysis, healthy young (n=20, 27 ± 3 years; 10 males, 10 females) and older (n=18, 70 ± 5 years; 10 males, 8 females) subjects performed an intermittent (12s/12s duty cycle) MVC exercise protocol on two separate occasions and the pathways of ATP synthesis were determined using MRS (Lanza *et al.*, 2007). During the first session blood flow was unoccluded but during the second session blood flow was occluded using a pressure cuff inflated at 220 mmHg, thus eliminating the ability of the muscle to generate ATP via oxidative metabolism and therefore measuring the degree to which ATP was produced via anaerobic glycolysis. The peak glycolytic flux during the unoccluded exercise protocol was lower in older adults (0.8 ± 0.1 mM $ATP.s^{-1}$) compared with the young group (1.4 ± 0.1 mM $ATP.s^{-1}$), $P>0.001$), such that the young group generated more ATP by glycolysis. During the ischaemic exercise condition, peak ATP generated by anaerobic glycolysis was similar in both young (1.3 ± 0.2 mM $ATP.s^{-1}$) and older (1.4 ± 0.2 mM $ATP.s^{-1}$) groups, which implied that glycolytic function was not impaired in older adults. Interestingly, it was concluded that older adults show a preference rather than a reliance on oxidative metabolism during unoccluded exercise. A significant correlation between force reduction and the accumulation of the metabolite $H_2PO_4^-$ was very apparent, which is similar to previous findings of Kent-Braun *et al.*, (2002). Furthermore, analysing a subgroup of

individuals (16 older and 16 younger) revealed that older adults displayed a higher metabolic economy ($Ns^{-1}.cm^{-2}$.mM ATP) during the unoccluded exercise protocol.

More recently, the lower energy cost of skeletal muscle contractions in older humans has been examined again using the technique of MRS but also using tetanic and twitch techniques (Tevald *et al.*, 2010). Young men displayed greater fatigue than older men during the 25 Hz contraction. The energy cost of a twitch (older; 0.13 ± 0.04 mM ATP/twitch, young; 0.18 ± 0.06 mM ATP/twitch; P=0.045) and tetanic contractions at 25 Hz were 27% and 26% lower (P=0.01) for older adults (1.5 ± 0.4 mM $ATP.s^{-1}$) compared with young adults (young; 2.0 ± 0.2 mM $ATP.s^{-1}$). Energy costs during a 90 s tetanus at a frequency recorded at 50% peak force (older; 10.9 ± 2.0 Hz, young; 14.8 ± 2.1 Hz) was 49% lower in older (1.0 ± 0.2 mM ATP/s) compared with young (1.9 ± 0.2 mM ATP/s; P<0.001) adults. The energy cost of a twitch was related to the maximal rate of force relaxation, which was slower in the older group compared with the young group (Tevald *et al.*, 2010).

Overall, these results indicate that the way in which ATP is synthesised to meet the demands of the fatiguing exercise protocol has a major role in the ability of older adults to resist fatigue compared with young adults.

1.7.2.4 Association between muscle fatigue and task specificity in ageing

The task dependency of muscle fatigue shows that the site of impairment of muscle contraction is dependent on the task that is being performed. The details of the task that influence the mechanisms that contribute to the age-associated development of fatigue include the type (i.e. voluntary vs. electrical stimulation, isometric vs. dynamic, sustained vs. intermittent) and the intensity (high versus low) of the exercise as well as the muscle groups involved. This will be discussed below.

1.7.2.5 Influence of the contraction type being performed during the exercise protocol

The type of muscle contraction (sustained vs. intermittent) has an influence on the amount of fatigue that develops during different tasks. For instance, during a sustained 35% MVC protocol using the elbow flexors, older subjects displayed greater endurance (assessed using time to failure) compared with young subjects (young: 179.6 ± 51.5 s, older: 471.7 ± 260.8 s) (Bilodeau *et al.*, 2001). However, in a different study no significant age-related difference in muscle endurance (time to failure, young: 4.1 ± 1.2, older: 4.9 ± 2.2 min) or fatigue (% force decrements, young: 57 ± 2%, older: 59 ± 6%) was evident between older (n=7, 84 ± 2 yr) and young (n=7, 24 ± 2 yr) men during an intermittent isometric submaximal exercise performed at a moderate to high intensity (60% MVC) for the same muscle group (Allman & Rice, 2001).

The duty cycle employed in studies is an additional factor that may influence the degree to which older adults fatigue during exercise. For instance, when protocols use duty cycles of 50% or less (work periods shorter than rest periods), an increase in fatigue resistance seems to be evident with age (Bemben *et al.*, 1996; Kent-Braun *et al.*, 2002). In contrast, when work periods are longer than rest periods (> 50% duty cycle), studies tend to show that there is no definite effect of age on muscle fatigue (Cupido *et al.*, 1992; Stackhouse *et al.*, 2001). Indeed, a lower duty cycle may be beneficial to the more oxidative nature of older muscles (Kent-Braun *et al.*, 2002), as a larger rest period may allow for the replenishment of oxygen to the working muscle and this may enhance fatigue resistance in the older adult.

Also, the type of muscles being tested during various protocols may have a bearing on whether older adults have the ability to resist fatigue or demonstrate more fatigue compared with young individuals. For instance, older men fatigued less compared with young men during repeated maximal dynamic contractions of the ankle dorsiflexors. In contrast, studies using the knee extensor muscles cited no age-related alterations in fatigue resistance (Larsson *et al.*, 1979; Laforest *et al.*, 1990; Lindstrom *et al.*, 1997). The discrepancies between studies may be due to patterns of muscle

activity changing with age. Older adults may use the dorsiflexor muscles more in order to maintain posture and balance and during walking activities. On the contrary, the knee extensors are mainly involved in power orientated activities and thus may undergo a higher degree of disuse and in addition, ageing is associated with a decline in maximum power output (Lanza *et al.*, 2004).

Furthermore, studies which assessed voluntary activation during fatigue protocols using the technique of twitch interpolation in older and young adults found that older subjects did not display any age-associated impairment in the level of voluntary activation in the elbow flexor, tibialis anterior or adductor pollicis muscles (Hicks & McCartney, 1996; Ditor & Hicks, 2000) during intermittent MVC fatigue protocols. However, there was an age-associated impairment in the level of voluntary activation in the quadriceps muscles (Stackhouse *et al.*, 2001).

Overall, no global physiological mechanism seems to be responsible for the development of muscle fatigue in young or old, male or female. Instead the factors and mechanisms that cause fatigue are dependent on the task being performed and thus, the dominant mechanism of fatigue is specific to the processes that are stressed during the fatiguing exercise. However, the kinetics of blood flow and thus the delivery of oxygen to the working muscle may have a major influence on the development of muscle fatigue within the ageing individual and this will be investigated throughout this thesis.

Table 1.4: A summary of studies comparing fatigue responses during exercise in older and young men and/or women. Y=young=older, m=men, w=women, n=number taking part.

Investigating Team	Participant information	Age (yr)	Activity/fitness Status Strength matched	Muscle/exercise	Protocol used	Main measurements taken	Outcome
Tevald *et al.*, (2010)	n=9 (YM) n=9 (OM)	26-4 72-5	Activity levels higher in Y (accelerometer) YM stronger than OM)	Ankle dorsiflexion	16 s MVC (voluntary) 60 s continuous stim 25 Hz, 90 s continuous stim at f_{50}	Energy cost of twitch using MRS (voluntary & electrically evoked)	Energy cost lower in O $T_{1/2}$ higher in O
Stackhouse *et al.*, (2001)	n=11 (YM) n=9 (YW) n=8 (OM) n=9 (OW)	18-32 65-84	Activity level not defined Y stronger than O (gender differences in strength not given)	Knee extension kicking exercise (Quadriceps)	Fatiguing 25 MVC's (5 s/2 s)	Central activation ratio (CAR) electrically evoked fatigue & non fatiguing conditions	Non fatigued: CAR lower in O Fatigued: CAR even greater in O
Lindstrom *et al.*, (1997)	n=14 (YM) n=8 (YW) n=8 (OM) n=8 (OW)	28-6 73-3	Activity levels similar Y/O (questionnaire) YM stronger than OM, M stronger than W)	Quadriceps Dynamic knee extension	100 repeated maximal dynamic knee extensions at 90° s^{-1}	Peak torque, MVC, Fatigue rate	No difference between Y & O in fatigue rates or reduction in muscle force. Endurance lower in O than Y.
Bilodeau *et al.*, (2001)	n= 5 (YM) n=5 (YW) n=5 (OM) n=4 (OW)	26-3 71-4	Activity levels similar Y/O (questionnaire) Y/O no strength differences (gender differences in strength not given)	Elbow flexion	Sustained submaximal (35% MVC) to failure	Neuromuscular propagation, voluntary activation, endurance time, rate of fatigue	No neuromuscular propagation failure, < change in EMG median frequency in O. Endurance time longer in O. Similar ROF in Y & O
Russ *et al.*, (2008)	n=8 (YM) n=8 (YW) n=9 (OW) n=7 (OM)	20-35 67-78	Activity levels similar Y/O (questionnaire) YM stronger than OM, M stronger than W)	Ankle dorsiflexion (1 leg)	Maximal isometric (7 s/3 s) for 5 mins	% force decrements Volitional & stimulated force, cMAP, contractile properties	Y fatigued more than O (pre MVC vs. post fatigue MVC) O showed longer ½ relaxation
Ditor *et al.*, (2000)	n=12 (YM) n=12 (OM) n=12 (YW) n=12 (OW)	25-27 72-65 24-27 70-59	Activity level not defined M stronger than W, YM stronger than OM	Thumb abduction (adductor pollicis)	Intermittent maximal MVC (5 s/2 s) for 3 mins	Voluntary fatigue index (% force decrements)	Y fatigued more than O M fatigued more than W OW fatigued more than OM
McNeil & Rice (2007)	n=12 (YM) n=12 (OM) n=12 (very OM)	22-33 60-69 80-90	Activity level not defined Very O weaker than O. YM similar strength to OM	Ankle dorsiflexion	25 maximal contractions (20% MVC at 25° ROM	Power output at 20% MVC, fatigue induced changes in EMG	O fatigued more than Y Fatigue increased with age
Callahan *et al.*, (2010)	n=8 (YM) n=8(YW) n=8 (OW) n=8 (OM)	21-35 65-80	Activity levels similar Y/O (accelerometer) YM stronger than OM (gender differences not defined)	Knee extensor exercise Quadriceps	1.Isometric intermittent maximal MVC (5 s/5 s) for 4 mins 2. Dynamic maximal contractions at 120° s^{-1} for 4 mins	CAR (electrically stimulated & voluntary contractions). Torque production.	Isometric: O displayed higher MVC torque. Similar CAR. Dynamic: No difference in torque maintenance between O & Y. Similar CAR.

Investigating Team	Participant number	Age (yr)	Activity/fitness Status Strength matched	Muscle used	Exercise type, protocol and muscles used	Main measurements taken	Outcome
Bemben *et al.*, (1996)	n=153 (Y/O M)	20-74	Activity levels differed Y/O (questionnaire) YM stronger than OM	Finger flexion Thumb abduction Dorsiflexion Plantar flexion	11 Isometric intermittent (2 s/5 s) maximal contractions	% total impulse (energy expended) % force decrements	O lower energy expenditure % force decrements greater for Y than O for plantar flexors only.
Rawson (2009)	n=16 (YM) n=19 (OM)	21-2 66-6	Activity levels not defined Y stronger than O	Quadriceps Knee extension	5 sets of 30 isokinetic exercise at $180°.s^{-1}$	Relative fatigue Absolute fatigue	Relative and absolute fatigue greater in Y Y higher torque for all 5 sets
Kent-Braun *et al.*, (2002)	n=10 (YM) n=10 (YW) n=11 (OM) n=10 (OW)	25-45 65-85	Activity levels similar Y/O (accelerometer) Y & O similar strength (men stronger than women)	Ankle dorsiflexion (one leg)	16 min of incremental intermittent (4 s/6 s) (begin at 10% MVC, increased by 10% every 2 min)	CAR Peripheral (cMAP) Metabolites (MRS) Post exercise MVC/pre exercise MVC	No difference between Y & O in CAR or cMAP. Greater P_i & $H_2PO_4^-$ in Y than O O fatigued less than Y (Post/pre exercise MVC)
Lanza *et al* (2004)	n=9 (YM) n=9 (OM)	22-30 68-76	Activity levels similar Y/O (accelerometer) Y & O similar strength for isometric exercise only.	Ankle dorsiflexion (one leg)	1. Intermittent isometric (5 s/5 s) for 3 min 2. Intermittent dynamic 90 at $90°.s^{-1}$	CAR Tetanic torque Contractile properties cMAP	O fatigued less than Y during both protocols, no age difference in CAR or peripheral activation failure. In both protocols $t_{1/2}$ less in O
Lanza *et al.*, (2007)	n=10 (YM) n=10 (YW) n=11 (OM) n=8 (OW)	24-30 65-75	Activity levels similar Y/O (accelerometer) Y & O similar strength (gender differences in strength not given)	Ankle dorsiflexion	Maximal muscle contractions 6 MVC's (12 s/12 s) 1. Free flow 2. Ischaemia	CAR Glycolytic flux Metabolism (MRS)	O fatigued less than Y while glycolytic flux was lower in O under both free flow & ischemic conditions.
Hunter *et al.*, (2005)	n=8 (YM) n=8 (OM)	18-31 67-76	Activity levels similar Y/O (questionnaire) Y & O similar strength	Elbow flexion	Sustained isometric contraction at 20% MVC to failure	Time to failure EMG, torque fluctuation	O fatigued less than Y W fatigued less than M EMG, Torque fluctuations greater for Y
Hunter *et al.*, (2004)	n=14 (YM) n=13 (YW) n=10 (OM) n=8 (OW)	18-35 65-80	O less active than Y (questionnaire) Y stronger than O	Elbow flexion	Isometric sustained contraction at 20% MVC to failure	Time to failure	O fatigued less than Y W fatigued less than M

1.8 Aims

The relationship between exercise performance and blood flow responses in ageing have been investigated in recent research studies using a variety of exercise models and modes (Martin *et al.*, 1991, Proctor *et al.*, 1998, 2003, 2004, Donato *et al.*, 2006, Parker *et al.*, 2008). However, there is still conflicting views regarding age and gender related differences in blood flow responses during exercise maybe in part due to the large variations in exercise protocols and modes between studies. Most studies have employed cycling or knee extension exercise modes and have consistently showed that older women have attenuated blood flow and vascular conductance responses during submaximal and graded exercise (Proctor et al., 2003, 2004). However, there are contrasting reports amongst aged men, with some studies reporting maintained haemodynamic responses while others have found attenuation blood flow responses compared with young individuals. However, calf plantar flexor exercise, which is an essential activity for walking and posture stabilization has not yet been investigated. Furthermore, plantar flexion exercise is vital for older adults when carrying out their activities of daily living. Hence, to further explore this, the aim of the first study (chapter 2) was to examine haemodynamic responses and muscle performance during an incremental isometric plantar flexion exercise performed to maximal exertion in older compared with young men and women of similar activity levels.

In addition to the apparent ageing induced reduction in blood flow responses during steady state submaximal exercise, the ability of older adults to increase muscle blood flow at the onset of exercise may also be impaired. Recently, in the only published study exploring age induced effects on blood flow kinetics, DuManoir *et al.*, (2010) directly measured femoral artery blood flow responses during knee extension exercise in young versus aged subjects and reported that blood flow kinetics were slowed in older compared with young participants during moderate absolute intensities but not during moderate intensities relative to each subjects lactate threshold. In light of this, the aim of the second study (chapter 3) was to examine the age-associated effects on

the rate at which the vascular conductance (VC) response increases (VC kinetic response) at the onset of constant-force intermittent contraction exercise performed at low, moderate and high relative exercise intensities (30%, 45%, 60% and 70% MVC) in older compared with young men and women.

Despite deleterious age-associated haemodynamic alterations, the majority of previous research findings demonstrate an age-related maintenance or even enhancement of fatigue resistance and muscular endurance (Ditor *et al.*, 2000, Bilodeau *et al.*, 2001, Stackhouse *et al.*, 2001, Kent-Braun *et al.*, 2002, Hunter *et al.*, 2004, 2005, Russ *et al.*, 2008). Thus, there are a number of favourable age-related compensatory mechanisms that play a role in maintaining performance and fatigue resistance during exercise despite reductions in strength and deleterious haemodynamic alterations. These include differences in muscle fibre type size and distribution as well as adaptations in muscle metabolism; which can favour the degree to which older adults perform everyday tasks and prevent the onset of fatigue. However, the relationship between haemodynamic alterations and performance during fatiguing exercise has not been simultaneously examined employing the same exercise protocol in the same individuals. Hence, the aim of the third study (chapter 4) was to examine fatigue profiles (i.e. rate of fatigue) following the same submaximal relative exercise intensities (30%, 45%, 60% and 70% MVC) employed in our previous study (chapter 3) where blood flow and vascular conductance were determined in the same young and older individuals.

1.9 Hypotheses

The primary hypothesis was that older adults would be able to augment blood flow and display similar vascular conductance responses to young subjects during a relative incremental isometric plantar flexion exercise performed to maximal exertion. The second hypothesis was that vascular conductance kinetics would be slower in older adults compared with young adults again during plantar flexion exercise performed at constant-force exercise intensities of 30, 45, 60 and 70% MVC. The third hypothesis was that despite older adults displaying an impaired kinetic

response, they would have an enhanced fatigue resistance and greater muscular endurance compared with young adults.

Chapter 2:

2.1. Introduction

Peak exercise capacity declines with advancing age (Proctor *et al.*, 1998; Doherty, 2003). Initial studies examining the physiological basis of age-related reductions in exercise tolerance focused primarily on the age-associated declines in muscle mass (Mazzeo & Tanaka, 2001). However, more recently, an age-related reduction in blood flow to the active skeletal muscles has been implicated as an important potential underlying cause of reduced exercise tolerance in older adults (Proctor *et al.*, 2003a; Proctor *et al.*, 2004). Furthermore, a reduction in the blood flow kinetic response may also result in premature fatigue and thus, a decline in performance. Previous studies reveal that normally active older women consistently display an attenuation in both exercising leg blood flow and vascular conductance during various exercise protocols including single leg graded knee extension exercise (Parker *et al.*, 2008) and graded dynamic leg cycling exercise (Proctor *et al.*, 2004). However, age-related haemodynamic responses are less consistent among men. It has been shown that both sedentary and chronically endurance trained older men demonstrated a reduction in absolute blood flow during cycling (Beere *et al.*, 1999; Poole *et al.*, 2003; Proctor *et al.*, 2003a) and knee extensor exercise compared with young men (Magnusson *et al.*, 1994; Lawrenson *et al.*, 2004). In contrast, normally active older men displayed a preservation in peak and submaximal leg blood flow and vascular conductance responses compared with young men during graded single knee extension exercise performed to maximal exertion (Parker *et al.*, 2008), as well as during both graded and constant-load bouts of leg cycling performed at the same submaximal exercise intensities (Proctor *et al.*, 2003b).

The vast majority of the above-mentioned studies have focused on either whole body cycling or knee extension exercise when examining haemodynamic responses between young and older participants. However, haemodynamic responses during calf plantar flexion exercise, an activity fundamental for walking and posture stabilization, have not to our knowledge been examined. Thus, the aim of this first study was to examine the age-related peak and exercising leg blood flow and vascular

conductance responses during an incremental plantar flexion exercise performed to maximal exertion. The main hypothesis was that older adults would be able to augment blood flow and display similar vascular conductance responses to young subjects during a relative incremental isometric plantar flexion exercise performed to maximal exertion.

2.2 Materials and methods

2.2.1 Subjects

Fifteen young men, 8 young women, 13 older men and 10 older women were recruited for the study.

2.2.1.1 Subject recruitment

Older subjects were recruited from Senior Citizens Clubs around Dublin city, whereas young subjects were recruited at Trinity College Dublin. Subjects were given an information sheet (Appendix I), which outlined the study procedures and the possible risks and benefits of participating. Subjects provided written informed consent prior to being accepted onto the study. The study was conducted according to the Declaration of Helsinki and was approved by the Faculty of Health Science Research Ethics Committee, Trinity College Dublin.

2.2.1.2 Subject inclusion and exclusion criteria

Participants were non-smokers, non-obese (body mass index $\leq$ 30 $kg.m^{-2}$) and were free of signs, symptoms and clinical evidence of cardiovascular disease, chest pains, diabetes, high blood pressure, asthma, musculoskeletal disease and anaemia as assessed by medical history questionnaire (Appendix II). In addition, subjects were excluded from the study if they were taking beta-blockers, calcium channel blockers or any other anti-hypertensive medication. All of the older female subjects were post-menopausal and were not taking hormone replacement therapy for at least 12 months prior to the study. All subjects were normally active according to scores on the Low-Level Physical Activity Recall (LOPAR) questionnaire (Appendix III) (Hiatt *et al.*, 1995; Regensteiner *et al.*, 1996). None of the subjects participated in moderate to high intensity aerobic exercise for more than 3 days per week or regular lower body resistance training for more than 2 days per week during the past 12 months. Also, subjects didn't take part in a regular exercise programme for at least 6 months prior to the commencement of the study.

2.2.2 Medical assessment

All subjects underwent a medical examination (Appendix IV) by a qualified Medical Practitioner at the cardiovascular laboratory of the Department of Physiology, Trinity College Dublin. This included a physical examination where a fasting blood sample was taken.

2.2.2.1 Blood analyses

A fasting venous blood sample was taken from each subject for determination of cholesterol, glucose, haemoglobin, white blood cell, red blood cell and % haematocrit (see equipment and measurement section 2.2.4). This was to clarify that all subjects had similar profiles.

2.2.3 Experimental design

2.2.3.1 Experimental protocol overview

Subjects were required to attend the cardiovascular laboratory in the Department of Physiology at Trinity College Dublin on two occasions to perform a number of physiological tests (Appendix V). On visit one, subjects were familiarised with the custom-built calf ergometer and exercise protocol (see equipment and measurements section 2.2.4). Then, on visit two, they performed a single leg (right) graded isometric calf plantar flexion exercise to failure. During the test, limb blood flow, blood pressure and heart rate measurements were taken. Subjects were required to refrain from alcohol, caffeine and strenuous physical activity in the proceeding 24 hours prior to the testing sessions.

2.2.3.2 Visit 1; Subject familiarization procedure and maximum voluntary contraction (MVC)

Subjects performed three to four low intensity intermittent isometric plantar flexions of the right calf muscle in the horizontal position. The contraction/relaxation ratio was 2 s/4 s. Then, the procedure for performing the incremental exercise test was explained to each of the subjects. The subjects practised the incremental exercise test for approximately 10-15 min (contraction/relaxation ratio of 2 s/4 s). They initially began exercising in the supine or horizontal posture at a force of 50 N for four to five

contractions. When comfortable with the contraction/relaxation rhythm, the force was then increased to 150 N. Then the force was further increased to 200 N and 300 N. The exercise was continued until the subject felt comfortable with the procedure. The entire procedure was then repeated at an inclination of 67°.

Subjects were then familiarised with the procedure for performing their maximum voluntary efforts in the upright (67°) posture. They were required to exert the greatest amount of force possible on the force-plate while plantar flexing the right foot. Each contraction was sustained for approximately 3 s and the procedure was repeated six times with a 1 minute rest period between contractions. The highest force produced was recorded as the MVC.

2.2.3.3 Visit 2; Leg vascular conductance during the incremental exercise test to failure

Each subject performed an incremental intermittent plantar flexion isometric exercise (6 s duty cycle; 2 s contraction and 4 s relaxation) test to failure on a calf ergometer in the upright posture at a tilt angle of 67°. The upright posture was employed in this study as it exemplifies the haemodynamic conditions under which the plantar flexor muscles work during every day activities such as walking. A tilt angle of 67° was chosen instead of the fully upright angle of 90° previously employed (Egana & Green, 2005) as subjects complained of discomfort in the pelvic region, a factor that may influence study outcomes. The incremental exercise test for all subjects began at a force of 100 N. Each subject exercised at this force for 2 minutes. The force was then increased by 200 N for young men, 150 N for older men and young women, and 100 N for older women every 2 minutes. Failure occurred when the subject could not maintain the required force for two consecutive contractions (Fig. 2.1). The peak force achieved was defined as the highest force sustained for at least 1 minute (10 consecutive contractions). The different incremental exercise protocols were designed to produce similar times to failure for all four groups. During the incremental graded exercise test, calf blood flow was measured non-invasively using the technique of venous occlusion plethysmography (see equipment and measurement section 2.2.4).

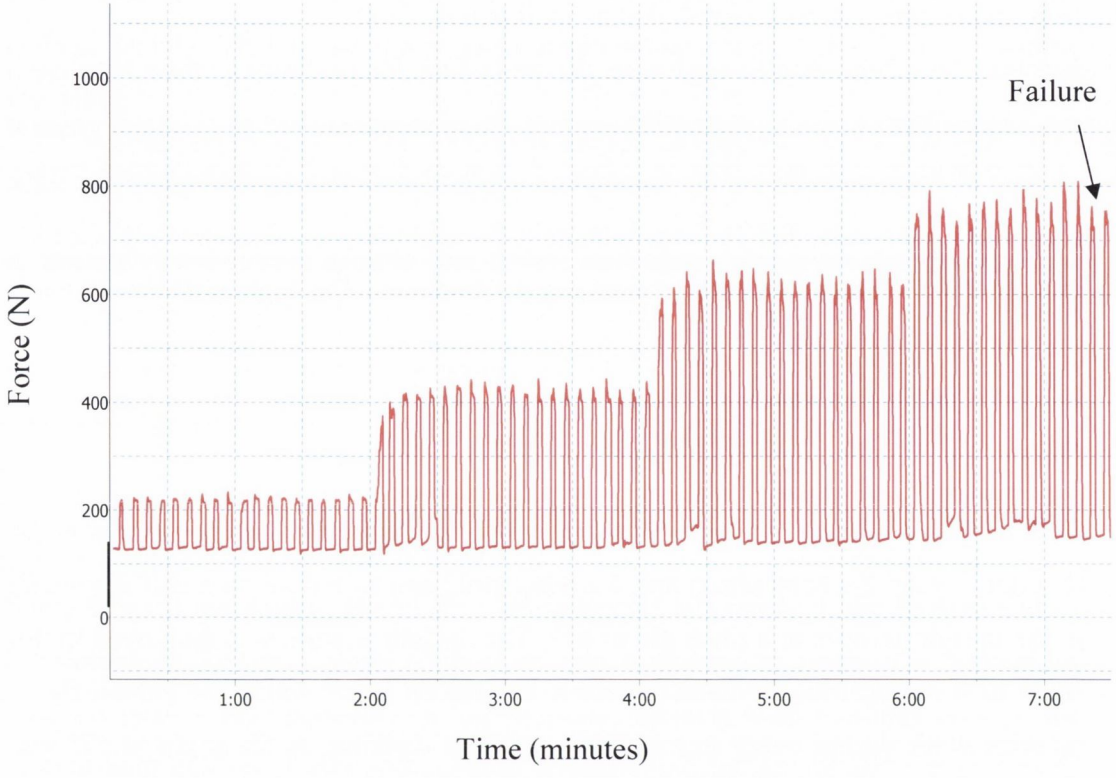


Figure 2.1 *Incremental exercise test to failure for a young male individual. The subject initially reaches a force of 100N (0-2 min). This is increased by 200N for a further 2 minutes and continued until failure (displayed at 800N). Failure occurs when the subject can no longer maintain the force of 800N for two consecutive contractions.*

2.2.4 Equipment and measurements

2.2.4.1 Calf ergometer and exercise model

The calf ergometer consisted of a counterbalance tilt-table with two immobile force-plates, each connected to a strain gauge (Figure 2.2). The right foot was strapped into a leather boot, which was attached to the force plate and the left leg was supported on a padded platform that lay approximately 30 cm anterior to the heel of the exercising foot. All blood flow measurements were obtained from the right leg. Subjects were fixed to the table using a harness to prevent upward displacement of the body during plantar flexion of the foot while in the upright position (67°). The 67° tilt angle was

chosen instead of the upright angle of 90° used previously (Egana & Green, 2005) as subjects complained of discomfort in the pelvic region, a factor that may influence study outcomes. The majority of body weight was supported by a seat, which was raised to crotch level and thus the force exerted onto the force-plate was mainly attributed to the plantar muscles of the right leg. Furthermore, the seat prevented accessory muscles adding further to the force production. The seat height was recorded for each subject and was used in subsequent exercise tests. Plantar flexion of the foot resulted in the application of force to the plate. Forces exerted on the foot-plate by the subject could be seen as a trace on a visual display monitor (Chart™ v5.5.4, AD Instruments). A metronome was used to maintain a contraction/relaxation ratio of 2 s to 4 s (6 s duty cycle). The force that was applied to the force plate was amplified (RS stock no. 846-147) and sampled at 40 Hz. The data was processed using a Power Lab analog-to-digital converter (ML 795, AD Instruments).

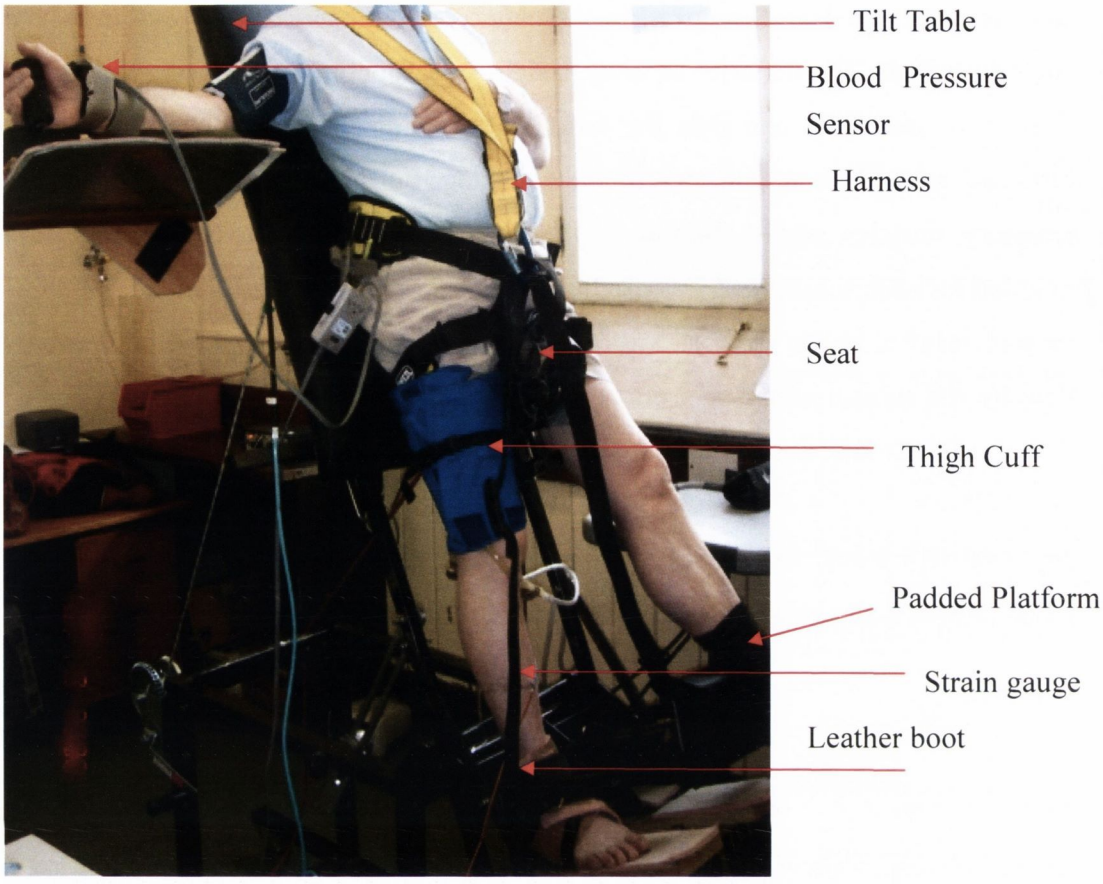


Figure 2.2. *Experimental set-up. The subject (older man) was harnessed to the calf ergometer and his body weight supported by the seat. His right foot was strapped into the leather boot, while the left foot rested on the padded platform. The thigh cuff was placed on the right leg and inflated to 55 mmHg during the incremental exercise test. A strain gauge, placed on the widest part of the calf was used to measure the change in calf girth over time. A blood pressure (BP) sensor, placed on the right arm, measured beat-to-beat BP (see equipment and measurements section 2.2.4.2 for further details).*

The calf ergometer was calibrated manually prior to each testing day in the upright position. Firstly, the unloaded force-plate was set to 0 V. A number of previously calibrated weights, ranging from 0-80 kg, were then placed one at a time on the force-plate and the corresponding voltage (V) was recorded from the computer trace. Once all of the weights were placed on the force-place, they were then removed again one at a time and the corresponding decreasing voltage was recorded from the force-trace. The forces in V were then converted to mV and graphs depicting the linear relationship between weight (kg) and ascending forces (mV) and weight (kg) and descending forces (mV) were created in a spreadsheet (Excel, Windows 2000). The mV at 20 kg and 80 kg were used to convert mV readings in PowerLab (ML 795, AD Instruments) to newtons (N). The mean ascending and descending V values at 20 kg and 80 kg and the co-efficient of variation can be seen in Appendix VI.

2.2.4.2 Blood flow measurement

Leg blood flow at rest (supine position) and during exercise (upright position, at tilt angle of 67°) was measured non-invasively using the technique of venous occlusion plethysmography (Egana & Green, 2005).

The subject rested in the supine position and a thigh cuff (Hokanson, USA) was fixed securely using velcro fasteners around the upper right thigh. A mercury-in-Silastic strain gauge measuring 2 cm less than the circumference of the calf was placed around the widest calf girth (Figure 2.3) and connected to the plethysmograph. The thigh cuff was then rapidly inflated to 40 mmHg for 10 s. This allowed arterial inflow but occluded the venous return, which caused an increase in calf blood volume and calf girth. Resting calf blood flow was calculated as the rate of change in calf girth divided by time.

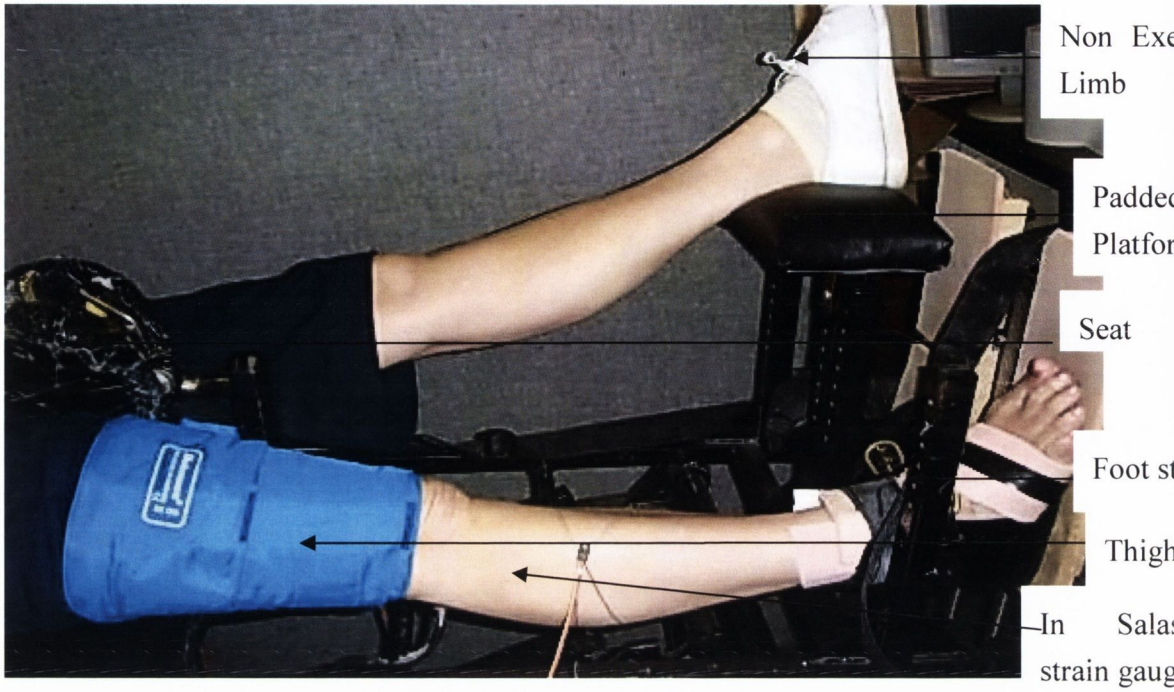


Figure 2.3 *Venous occlusion plethysmography experimental set up in the horizontal position*

The subject was then rapidly (within 3 s) tilted from the horizontal to the upright (67° tilt angle) while the thigh cuff was inflated (< 1 s) to 55 mmHg. This tight pressure was maintained for the duration of the graded exercise. The subject began to exercise at the required intensity at a contraction/relaxation ratio of 2 s / 4 s. Leg blood flow was evaluated during the 4 s relaxation period between contractions. During the 4 s relaxation phase, the blood volume in the calf muscle increases due to a rise in arterial inflow as venous outflow is occluded due to the pressure of the cuff (Fig. 2.4). The overall result is an increase in calf girth during the relaxation phase between muscle contractions. This was detected as an increase in strain gauge length. During the 2 s contraction phase, the blood was expelled back into the circulation and a decrease in strain gauge length was detected. The calf girth was recorded at the start and end of the relaxation phase and blood flow was calculated as the rate of change in calf girth divided by time. The change in the leg volume was measured and accepted only when the force had returned to within 10 N of a stable base value, which corresponded to the relaxed phase (Figure 2.4). This minimal base force had to be maintained for at least 3 s. Leg blood flows ($ml.100ml^{-1}.min^{-1}$) were converted to millilitres per minute using an estimate of each subject's leg volume obtained from

the anthropometric measurements of the leg (see section 2.2.4.4). Leg vascular conductance for each of the increments during the graded test was calculated as leg blood flow divided by mean arterial pressure (MAP) added to the estimated hydrostatic component acting at the midpoint of the calf (see equipment and measurement section 2.2.4.9).

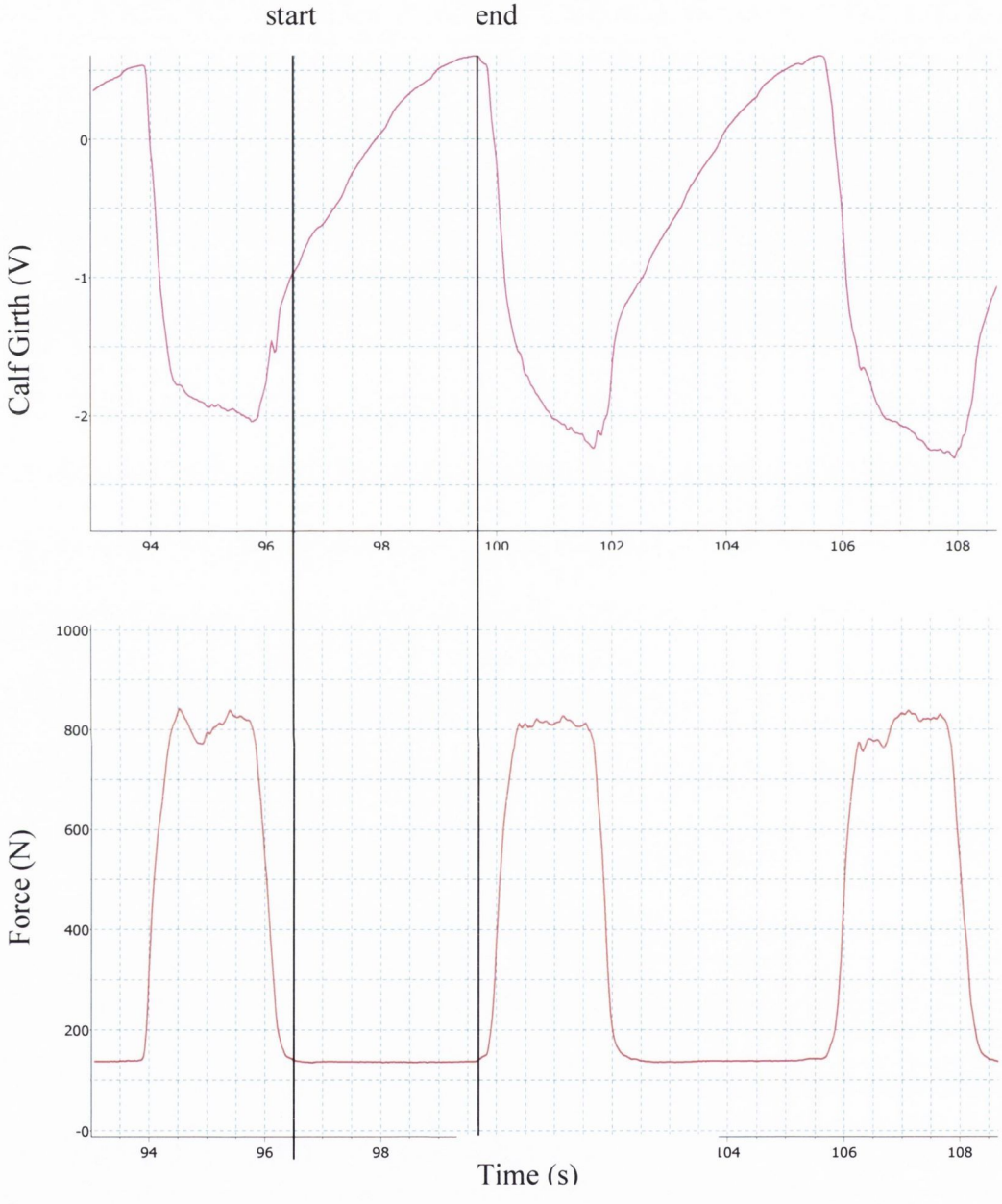


Figure 2.4 *Force and calf girth traces from one subject during the incremental graded plantar flexion exercise at a body tilt angle of 67°. The two vertical bold lines indicate where the force trace has returned to a stable baseline, which corresponds to the relaxation phase. The relaxation begins at the left line (start) and ends (end) at the right line. The rate of change in calf girth (detected by the change in strain gauge length) during the relaxation phase is indicative of change in blood flow over time.*

2.2.4.3 Body mass, height and body mass index (BMI)

Body mass (kg) was measured using a platform beam scales (AVERY, England). Height (cm) was measured using a Seca^{TM} stationmetre (Seca Ltd., Germany). Body mass index (BMI) was determined by dividing the body mass (kg) of each subject by his or her height squared (m^2) (BMI = $kg.m^{-2}$).

2.2.4.4 Leg volume measurements

Leg volume of the exercising leg was calculated using the regression equation for the prediction of volume and masses of tissue (Clarys & Marfell-Jones, 1986) as follows: (Tibiale – malleolar length x [38.20851 + maximum calf girth - (π x (medial calf skinfold/10))] x 80.24423 - 2467.9)

Tibiale – malleolar length (cm) was measured from the tibial plateau to the centre of the medial malleolus using a measuring tape. Maximal calf girth (cm) was measured as the widest point of the calf in a relaxed state also using a measuring tape. Medial calf skinfold (mm) was measured from the medial aspect of the leg using a skinfold caliper (Harpenden calipers).

2.2.4.5 Blood analyses

Once cleared to take part in the study, fasting venous blood samples were taken from each of the subjects. Blood samples were collected into 6-ml vacutainers. Whole blood samples were analysed for haemoglobin (Hb), white blood cells (WBC), red blood cells (RBC) and % haematocrit (Hct) using a haematological particle analyser (Coulter Ac.T diff: Counter Ltd., UK). Fasting blood glucose was analysed using the Accutrend® GC (BM-Accutrest, Boechringer Mannheim, Germany) and cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and triglycerides were analysed using Cardio Chek P·A system analyser (Polymer Technology Systems, Inc., Indianapolis).

2.2.4.6 Low-level physical activity recall (LOPAR): A measurement of habitual physical activity.

Activity levels for each subject were determined using the Low-Level Physical Activity Recall (LOPAR) (Appendix III). The LOPAR questionnaire was administered by the Investigator on the initial day of testing. Subjects were asked to recall their daily activities for the previous 7 days. The LOPAR questionnaire comprises of three categories; work, household chores and recreational activities. Within each category, each activity is rated using a metabolic (MET) hour equivalent. One MET equals 3.5 $ml.kg^{-1}.min^{-1}$ of oxygen consumption. The Investigator calculated the amount of time (hours) spent during each activity for all three categories. The total MET hours (both awake and asleep) per week was then calculated for each subject (Regensteiner *et al.*, 1998; Brandenburg *et al.*, 1999).

2.2.4.7, Blood pressure, heart rate and vascular conductance

2.2.4.8 Blood pressure

Blood pressure was measured beat-to-beat using either (a) arterial applanation tonometry (COLIN, CBM7000) or (b) volume-clamp method (finometer).

(a) Arterial applanation tonometry (COLIN, CBM7000)

Brachial blood pressure (BP) was measured beat-to-beat at rest and during exercise tests using applanation tonometry of the right radial artery (COLIN, CBM7000, Japan). An oscillometric cuff was placed around the upper right arm. A tonometric blood pressure (TBP) sensor was secured to the right wrist over the subject's radial artery. This sensor contains piezoelectric pressure transducers, which were held down over the radial artery by a pneumatic pump. This sensor was fastened with a wristband to counteract any movement of the subject's wrist.

(b) Volume-clamp method (Finometer®)

Blood pressure (BP) was measured at rest and exercise using the finometer (medical systems) continuous beat-to-beat finger blood pressure, which is based on the volume clamp method (Penaz, 1963). The diameter of the artery under a cuff wrapped around the finger is kept constant (clamped). Changes in arterial diameter, detected by means of an infrared photo-plethysmograph built in the finger cuff, are opposed by a fast pressure servo controller that changes pressure in an inflatable air bladder, which is

also mounted in the finger cuff. The plethysmograph consists of a light source (LED; light emitting diode, emits infrared light) and a light detector (an infrared photodiode). A sudden rise in finger intra-arterial pressure during systole causes an increase in arterial diameter, which is detected as an increase in light absorption and thus a decrease in the signal detected by the plethysmograph. During the diastolic phase of the beat, when blood pressure declines gradually, the blood is expelled from the artery and as a result the amount of light detected by the photodiode will increase again.

For both the COLIN and finometer, mean arterial pressure was calculated as;

Diastolic BP + [0.333 (Systolic BP – Diastolic BP)].

The finometer unit was acquired by the research team at the end of the first year of my PhD and as such only 26 % of the subjects were tested using the volume-clamp method. A pilot reliability study showed that both methods obtained similar MAP responses (ICC = 0.8) (Appendix VII).

2.2.4.9 Heart rate

Heart rate was measured beat-by-beat using either applanation tonometry (COLIN, CBM7000, Japan) or the volume-clamp method (Finometer®) at rest and during the incremental graded calf exercise test.

2.2.4.10 Vascular conductance

Leg (calf) vascular conductance at rest was calculated using the following equation

$$VC = BF/MAP$$

Leg (calf) vascular conductance during exercise was calculated using the following equation:

$$VC = [BF/(MAP + HP)] \times 10$$

where BF is blood flow, MAP is mean arterial pressure (mmHg) and HP is hydrostatic pressure. Estimates of leg blood flow normally expressed relative to resting limb volume ($ml.100ml^{-1}.min^{-1}$) were converted to $ml.min^{-1}$ using an estimate of each subject's leg volume (measurement section 2.2.4.4). MAP was calculated as diastolic BP + [0.333 (Systolic BP – Diastolic BP)]. HP was attained using the equation ρgh, where ρ is the density of the blood (haematocrit), g is acceleration due to gravity (9.81 ms^{-2}), and h is the vertical distance between the heart and the midpoint of the calf. Final VC values obtained were multiplied by 10 for clarity.

2.2.5 Data analyses

To examine age by gender interactions, data are expressed as each subjects workload normalised to his or hers peak force (percentage of maximal workload) to account for differences in workload increases used for the four groups. Individual linear regression equations were produced from each subject's blood flow and vascular conductance responses as follows;

$$y = ax + b$$

where, y is the blood flow or vascular conductance response, a is the slope of the blood flow or vascular conductance response, b is the intercept and x is the force (% max). The R^2 values were calculated for each individual's response. The slopes (a) of the hyperaemic and vasodilatory responses were then compared between the four groups.

2.2.6 Statistical analysis

All anthropometrical, haematological and peak physiological data were compared between young and older men and women using a two-way, (gender x age) ANOVA. A two-way ANOVA was also used to compare the slope of the blood flow and vascular conductance responses during the incremental exercise test between each of the groups. Differences were located using a Tukey's *post hoc* test. A three-way, repeated measures ANOVA (gender x age x time, with time as the repeated measure) was used to compare MAP and HR responses at different time points during the test. Differences were located using a Bonferroni *post hoc* test. The level of significance was set at $P<0.05$. All results are given as mean ± standard deviation (SD). The statistical software Sigma Stat, (USA) was used for all two-way ANOVA statistical analyses, while for three-way repeated measures ANOVA, data desk® 6.1 was used.

2.3 Results

2.3.1 Subjects

2.3.1.1 Physical characteristics

Physical characteristics and activity levels for the four groups can be seen in Table 2.1. Men were taller than women (main effect = gender) and young subjects were taller than older subjects (main effect = age) with no interaction between gender and age. Males had a greater body mass than females (main effect = gender), whereas older subjects had a higher BMI than their young counterparts (main effect = age). No differences in either leg volume or activity levels were detected between the groups. Individual anthropometrical data can be seen in Appendix VIII.

Table 2.1. Physical characteristics and activity levels for young and older men and women (mean ± SD).

* Significantly different ($P<0.05$) compared with women of the same age group. † Significantly different ($P<0.05$) compared with young within the same gender.

	Young men n=15	Older men n=13	Young women n=8	Older women n=10
Age (yr)	24±2	70±7†	23±1	64±7†
Height (cm)	179±7*	170±7†*	167±4	158±7†
Body mass (kg)	73.5±12.3*	75.1±9.0*	58.4±4.2	68.6±9.0
BMI ($kg.m^{-2}$)	23.0±3.5	26.1±2.0†	21.6±1.5	27.7±4.1†
Leg volume (mL)	2839±766	2881±474	2353±236	2760±623
Activity level ($MET\ h.week^{-1}$)	181±29	190±38	210±42	206±44

2.3.1.2 Haematology

Haematological values for the four groups can be seen in Table 2.2. Older individuals displayed higher total cholesterol, low density lipoprotein and total cholesterol/HDL compared with young subjects (main effect = age). Triglyceride values were also higher in older compared with young subjects (main effect = age) but there was a significant interaction between age and gender so that triglycerides were also higher in older men compared with older women but not between young men and women. Males displayed higher haematocrit and haemoglobin levels compared with females (main effect = gender). In addition, for haemoglobin there was a significant interaction between age and gender so that values were also greater for older women compared with young women but not for older and young men. There was no significant age or gender effect for either HDL or fasting plasma glucose. Individual haematological data can be seen in Appendix IX.

Table 2.2. Haematological results for young and older men and women (mean ± SD).

* Significantly different ($P<0.05$) compared with women of the same age group. † Significantly different ($P<0.05$) compared with young within the same gender.

	Young men n=15	Older men n=13	Young women n=8	Older women n=10
Haemoglobin ($g.dL^{-1}$)	15.4±0.9*	14.7±1.0*	13.5±0.8	13.7±1.0†
Haematocrit (%)	45.6±2.3*	44.5±2.4*	39.7±1.9	40.2±2.8
Plasma Glucose ($mmol.L^{-1}$)	4.7±0.8	4.6±1.1	4.9±0.5	4.8±0.4
Total Cholesterol ($mmol.L^{-1}$)	3.7±0.6	4.7±0.8†	3.9±0.9	4.9±0.5†
High Density Lipoprotein ($mmol.L^{-1}$)	1.1±0.3	1.0±0.3	1.2±0.3	1.1±0.2
Triglycerides ($mmol.L^{-1}$)	1.3±0.1	2.1±0.7*†	1.3±0.0	1.5±0.4†
Low Density Lipoproteins ($mmol.L^{-1}$)	2.3±0.7	3.2±0.7†	2.4±0.6	3.5±0.6†
Total Cholesterol/High Density Cholesterol ($mmol.L^{-1}$)	3.6±1.5	4.8±1.5†	3.1±0.3	4.6±1.2†

2.3.2 Peak responses during the calf incremental test to failure

2.3.2.1 Number of increments and time to failure

There was no significant age or gender effect in the number of work rates taken to reach peak force (Table 2.3) such that the exercise protocols were of similar duration (Fig. 2.5). Individual data can be seen in Appendix X.

Table 2.3. Number of increments for young and older men and women (mean ± SD).

Group	Young men	Older men	Young women	Older women
No of increments	5.1±1.0	5.0±1.0	4.9±1.0	6.1±1.0

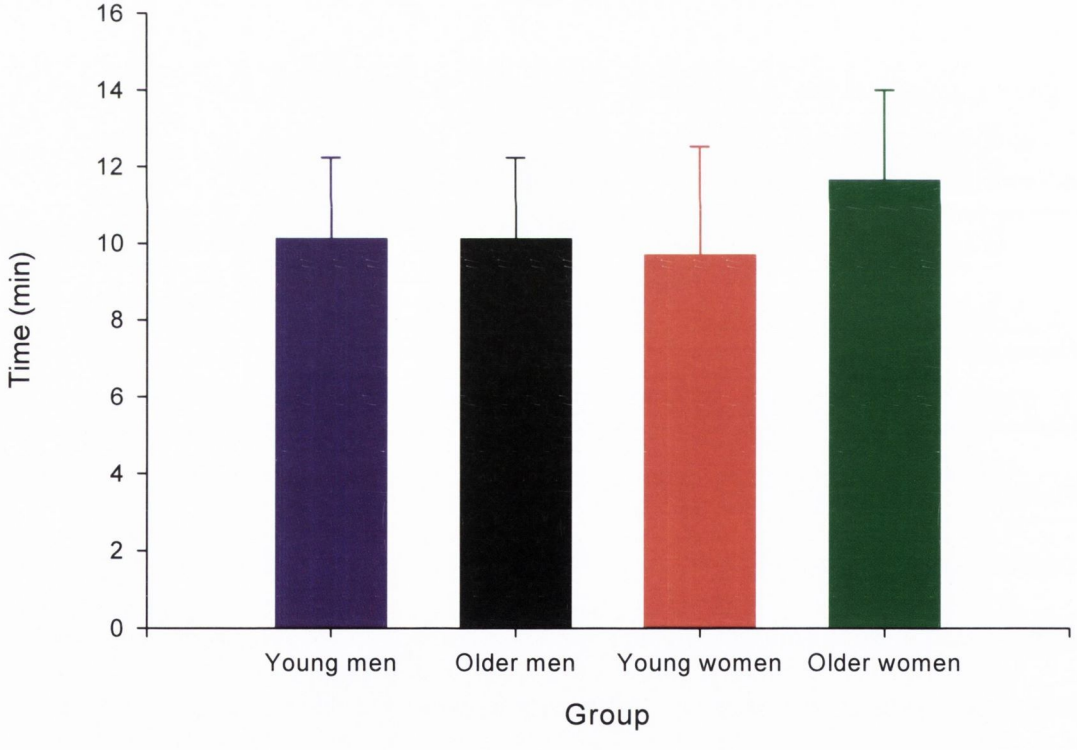


Figure 2.5. Time to failure (mean ± SD) during the calf incremental test for young and older men and women.

2.3.2.2 Peak force and MVC

Men displayed a higher peak force at the end of the incremental test compared with women (main effect = gender), while young individuals achieved a higher peak force than older subjects (main effect = age) (Fig. 2.6). No significant interaction between age and gender was observed. Men displayed a MVC compared with women (main effect = gender), while young individuals achieved a higher MVC than older subjects (main effect = age) (Fig. 2.7). Individual data can be seen in Appendix XVIII.

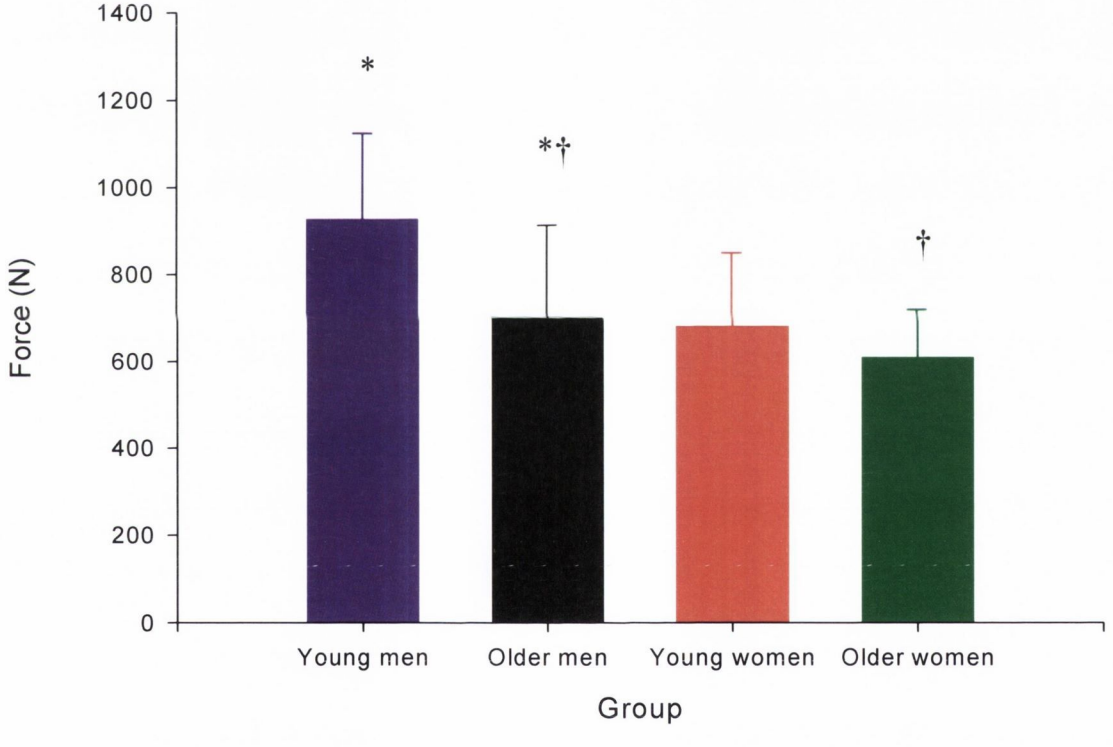


Figure 2.6. Peak force (mean ± SD) achieved during the calf incremental test for young and older men and women. * Significantly different ($P<0.05$) compared with women of the same age group. † Significantly different ($P<0.05$) compared with young within the same gender.

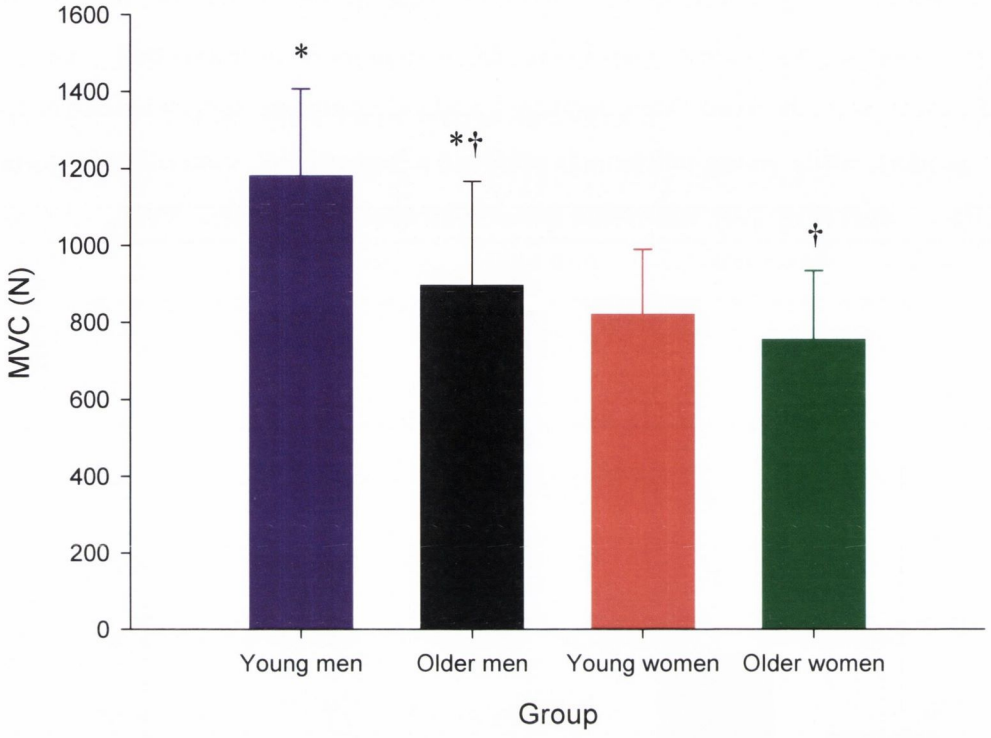


Figure 2.7. Maximum voluntary force (mean ± SD) achieved for young and older men and women. * Significantly different (P<0.05) compared with women of the same age group. † Significantly different (*P*<0.05) compared with young within the same gender.

2.3.2.3 Peak blood flow and peak vascular conductance

All four groups demonstrated similar peak hyperaemic and peak vascular conductance responses (Table 2.4). When estimates of leg volume were used to calculate blood flow and vascular conductance responses, peak blood flow and vascular conductance responses in men and women were not altered (Table 2.4). Individual data can be seen in Appendix XII.

Table 2.4. Peak blood flow and peak vascular conductance responses (mean ± SD) for young and older men and women.

	Young men	Older men	Young women	Older women
Peak Blood Flow ($ml.min^{-1}.100ml^{-1}$)	34.4±8.8	34.5±16.7	32.3±12.1	35.9±10.1
Peak Blood Flow ($ml.min^{-1}$)	97.1±32.8	97.8±49.7	81.6±32.9	93.7±24.7
Peak Vascular Conductance ($ml.min^{-1}.100ml^{-1}.mmHg^{-1}.10$)	2.1±0.5	1.9±1.0	2.0±0.7	2.1±0.6
Peak Vascular Conductance ($ml.min^{-1}.mmHg^{-1}.10$)	58.4±18.9	54.3±27.7	50.9±18.8	53.9±15.4

2.3.3. Cardiovascular responses to graded calf exercise

2.3.3.1 Leg blood flow and leg vascular conductance responses to graded calf exercise

To examine age by gender interactions in response to graded exercise, data are presented as each subject's workload normalised to his or her peak force (percentage of maximal workload) to account for differences in workload increases used for the four groups. Leg blood flow and leg vascular conductance responses (mean ± SD) expressed at percentage of maximal force (% max) in young and older men and women are illustrated in Fig. 2.8a and Fig. 2.8b. There were no age or gender differences in the hyperaemic responses (when comparing the slope of calf blood flow versus force (% max)) or in the vasodilatory responses (when comparing the slope of leg vascular conductance versus force (% max)) between the four groups

(Table 2.5). Blood flow and vascular conductance responses (mean ± SD) calculated using estimates of leg volume expressed at percentage of maximal force (% max) in young and older men and women are illustrated in Fig. 2.8c and Fig. 2.8d. There were no age or gender differences in the hyperaemic (when comparing the slope of calf blood flow versus force (% max)) or the vasodilatory responses (when comparing the slope of leg vascular conductance versus force (% max)) between the four groups (Table 2.5). Individual data can be seen in Appendix XIII.

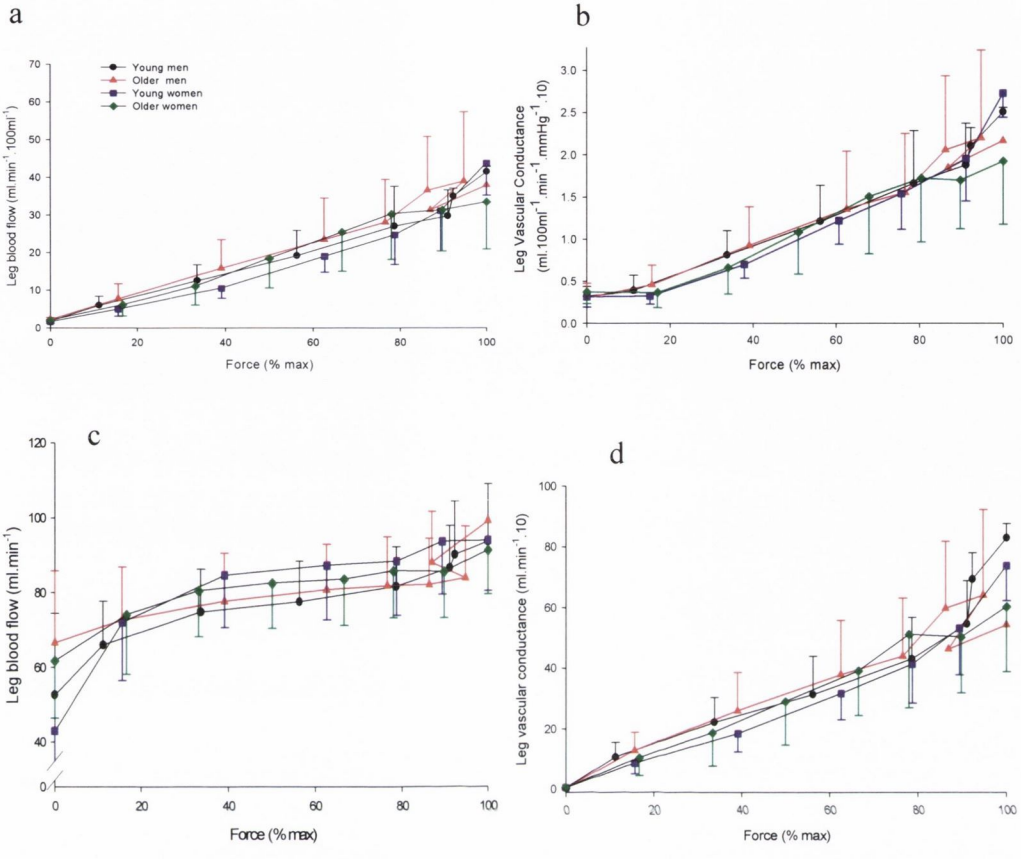


Figure 2.8. Leg blood flow (a) and vascular conductance (b) responses expressed as group means (± SD) at percentage of maximal force (% max) in young and older men and women. Leg blood flow (c) and (d) leg vascular conductance responses (mean ± SD) normalised to estimated leg muscle mass and expressed as group means ± SD at percentage of maximal force (% max) in young and older men and women. Graphically, portraying group averages of relative workloads (% max), yielded the following sample size: for young men, sample size was n=15 until 78.6%, n=11 at 91%, n=4 at 92.3% and n=2 at 100% force, (%max); for older men, sample size was n=13 until 62.5%, n=11 at 76.6%, n=8 at 86.3%, n=5 at 94.8%, n=1 at 87%, n=1 at 100% force, (% max); for younger women, sample size was n=8 until 60.7%, n=7 at 75.7%, n=6 at 91.2%, n=3 at 100% force (% max); for older women, sample size was n=10 until 67.9%, n=8 at 80.4%, n=7 at 89.9%, n=5 at 100% force (% max). Please note that these dropouts are an artefact of graphical representation only; statistical comparisons were achieved by fitting curves to each individual's haemodynamic responses versus the range of percent maximal workload attributable to each workload increase.

Table 2.5. The mean (± SD) slope between the leg blood flow and vascular conductance versus the percentage of maximal workload (% max) in young and older men and women.

* Significantly different ($P<0.05$) compared with women of the same age group. † Significantly different ($P<0.05$) compared with young within the same gender.

	Young men n=15	Older men n=13	Young women n=8	Older women n=10
Leg Blood Flow ($ml.min^{-1}.100ml^{-1}$) relative to % max	0.32±0.12	0.31±0.17	0.32±0.12	0.38±0.14
Leg Blood Flow ($ml.min^{-1}$) relative to % max	9.0±3.6	8.7±4.8	8.5±3.7	9.6±2.2
Vascular Conductance ($ml.min^{-1}.100ml^{-1}.mmHg^{-1}.10$) relative to % max	0.020±0.007	0.017±0.010	0.020±0.007	0.021±0.009
Vascular Conductance ($ml.min^{-1}.mmHg^{-1}.10$) relative to % max	0.52±0.2	0.48±0.3	0.52±0.2	0.60±0.2

2.3.3.2 Heart Rate

All four groups demonstrated similar heart rate responses at rest, at the beginning of exercise and at the end of the exercise protocol (Table 2.6). Heart rate responses (mean ± SD) expressed at percentage of maximal force (% max) in young and older are shown in Figure 2.9. When comparing the slopes of the heart rate responses relative to percent max force (% max), greater heart rate responses were displayed by young individuals compared with older individuals (main effect = age) (Table 2.7). Individual HR responses can be seen in Appendix XIV.

Table 2.6. Heart rate responses for young and older men and women.

	Heart rate ($beats.min^{-1}$)			
	Young men n=15	Older men n=13	Young women n=8	Older women n=10
Rest	66±12	73±14	72±16	74±16
Onset	78±12	76±13	82±16	77±10
End exercise	98±15	85±12	92±13	90±11

Table 2.7. The slope of heart rate responses expressed as group means (± SD) at percent of maximal workload (% max) in young and older men and women.

Heart rate (% max)			
Young men n=15	Older men n=13	Young women n=8	Older women n=10
0.18±0.11	0.09±0.04†	0.12±0.05	0.11±0.05†

2.3.3.3 Mean Arterial Pressure

All four groups demonstrated similar mean arterial pressure responses at rest, at the beginning of exercise and at the end of the exercise protocol (Table 2.8). MAP responses (mean ± SD) expressed as a percentage of maximal force (% max) in young and older men and women are shown in Figure 2.9. When comparing the slopes of the MAP response relative to the percentage of maximal workload (% max), there was no significant difference among the four groups (Table 2.9). Individual MAP responses can be seen in Appendix XV.

Table 2.8. Mean arterial pressure (mean ± SD) for young and older men and women.

	MAP (mmHg)			
	Young men	Older men	Young women	Older women
Rest	82±3	98±13	79±11	85±13
100N	86±8	105±14	87±17	106±18
End exercise	95±11	112±11	93±15	114±14

Table 2.9. The slope of the MAP response expressed as group means (± SD) at percent of maximal workload (% max) in young and older men and women.

* Significantly different ($P<0.05$) compared with women of the same age group. † Significantly different ($P<0.05$) compared with young within the same gender.

MAP (% max)			
Young men	Older men	Young women	Older women
0.13±0.09	0.13±0.13	0.14±0.04	0.15±0.08

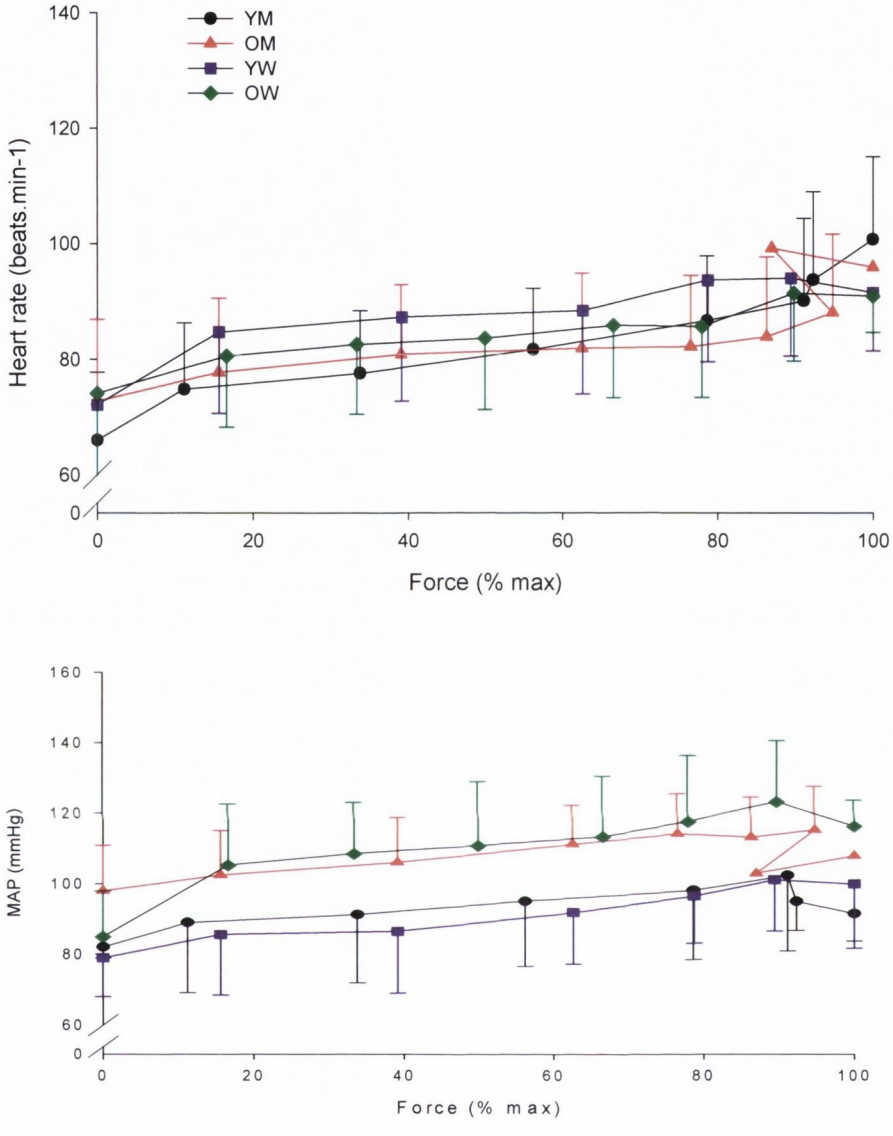


Figure 2.9. (A) Heart rate and (B) MAP expressed as group means (± SD) at percent of maximal workload (% max) in young and older men and women. Graphically, portraying group averages of relative workloads (% max), yielded the following sample size: for young men, sample size was n=15 until 78.6%, n=11 at 91%, n=4 at 92.3% and n=2 at 100% force (% max); for older men, sample size was n=13 until 62.5%, n=11 at 76.6%, n=8 at 86.3%, n=5 at 94.8%, n=1 at 87%, n=1 at 100% force (% max); for younger women, sample size was n=8 until 60.7%, n=7 at 75.7%, n=6 at 91.2%, n=3 at 100% force (% max); for older women, sample size was n=10 until 67.9%, n=8 at 80.4%, n=7 at 89.9%, n=5 at 100% force (% max). Please note that these dropouts are an artefact of graphical representation only; statistical comparisons were achieved by fitting curves to each individual's heart rate and MAP responses versus the range of percent maximal workload attributable to each workload increase.

2.4 Discussion

The main findings from the present study are as follows;

1. Peak hyperaemic and peak vascular conductance responses were similar between the four groups.
2. Similarly, the slopes of both the hyperaemic and vasodilatory responses expressed as a percentage of maximum workload were not different for young and older men and women.
3. Peak performance (peak force) was higher in men compared with women and in young compared with older individuals. When comparing peak forces as percentage of MVC, there were no differences between the four groups.

2.4.1 Leg blood flow and vascular conductance responses in men

Older men demonstrated similar peak hyperaemic and peak vascular conductance responses compared with young men expressed in absolute values and when using estimates of leg volume. In order to examine the effects of age and gender on the increase in muscle blood flow and vascular conductance during the isometric incremental plantar flexion exercise, the slopes of both the hyperaemic and vasodilatory responses expressed as percentage of maximal force (% max) were compared between the four groups. Similar to our results for peak responses, the present findings indicated no age differences in the hyperaemic or vasodilatory responses performed at submaximal workloads throughout the graded exercise test, when expressed in absolute values or when using estimates of leg volume.

The preservation of limb vascular conductance during exercise in older men is supported by previous research findings involving healthy young and older men during graded forearm dynamic handgrip exercise (Jasperse *et al.*, 1994), graded single knee extension exercise (Lawrenson *et al.*, 2003; Parker *et al.*, 2008) and single and two-legged dynamic knee extension exercise performed to failure (Magnusson *et al.*, 1994). In fact, some findings have indicated an augmented vascular conductance response in older men compared with young men during single knee extension exercise (Lawrenson *et al.*, 2003; Parker *et al.*, 2008). For instance,

Parker *et al.*, (2008) observed that the slope of both the hyperaemic and vasodilatory responses for the femoral artery plotted versus the % workload max were larger for the older compared with young men. In the same study, Parker and colleagues (2008) also observed that peak blood flow and vascular conductance responses were similar between older and young men.

A preservation of leg blood flow and vascular conductance was also evident in normally active older men compared with young men during both submaximal and incremental cycling exercise (Proctor *et al.*, 2003b). Leg blood flow and vascular conductance responses performed at the same absolute submaximal power output (20-100 W) and at a fixed systemic O_2 demand (1.1 $L.min^{-1}$) were similar between older and young men despite older men displaying lower CO responses (Proctor *et al.*, 2003b). Similarly, leg blood flow was preserved at lower power outputs (15 W and 30 W) during incremental cycling exercise in older compared with young men. In contrast, at higher power output of 99 W and at maximum workrates, leg vascular conductance was attenuated in the older group, and this was due to reduced leg blood flow and elevated MAP responses in the older men (Poole *et al.*, 2003). Moreover, older men also displayed attenuated leg blood flow and vascular conductance responses during incremental two legged cycling (Proctor *et al.*, 1998) and during knee extension constant-force exercise (Donato *et al.*, 2006).

Disparities between previous investigations indicating an attenuation in blood flow and the present findings, which found a preservation of leg blood flow, may be attributed to the fitness levels of the individuals being assessed. Recently, Parker *et al.*, (2008) reported that the most fit individuals demonstrated the greatest vascular conductance responses during exercise. Thus, it may be possible that subjects taking part in the present study were more physically active than those taking part in previous research studies indicating an attenuated blood flow and vascular conductance response (Proctor *et al.*, 1998; Poole *et al.*, 2003; Donato *et al.*, 2006). For instance, the older group of adults in the present study displayed a 19% lower peak force compared with the young group, whereas the older subjects in the study of Donato *et al.*, (2006) displayed a 50% lower workrate during knee extension exercise

compared with the young group. Similarly older subjects in the study of (Poole *et al.*, 2003), displayed a 56% greater peak force compared with young subjects. This would suggest a more sedentary profile of older subjects and/or fitter young subjects in these studies (Poole *et al.*, 2003; Donato *et al.*, 2006) compared with those of the present study.

In addition to the fitness levels of participants, differences in experimental and/or exercise modes and thus muscles used in different studies may also influence age-associated differences in leg blood flow and vascular conductance. For instance, Saltin *et al.*, (1998) showed that duing knee extension exercise peak blood flow reached 6 $L.min^{-1}$ at exhaustion compared to the findings of the present study of approximately 1 $L.min^{-1}$ at maximal workload. Also, we are unaware of a published study that used the plantar flexor exercise model to compare blood flow responses between older and young individuals and this makes the interpretation of our results more challenging.

2.4.2 Leg blood flow and vascular conductance responses in women

Findings from the present study revealed similar exercising peak hyperaemic and vasodilatory responses in older compared with young women during plantar flexion graded exercise. In addition the slopes of both the hyperaemic and vasodilatory responses expressed as percentage of maximal force were also similar between both groups of women. Our present results are in direct contrast to a number of recent studies, which reported attenuated blood flow and vascular conductance responses in older women compared with young women during dynamic single knee extension (Parker *et al.*, 2008) and during constant load and peak cycling exercise (Proctor *et al.*, 2003a; Proctor *et al.*, 2004).

For instance, (Parker *et al.*, 2008) reported that older women displayed a significantly attenuated peak leg blood flow and peak leg vascular conductance relative to young women during single leg knee kicking exercise. In addition, they also reported that the slopes of both the hyperaemic and vasodilatory responses of the femoral artery plotted versus the % workload max were significantly smaller for the older compared with young women. In addition, during peak cycling exercise, peak leg blood flow

and estimated leg vascular conductance were reported to be 29% and 38% lower in older compared with young women and this correlated with a 32% decline in peak leg O_2 demand in the older group (older: 0.81 ± 0.06L.min^{-1}, young: 1.18 ± 0.10 L.min^{-1}) (Proctor *et al.*, 2004).

Similarly, older women displayed an attenuated peak leg vascular conductance response during incremental cycling which was also evident during moderate intensity (50–60W) constant-load cycling exercise performed by the same older and young individuals (Proctor *et al.*, 2003a). However, at lower exercise intensities (20–40 W), older women displayed similar vascular conductance responses as their young counterparts, indicating the influence of exercise intensity on hemodynamic responses.

Discrepancies between our findings and previous studies (as was the case amongst men) may be related to differences in the fitness levels among participants and experimental and/or exercise models used. It is also important to stress that no previous studies have employed the plantar flexion model to measure the age-related blood flow responses to exercise among women.

2.4.3 Conclusion

In the present study, older adults displayed a significantly lower peak force during the incremental exercise test to failure compared with young adults. This has also been shown for different modalities for both upper and lower body exercise, including cycling (Poole *et al.*, 2003; Proctor *et al.*, 2003a), knee extension and handgrip exercise (Donato *et al.*, 2006). The reduction in performance displayed by the older group of adults compared with the young group in this study is not attributable to an attenuated vasodilatory capacity since peak calf blood flow and vascular conductance values were not different between older and younger groups. Thus, it is possible that the reduction in calf muscle performance displayed by the older group of adults may be related to a slower adaptation in pulmonary O_2 uptake elicited by a slower leg vascular conductance kinetic response. This potentially important factor will be examined in the next chapter (see chapter 3).

Chapter 3: Effect of ageing on leg vascular conductance dynamic responses during constant-force plantar flexion exercise

3.1 Introduction

Ageing is associated with an impaired exercise performance characterised by a reduced peak pulmonary oxygen uptake ($\dot{V}O_{2peak}$) (Proctor *et al.*, 2003). In addition, the rate of increase of $\dot{V}O_2$ ($\dot{V}O_2$ kinetics) at the onset of steady-state exercise is slowed in older compared with young individuals during moderate and heavy intensity cycling (DeLorey *et al.*, 2005; Ferreira *et al.*, 2005) as well as during moderate knee extension exercise (DuManoir *et al.*, 2010). This age-associated slowed $\dot{V}O_2$ kinetic response is either due to an impairment in active skeletal muscle blood flow (O_2 delivery) (Murias *et al.*, 2010), and/or an impairment in the O_2 extraction capacity by the myocyte, factors that will ultimately limit the rate of adjustment of muscle O_2 utilization.

The fact that the slowed VO_2 kinetic responses among the older subjects were accompanied by a faster adaptation of muscle deoxygenation (HHb) during moderate and heavy intensity exercise (DuManoir *et al.*, 2010; DeLorey *et al.*, 2005; Ferreira *et al.*, 2005), signifies a greater mismatch between muscle oxygen delivery and muscle oxygen utilisation during the on-transient of exercise in older compared with young adults. Thus, an attenuated microvascular blood flow response would require a greater increase in O_2 extraction for a given increase in muscle O_2 utilization. In fact, DuManoir *et al.*, (2010) reported that the slowed VO_2 kinetics in older compared with young subjects during knee extension exercise were accompanied by slower blood flow kinetics measured at the same absolute workloads.

To our knowledge, there is only one study in the literature (DuManoir *et al.*, 2010) reporting leg blood flow kinetic responses in ageing individuals and this study employed a knee extension exercise model at moderate intensities. Thus, to further explore this, the aim of our second study was to examine the age-associated leg vascular conductance (LVC) kinetic responses during constant-load plantar flexion exercise performed at a range of low, moderate and high submaximal relative

exercise intensities (30%, 45%, 60% and 70% MVC). The main hypothesis was that vascular conductance kinetics would be slower in older adults compared with young adults again during plantar flexion exercise performed at constant-force exercise intensities of 30, 45, 60 and 70% MVC.

3.2 Materials and methods

3.2.1 Subjects

Twelve young men, 12 young women, 10 older men and 10 older women were recruited for the study. All subjects were non-obese (body mass index <30 $kg.m^{-2}$), were non-smokers and were classified as normally active.

3.2.1.1 Subject recruitment

see chapter 2 section 2.2.1.1

3.2.1.2 Subject inclusion and exclusion criteria

see chapter 2 section 2.2.1.2

3.2.2 Medical assessment

see chapter 2 section 2.2.2

3.2.2.1 Blood analysis

see chapter 2 section 2.2.2.1

3.2.3 Experimental design

3.2.3.1 Experimental protocol overview

Subjects were required to attend the cardiovascular laboratory in the Department of Physiology on three occasions separated by at least 48 hours. Prior to each visit, subjects were required to refrain from alcohol, caffeine and exercise for 24 hours preceding the exercise test. During the initial visit, subjects were familiarised with the single-leg (right) constant force calf plantar flexion exercise and the protocol to be completed on the second and third day of testing.

On visit 2, subjects performed three bouts of intermittent (6 s duty cycle, 2s contraction, 4 s relaxation) constant force isometric plantar flexion exercise at two randomly selected intensities (six bouts in total) of 30%, 45%, 60% or 70% MVC in the upright position (tilt angle of 67°). Prior to each constant force bout, resting blood flow and blood pressure were simultaneously measured (chapter 1, section 2.2.4.5). In addition, arm and leg reactive hyperaemic responses were measured following a 5

min occlusion period.

On visit 3 subjects performed another three bouts of plantar flexion exercise at remaining two randomly selected intensities of 30%, 45%, 60% or 70% MVC (6 bouts in total). So, in total over the two day period (day 2 and 3) subjects completed 3 bouts of plantar flexion exercise at each of the four intensities.

All exercise bouts were limited to 6 min with a rest period of 10 min between each bout. The technique of venous occlusion plethysmography was used to assess resting and exercising blood flow (see chapter 2 section 2.2.4).

3.2.3.2 Visit 1; Subject familiarisation and maximum voluntary contraction (MVC)

See chapter 2, section 2.2.3.2

3.2.3.3 Visit 2; Leg vascular conductance kinetics and arm and leg reactive hyperaemia

a. Leg vascular conductance kinetics (I)

Resting right-leg vascular conductance (LVC) was measured in the supine position by simultaneously measuring resting leg blood flow and mean arterial pressure (MAP). Resting leg blood flow was determined using the technique of venous occlusion plethysmography. Three resting blood flow measurements were obtained over a 15 min period prior to commencement of each bout of the four constant force exercise tests. Resting leg vascular conductance was calculated as leg blood flow divided by mean arterial pressure.

Following 3 resting leg blood flow measurements, each subject performed a total of three 6 min bouts of intermittent (6 s duty cycle; 2 s contraction and 4 s relaxation) isometric plantar flexion exercise (tilt angle 67°) at two randomly selected intensities of either 30%, 45%, 60% or 70% MVC (six bouts in total), during which time leg blood flow and MAP were simultaneously measured during the 4 s relaxation phase of the 6 s duty cycle using the technique of venous occlusion plethysmography (chapter 2 section 2.2.4.2).

At the end of the initial 6 min exercise bout, the subject was lowered to the horizontal position and rested for 10 min where another two resting blood flow measurements were taken. Then two more 6 min exercise bouts were performed (10 min rest between bouts). Following a 15 min rest (supine position), subjects performed another three 6 min bouts at a different intensity with 10 min of rest between each bout.

b. Arm reactive hyperaemia

The subject rested on the tilt-table in the horizontal position with both arms fully extended at heart level. For a subgroup of subjects (chapter 1, section 2.2.4.7), a pneumatic cuff was placed around the left upper arm and the tonometric blood pressure sensor of the applanation tonometry apparatus (COLIN CBM700) was secured to the subject's forearm (wrist). For the remaining subjects, a cuff was wrapped around the finger of the left arm (Finometer), (chapter 1, section 2.2.4.7). From this beat-to-beat blood pressure and heart rate were recorded throughout the procedure. For all subjects, a second pneumatic cuff was placed around the right upper arm, which was also fully extended at the position of heart level. Blood flow was assessed using the method of venous occlusion plethysmography. A mercury in Silastic strain gauge which measured 2 cm less than the diameter of the widest girth of the right forearm was positioned around the circumference of the arm and secured using adhesive tape (Figure 3.1). While the subject rested in the horizontal position, resting blood flow measurements were taken by rapidly (<1 s) inflating the cuff of the right arm to 50 mmHg for 5 s and then deflating the cuff to 0 mmHg for 55 s. This was repeated four more times. Approximately 5 s following the last deflation, the cuff was inflated to between 200 - 220 mmHg for 5 min, which resulted in total ischaemia, restricting both venous and arterial blood flow. Following 5 min of total blood occlusion, the cuff was then released for 5 s and subsequently it was inflated to 50 mmHg for 5 s and then deflated to 0 mmHg for 5 s for a total of 5 min. Following this, blood flow was monitored only once every minute (by inflating the cuff to 50 mmHg for 5 s and then releasing the cuff for 55 s) for a further 5 min. Blood flow was determined by calculating the change in volume over time during the second cardiac cycle.

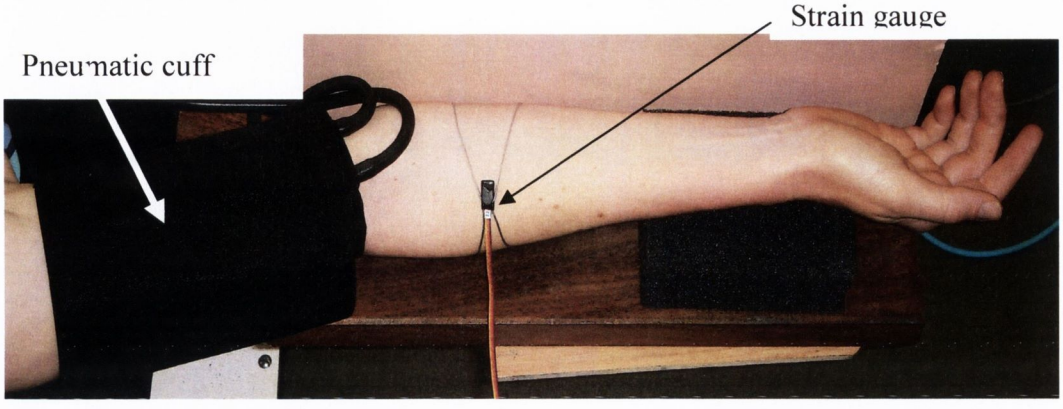


Figure 3.1 *Forearm reactive hyperaemia. A mercury in-Silastic strain gauge was placed at the widest forearm girth and a pneumatic cuff was placed on the right upper arm.*

c. Leg reactive hyperaemia

The procedure performed for the leg reactive hyperaemia test was similar to that of the arm reactive hyperaemia test, except the pneumatic cuff was placed around the right upper thigh. Similarly, a mercury in silastic strain gauge (measuring 2 cm less the diameter of the widest girth of the calf) was positioned around the widest girth of the right calf. Beat-to-beat blood pressure was continuously monitored from the right arm using the technique of applanation tonometry or the volume clamp method. Leg blood flow measurements (resting and following ischaemia) were determined using the same protocol as for the arm reactive hyperaemia test.

3.2.3.4 Visit 3; Leg vascular conductance kinetics (II)

Leg blood flow responses for the remaining two intensities were measured following the same procedure used during visit 2 (see section 3.2.3.4)

For all exercise bouts performed at all 4 intensities, LVC in the upright position was calculated as leg blood flow divided by MAP added to the estimated hydrostatic component acting at the midpoint of the calf (see chapter 2, section 2.2.4.9). The mean data from each of the three bouts for each intensity was used to calculate the vascular conductance kinetic responses for each individual (see below).

3.2.4 Equipment and measurements

3.2.4.1 Calf ergometer and exercise model see chapter 2, section 2.4.1

3.2.4.2 Blood flow measurements; see chapter 2, section 2.2.4.2

3.2.4.3 Body mass, height and BMI; see chapter 2, section 2.2.4.3

3.2.4.4 Leg volume measurements; see chapter 2, section 2.2.4.4

3.2.4.5 Blood analyses; see chapter 2, section 2.2.4.5

3.2.4.6 Low-level physical activity recall (LOPAR); see chapter 2, section 2.2.4.6

3.2.4.7 Blood pressure; see chapter 2, section 2.2.4.7

3.2.4.8 Heart rate; see chapter 2, section 2.2.4.8

3.2.4.9 Vascular conductance; see chapter 2, section 2.2.4.9

3.2.5 Data analysis

3.2.5.1 Leg Vascular Conductance Kinetics

An average of the VC kinetic response for each exercise intensity (30%, 45%, 60% and 70% MVC) was determined. Most of the individuals exhibited a biphasic pattern and thus a bi-exponential equation was fitted to the subject's mean vascular conductance response for each of the four intensities. The equation fitted was as

follows:

$LVC\ (t) = a + A_1 (1 - e^{-(t\text{-}TD1/\tau1)})U1 + A_2(1 - e^{-(t\text{-}TD2/\tau2)})U2$

Where *a* represents LVC at t=0. A_1 - A_2, TD_1 - TD_2 and τ_1, τ_2 represent the amplitude, time delay and time constant of the first and second phase. The parameters U1 and U2 are conditional expressions that limit the fitting of a particular phase to the period at and beyond the time delay associated with that phase. Data, which exceeded the 95% prediction intervals during an initial fit of a model, were omitted. No more than five data points were removed from the original time- series of the data. The models were fitted to the data using a weighted least squares non-linear regression procedure (LevMarqdt, TableCurve 2D, Jandel Scientific) (see Introduction, Fig 1.1).

The mean response time (MRT) which is the time required to achieve ~63% of the overall amplitude of the response from the baseline value (MacDonald *et al.*, 1998) was calculated as a weighted sum of the time delay and time constant of each phase,

$MRT = [A1/(A1 + A2)] (TD1 + \tau_1) + [A2/(A1 + A2)] (TD2 + \tau_2)$.

3.2.5.2 Curve fitting forearm and leg reactive hyperaemic response

Vascular conductance hyperaemic responses were curve fit using a mono-exponential function: $Y - A + b.e^{-cx}$ (Sigma Tablecurve 2D v5.01. U.S.A), where a is the baseline vascular conductance at t=0, A is the amplitude of the response and c is the decay constant. Data that exceeded the 95% prediction intervals during an initial fit of a model were excluded. No more than two data points were removed from the original time series of data. Peak vascular conductance responses recorded at the start of the hyperaemic responses were compared with the sum of baseline (a) and amplitude (Amp) values of the curve-fit.

3.2.6 Statistical Analysis

All anthropometrical and haematological data were compared between the young and older men and women using a two-way ANOVA (gender x age). A two-way ANOVA was also used to compare all vascular conductance kinetic parameters during the constant force exercise tests between the four groups. Differences were located using a Tukey's post hoc test. A three-way repeated measures ANOVA (gender x age x time) was used to compare MAP and HR responses at different time

points during the tests. Differences were located using a Bonferroni *post hoc* test. The level of significance was set at $P<0.05$. All results are given as mean ± standard deviation (SD). The statistical software Sigma Stat, (USA) was used for all 2 way, ANOVA statistical analyses, while for three-way, repeated measures ANOVA, data desk® 6.1 was used.

3.3 Results

3.3.1 Subjects

3.3.1.1 Physical characteristics and activity levels

Physical characteristics and activity levels for the four groups can be seen in Table 3.1. Men were taller than women (main effect = gender) and young subjects were taller than older subjects (main effect = age) with no interaction between gender and age. Men had a greater body mass than females (main effect = gender), while older subjects had a greater body mass and BMI than young subjects (main effect = age). No differences in either leg volume or activity levels were detected between the groups. Individual anthropometrical data can be seen in Appendix XVI.

Table 3.1. Physical characteristics and activity levels for young and older men and women (mean ± SD).

	Young men n=12	Older men n=10	Young women n=12	Older women n=10
Age (yr)	23±2	70±8†	22±1	64±7†
Height (cm)	179±7*	169±7*†	166±4	158±7†
Body mass (kg)	72.1±13.1*	75.0±8.2*†	58.8±4.0	68.6±9.0†
BMI ($kg.m^{-2}$)	22.6±3.7	26.2±2.3†	21.4±1.4	27.7±4.1†
Leg volume (ml)	2809±695	2779±434	2515±424	2760±623
Activity level ($MET\ h.week^{-1}$)	198±25	198±27	220±38	223±59

* Significantly different ($P<0.05$) compared with women of the same age group. † Significantly different ($P<0.05$) compared with young within the same gender.

3.3.1.2 Haematology

Haematological values for the four groups can be seen in Table 3.2. Older individuals displayed higher total cholesterol, high density cholesterol, low density lipoprotein, triglycerides and total cholesterol/HDL compared with young subjects (main effect = age). Males displayed higher haematocrit and haemoglobin levels compared with females (main effect = gender) with no interaction between age and gender. There was no significant age or gender effect for fasting plasma glucose. Individual haematological data can be seen in Appendix XVII.

Table 3.2. Haematological values for young and older men and women (mean ± SD).

* Significantly different ($P<0.05$) compared with women of the same age group. † Significantly different ($P<0.05$) compared with young within the same gender.

Group	Young men n=12	Older men n=10	Young women n=12	Older women n=10
Haemoglobin ($g.dL^{-1}$)	15.5±0.8*	15.0±0.8*	13.7±0.7	13.7±1.0
Haematocrit (%)	45.6±2.3*	45.4±1.8*	39.6±1.5	40.2±2.8
Plasma Glucose ($mmol.L^{-1}$)	4.8±0.7	4.6±1.1	5.0±0.4	4.8±0.4
Total Cholesterol ($mmol.L^{-1}$)	3.7±0.6	4.6±0.8†	4.1±0.9	4.9±0.5†
High Density Lipoprotein ($mmol.L^{-1}$)	1.1±0.3	1.3±0.3†	1.3±0.3	1.1±0.2†
Triglycerides ($mmol.L^{-1}$)	1.3±0.1	2.0±0.7†	1.3±0.0	1.5±0.4†
Low Density Lipoproteins ($mmol.L^{-1}$)	2.3±0.8	3.1±0.7†	2.5±0.6	3.5±0.6†
Total Cholesterol/HighDensity Cholesterol ($mmol.L^{-1}$)	3.6±1.6	4.9±1.6†	3.2±0.4	4.6±1.2†

3.3.2 Maximum voluntary contraction (MVC)

Men displayed a higher maximum voluntary force compared with women (main effect = gender), while young individuals achieved a higher maximum voluntary force than older subjects (main effect = age). No significant interaction between age and gender was observed (Fig. 3.2). Individual values data can be seen in Appendix XVIII.

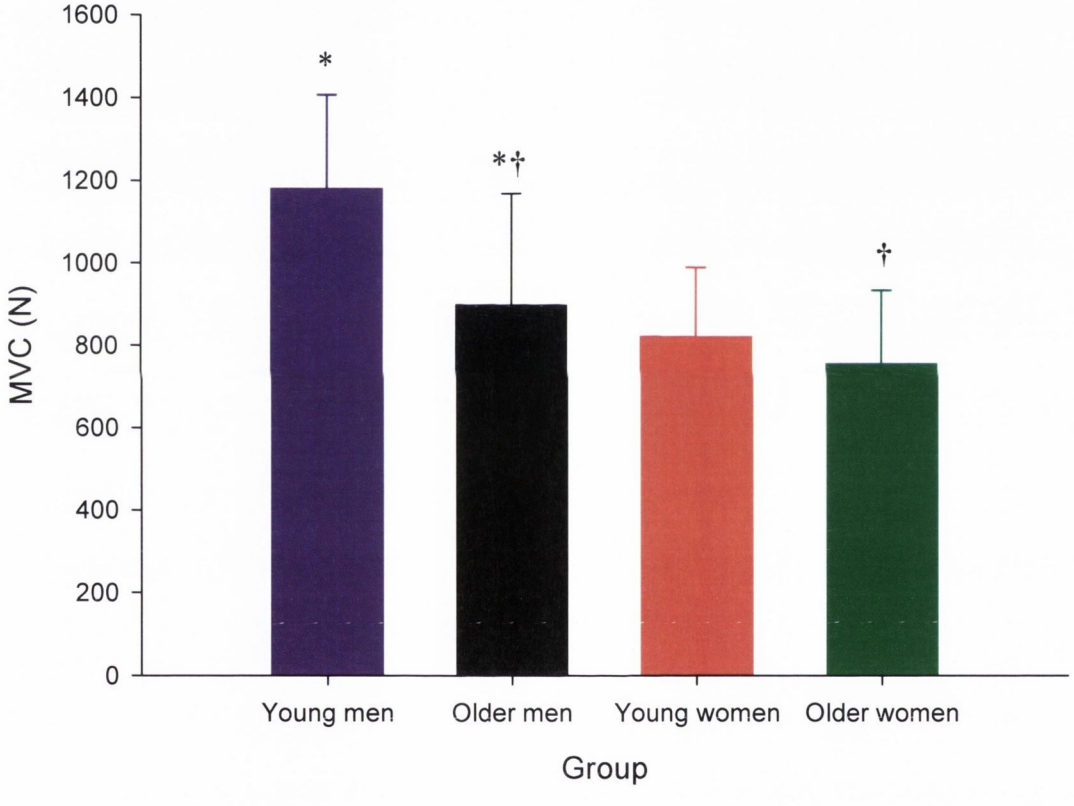


Figure 3.2. Maximum voluntary force (mean ± SD) achieved for young and older men and women.
* Significantly different (P<0.05) compared with women of the same age group. † Significantly different (*P*<0.05) compared with young within the same gender.

3.3.3 Resting calf blood flow and vascular conductance

Resting mean blood flow and vascular conductance values for the four groups can be seen in Table 3.3. Older individuals displayed higher resting blood flow responses ($ml.100ml^{-1}.min^{-1}$) compared with young subjects (main effect = age), while men displayed higher resting blood flow responses compared with women (main effect = gender). There was no significant age or gender effect for resting vascular conductance ($ml.100ml^{-1}.min^{-1}.mmHg^{-1}$).

Similarly, when estimates of leg volume were used to calculate blood flow ($ml.min^{-1}$) and vascular conductance ($ml.min^{-1}.mmHg^{-1}$), older individuals had a greater resting blood flow response compared with young individuals (main effect = age) with no difference in vascular conductance between groups (Table 3.3). Individual data can be seen in Appendix XIX.

Table 3.3. Resting mean blood flow and vascular conductance for young and older men and women (mean ± SD). * Significantly different ($P<0.05$) compared with women of the same age group. † Significantly different ($P<0.05$) compared with young within the same gender.

	Young men n=12	Older men n=10	Young women n=12	Older women n=10
Blood flow ($ml.100\ ml^{-1}.min^{-1}$)	1.9±0.5*	2.4±0.5*†	1.7±0.4	2.1±0.3†
Blood flow ($ml.min^{-1}$)	52.8±22.0	67.8±20.7†	43.0±11.0	60.0±15.1†
Vascular conductance ($ml.100ml^{-1}.min^{-1}.mmHg^{-1}$)	0.022±0.005	0.025±0.007	0.024±0.006	0.025±0.007
Vascular conductance ($ml.min^{-1}.mmHg^{-1}$)	0.60±0.21	0.72±0.26	0.60±0.15	0.75±0.26

3.3.4 Vascular conductance kinetics during constant load plantar flexion exercise at 30%, 45%, 60% and 70% MVC

The mean leg vascular conductance kinetic parameter estimates, as well as mean response time (MRT) and end-exercise values for young and older men and women during constant load plantar flexion performed at intensities of 30%, 45%, 60% and 70% MVC can be seen in Table 3.4. Throughout the three exercise bouts of each of

the four exercise intensities no systematic change in VC responses were observed. An example of the leg vascular conductance data for one young man during the three bouts of plantar flexion exercise at 30%, 45%, 60% and 70% MVC can be seen in Fig. 3.3. Representative individual vascular conductance responses, as well as the responses predicted from the fitting of the biphasic function can be seen in Figure 3.4 for 30% and 45% MVC and Fig. 3.5 for 60% and 70% MVC.

The mean response time (MRT) and the time constant for the second phase (τ_2) were faster in young individuals compared with older individuals during all exercise intensities (Table 3.4). Also, at all intensities, men displayed a greater end-exercise amplitude (A_{360}) compared with women, and older individuals displayed greater resting vascular conductance (a) responses compared with younger subjects. Younger individuals displayed a greater amplitude for the primary phase (A_1) compared with older subjects at 45% MVC only. The amplitude of the primary phase (A_1) was greater in men compared with women only at 70% MVC. In addition, there was an interaction between age and gender so that older men displayed a greater primary phase time delay (TD_1) and time constant (τ_1) compared with older women at 30% MVC only, but no differences were observed between young men and women. The amplitude of the secondary phase (A_2) was greater in men compared with women at exercise intensities of 45%, 60% and 70% MVC. No differences in the adjusted R^2 values were apparent between the 4 groups during all intensities, signifying similar biexponential equation fits (Table 3.4). Individual data can be seen in Appendix XX.

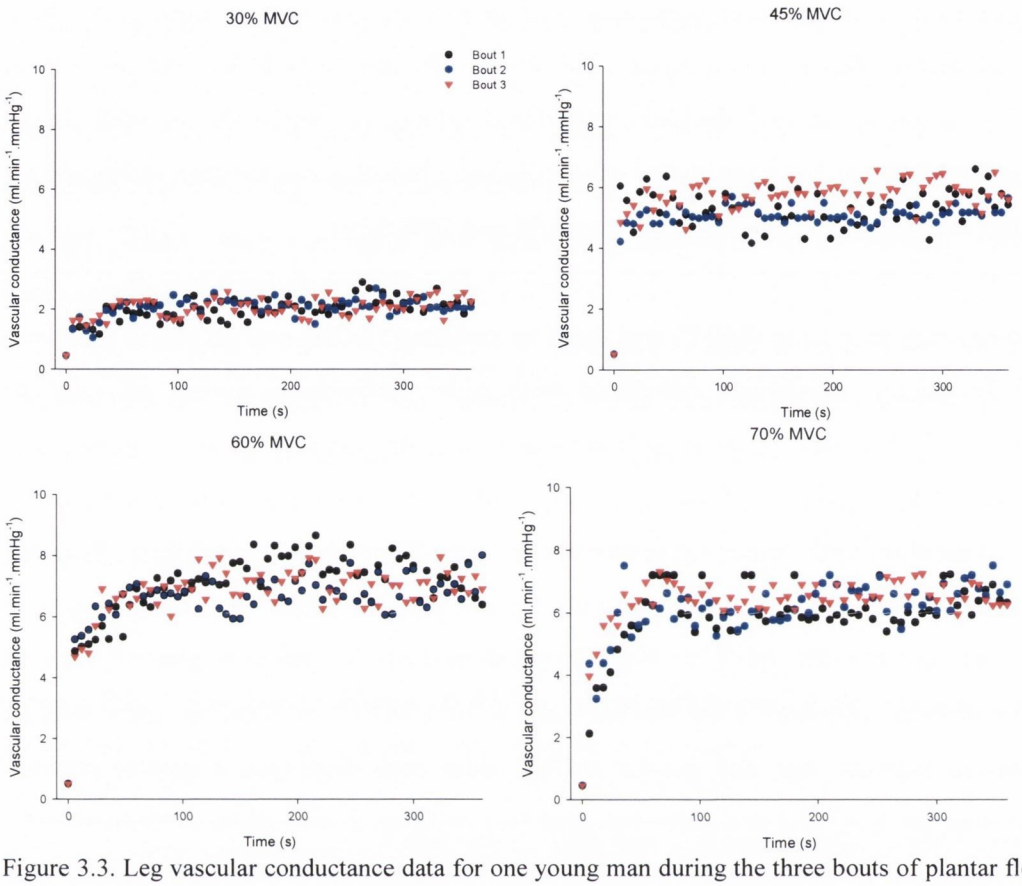


Figure 3.3. Leg vascular conductance data for one young man during the three bouts of plantar flexion exercise at 30%, 45%, 60% and 70% MVC.

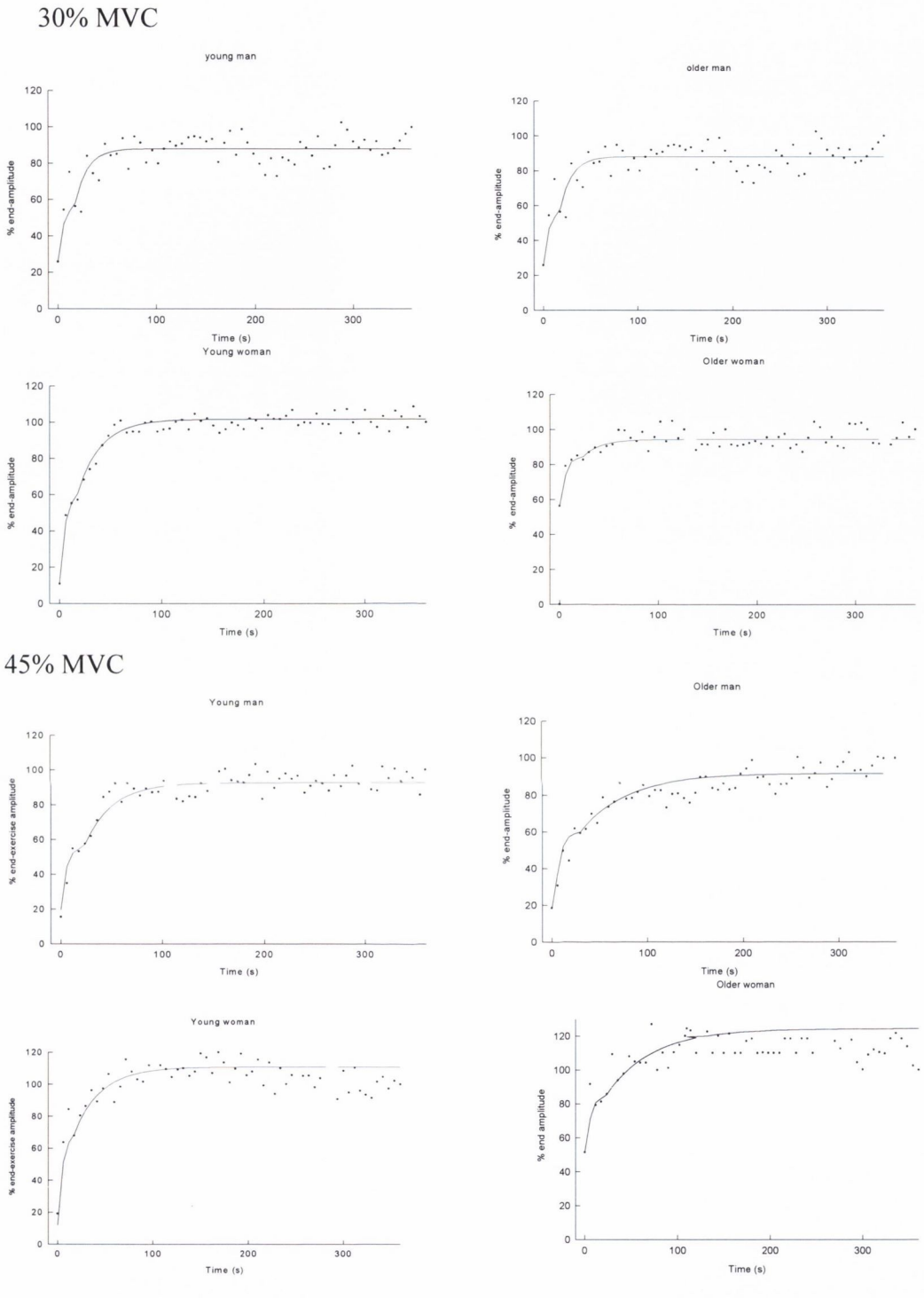
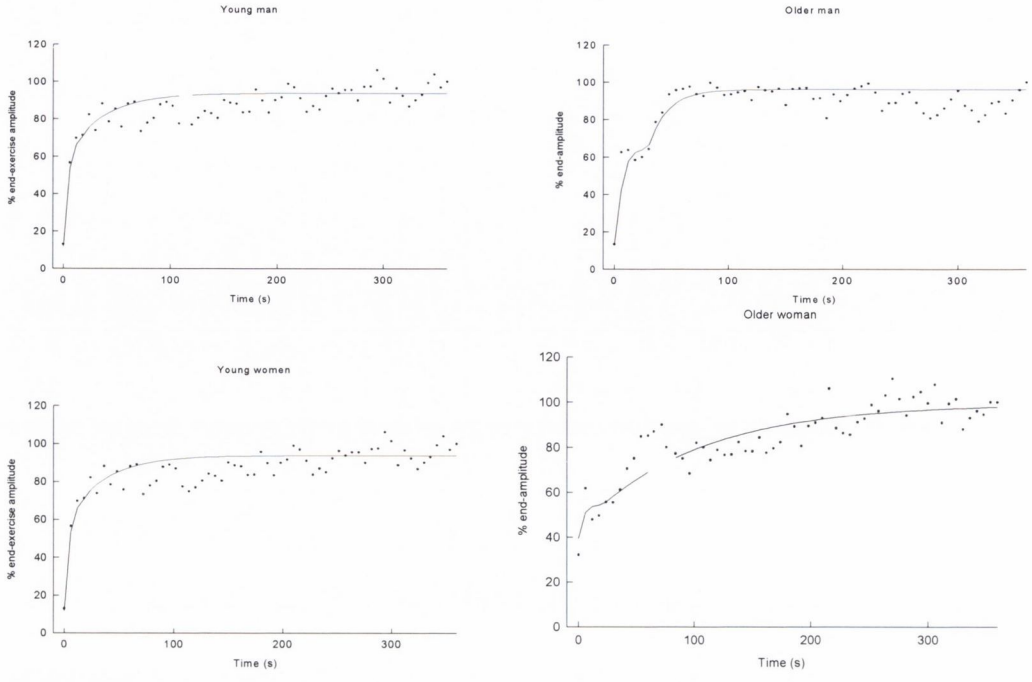


Figure 3.4. Leg vascular conductance responses (•) and biexponential curve fit (-) for a representative subject from each group performed at exercise intensities of 30% MVC (upper four panels) and 45% MVC (lower four panels). The y-axes have been converted to % end amplitude for better clarity.



70% MVC

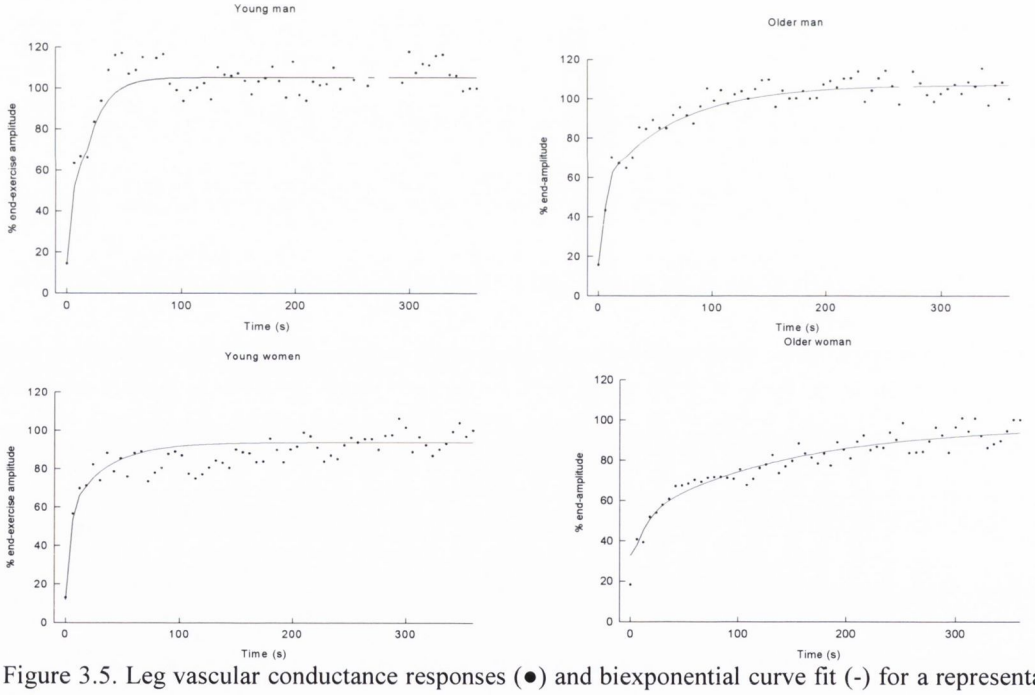


Figure 3.5. Leg vascular conductance responses (●) and biexponential curve fit (-) for a representative subject from each group performed at exercise intensities of 60% MVC (upper four panels) and 70% MVC (lower four panels). The y-axes have been converted to % end amplitude for better clarity.

Table 3.4. Mean LVC kinetic parameters performed at 30%, 45%, 60% and 70% MVC for older and young men and women.

* Significantly different ($P<0.05$) compared with women of the same age group. † Significantly different ($P<0.05$) compared with young within the same gender.

30% MVC	Young men n=12	Older men n=10	Young women n=12	Older women n=10
a ($L.min^{-1}mmHg^{-1}$)	0.60±0.20	0.75±0.20†	0.58±0.17	0.68±0.17†
A_1 ($L.min^{-1}mmHg^{-1}$)	1.39±0.71	1.11±0.72	0.91±0.72	0.71±0.50
TD_1 (s)	1.3±1.5	2.5±1.2*	2.2±2.1	0.6±1.1
τ_1 (s)	1.4±0.7	1.9±0.5*	1.5±0.9	1.0±0.6
A_2 ($L.min^{-1}mmHg^{-1}$)	0.70±0.25	1.09±0.89	0.75±0.36	0.53±0.40
TD_2 (s)	17.7±3.4	17.1±5.3	17.5±3.4	16.6±3.2
τ_2 (s)	13.9±9.8	40.6±33.5†	14.3±7.3	31.2±15.9†
A_{360} ($L.min^{-1}mmHg^{-1}$)	2.69±0.76*	2.93±1.36*	2.24±0.97	1.92±0.65
MRT (s)	13.4±5.7	33.5±28.5†	19.3±12.9	25.0±21.1†
Adj R^2	0.67±0.12	0.72±0.14	0.71±0.14	0.61±0.19
45% MVC				
a ($L.min^{-1}mmHg^{-1}$)	0.52±0.16	0.75±0.23†	0.62±0.17	0.68±0.22†
A_1 ($L.min^{-1}mmHg^{-1}$)	2.23±0.87	1.63±0.85†	1.73±1.21	1.07±0.69†
TD_1 (s)	1.7±2.0	2.2±1.4	1.7±1.9	1.8±1.4
τ_1 (s)	1.6±0.8	2.0±0.5	1.8±0.7	1.5±0.5
A_2 ($L.min^{-1}mmHg^{-1}$)	1.06±0.47*	1.44±0.64*	0.81±0.34	0.88±0.46
TD_2 (s)	16.5±3.1	14.9±2.0	16.3±3.2	17.6±5.4
τ_2 (s)	13.8±8.7	31.8±17.1†	17.0±10.2	39.9±15.7†
A_{360} ($L.min^{-1}mmHg^{-1}$)	3.81±0.95*	3.81±1.36*	3.16±1.36	2.62±0.86
MRT (s)	13.1±8.0	26.7±15.8†	16.8±13.4	30.3±18.1†
Adj R^2	0.72±0.13	0.68±0.13	0.76±0.08	0.75±0.13
60% MVC				
a ($L.min^{-1}mmHg^{-1}$)	0.56±0.21	0.73±0.17†	0.60±0.16	0.70±0.24†
A_1 ($L.min^{-1}mmHg^{-1}$)	2.55±0.84	2.52±1.01	2.52±1.42	1.79±0.90
TD_1 (s)	1.3±1.0	1.9±1.4	2.5±1.6	1.7±1.0
τ_1 (s)	1.9±0.9	1.7±0.5	1.6±0.9	1.4±0.5
A_2 ($L.min^{-1}mmHg^{-1}$)	1.68±0.64*	1.93±0.98*	1.25±0.48	1.11±0.62
TD_2 (s)	14.4±3.0	14.1±2.7	15.2±3.3	15.5±3.7
τ_2 (s)	21.1±10.6	37.4±10.7†	20.4±6.6	48.9±20.4†
A_{360} ($L.min^{-1}mmHg^{-1}$)	4.79±1.07*	5.18±1.57*	4.38±1.76	3.59±0.86
MRT (s)	16.2±5.9	24.5±9.5†	15.8±7.5	29.9±22.6†
Adj R^2	0.77±0.10	0.79±0.06	0.79± 0.12	0.70±0.19
70% MVC				
a ($L.min^{-1}mmHg^{-1}$)	0.56±0.25	0.76±0.16†	0.58±0.19	0.71±0.21†
A_1 ($L.min^{-1}mmHg^{-1}$)	2.91±0.73*	3.51±1.67*	2.84±1.42	2.10±0.73
TD_1 (s)	2.0±1.3	1.6±1.1	1.9±1.5	2.3±0.8
τ_1 (s)	1.7±0.7	1.7±0.6	1.5±0.6	1.5±0.3
A_2 ($L.min^{-1}mmHg^{-1}$)	2.04±0.71*	2.65±1.41*	1.40±0.74	1.62±0.86
TD_2 (s)	13.3±1. 8*	12.5±1.7*	14.0±2.6	14.7±3.0
τ_2 (s)	26.6±10.6	48.5±11.6†	22.8±3.5	59.2±23.5†
A_{360} ($L.min^{-1}mmHg^{-1}$)	5.51±0.80*	6.91±2.53*	4.82±1.94	4.41±0.83
MRT (s)	19.3±7.6	28.5±11.2†	14.7±4.1	36.1±20.5†
Adj R^2	0.74±0.08	0.79±0.09	0.79±0.09	0.78±0.07

3.3.5 Mean arterial pressure responses

Mean arterial pressure responses recorded at rest and at every minute during the calf plantar flexion exercise for each of the four exercise intensities for each group are displayed in Table 3.5. All four groups demonstrated similar mean arterial pressure responses at rest, minute 1 to minute 5 and at end exercise for 45% and 60% MVC. For 30% MVC, young men displayed a lower mean arterial pressure response for all time points compared with older men. Similarly, young women displayed lower mean arterial pressure response compared to young men at minute 1 to minute 5 and at end exercise. Young men displayed higher mean arterial pressure at end exercise only compared with young women, while older men displayed higher mean arterial pressure at rest only compared with older women. For 70% MVC, young men displayed lower mean arterial pressure responses compared with older men at rest, minute 4, minute 5 and at end exercise. Similarly, young women displayed lower mean arterial pressure responses compared with older women at minute 1 to minute 5 and at end exercise. Young men displayed higher mean arterial pressure responses compared with young women at minute 5 and end exercise only. Mean arterial pressure responses for each group performed at each intensity can be seen in Appendix XXI.

Table 3.5. Group averages for mean arterial pressure responses for young and older men and women (mean ± SD). * Significantly different ($P<0.05$) compared with women of the same age group. † Significantly different ($P<0.05$) compared with young within the same gender.

	MAP(mmHg)			
	Young men n=12	Older men n=10	Young women n=12	Older women n=10
30% MVC				
Rest	84±7	100±10*†	80±7	86±14
Min 1	86±11	98±9†	78±10	96±10†
Min 2	86±12	99±7†	80±13	96±11†
Min 3	87±12	102±7†	80±12	101±15†
Min 4	89±11	99±7†	81±11	99±15†
Min 5	89±10	102±12*†	80±12	100±15†
End exercise	89±12*	101±12†	79±12	99±14†
45% MVC				
Rest	88±8	100±11	79±9	85±12
Min 1	86±12	97±13	79±12	100±5
Min 2	86±14	102±10	79±10	103±5
Min 3	88±12	100±10	81±10	104±4
Min 4	89±11	100± 13	82±10	104±10
Min 5	88±12	100± 13	82±11	103±10
End exercise	90±10	101± 15	80±10	103±8
60% MVC				
Rest	88±6	94±6	82±8	87± 12
Min 1	91±11	99±22	82±13	99± 6
Min 2	88± 14	103±18	83±14	103±6
Min 3	90±14	104 ±13	84±13	105±7
Min 4	89±12	109±14	84±13	105±7
Min 5	91±10	108±14	83±14	104±8
End ex	91±13	111±17	83±15	106±8
70% MVC				
Rest	87±13	97±8†	79±5	85±14
Min 1	87±14	95±9	80±13	99±7†
Min 2	91±13	98±5	81± 13	101±9†
Min 3	92±13	102±7	83±13	103±10†
Min 4	92±13	104±6†	83±12	105±8†
Min 5	93±13*	105±7†	84±12	106±7†
End ex	94±13*	107±10†	85±16	105±9†

3.3.6 Heart rate responses

Heart rate responses recorded at rest, and at every minute during the calf plantar flexion exercise for each of the four exercise intensities for each group are displayed in Table 3.6. All four groups demonstrated similar heart rate responses at rest, minute 1 to minute 5 and at the end exercise protocol. Individual HR responses can be seen in Appendix XXII.

Table 3.6. Mean heart rate responses for young and older men and women (mean ± SD) performed at intensities of 30% and 45% MVC.

	HR (beats.min^{-1})			
Group	Young men n=12	Older men n=10	Young women n=12	Older women n=10
30% MVC				
Rest	70±12	70±11	74±14	75±11
Min 1	74±11	72±9	80±10	78±15
Min 2	74±10	74±10	81±10	81±15
Min 3	77±11	74±9	83±10	79±11
Min 4	76±11	74±8	84±9	80±13
Min 5	77±12	75±8	83±10	80±14
End exercise	78±12	73±8	83±11	79±11
45% MVC				
Rest	74±15	76±8	75±12	75±10
Min 1	78±13	76±8	82±10	76±8
Min 2	79±16	77±9	82±11	78±9
Min 3	79±14	78±9	83±11	78±11
Min 4	80±15	78±9	83±11	75±9
Min 5	81±15	78±9	84±11	76±11
End exercise	81±12	77±8	83±11	82±21
60% MVC				
Rest	74±14	74±13	78±15	80±13
Min 1	82±14	77±10	83±9	80±10
Min 2	82±13	80±12	84±11	79±11
Min 3	85±16	81±12	84±11	79±10
Min 4	86±15	81±12	83±12	80±10
Min 5	85±13	81±12	84±14	75±12
End exercise	86±14	81±12	85±14	79±11
70% MVC				
Rest	76±16	74±13	73±11	81±10
Min 1	87±16	77±13	83±9	81±10
Min 2	88±16	81±10	84±9	82±10
Min 3	90±18	82±10	85±10	83±11
Min 4	92±18	82±10	87±11	83±13
Min 5	91±19	83±10	85±12	86±11
End exercise	92±21	82±9	84±16	87±10

3.3.7 Arm vascular conductance responses (reactive hyperaemia)

The mean forearm reactive vascular conductance (VC) kinetic parameters for the four groups can be seen in Table 3.7. Representative individual reactive vascular conductance responses, as well as the responses predicted from the fitting of the monophasic function can be seen in Figure 3.6. Individual arm vascular conductance kinetic parameters can be seen in Appendix XXIII. No differences in baseline VC (a), amplitude (A) or decay constant were detected between the four groups. In addition, there were no differences between the experimentally measured peak VC and the predicted sum of the baseline and the amplitude (A + a).

Table 3.7. Mean forearm reactive vascular conductance kinetic parameters for young and older men and women (mean ± SD).

	Young men n=12	Older men n=11	Young women n=11	Older women n=9
a ($ml.100ml^{-1}.mmHg^{-1}.min^{-1}.10^{-1}$)	2.6±1.0	2.7±1.1	2.1±1.2	3.0±1.6
A ($ml.100ml^{-1}.mmHg^{-1}.min^{-1}.10^{-1}$)	22.7±8.0	18.0±6.9	18.2±2.7	18.0±5.9
Decay constant (s)	0.046±0.018	0.047±0.043	0.049±0.011	0.042±0.025
Peak VC ($ml.100ml^{-1}.mmHg^{-1}.min^{1}.10^{1}$)	23.4±8.7	20.7±8.6	18.0±2.0	20.4±8.3
A + a ($ml.100ml^{-1}.mmHg^{-1}.min^{-1}.10^{-1}$)	25.4±8.5	20.6±7.3	20.3±2.7	21.4±6.5

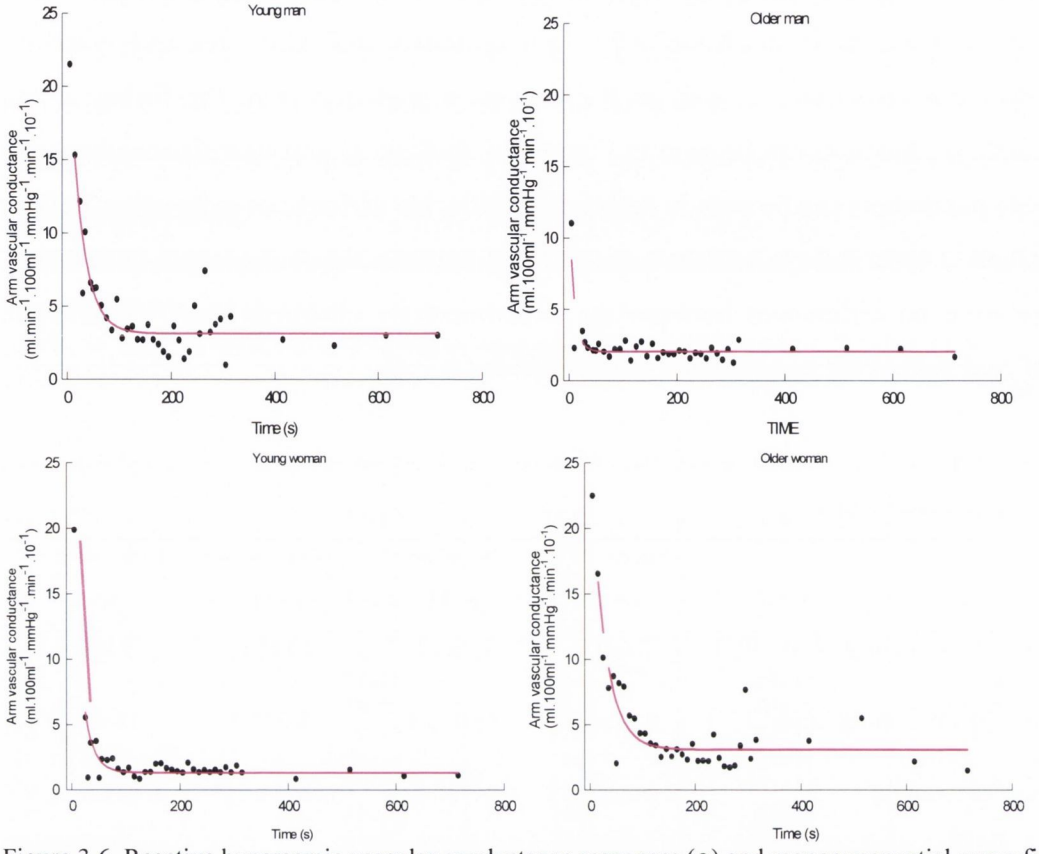


Figure 3.6. Reactive hyperaemic vascular conductance responses (●) and monoexponential curve fit (-) for a representative subject from each group

3.3.8 Leg vascular conductance response (reactive hyperaemia)

The mean leg reactive vascular conductance (VC) kinetic parameters for the four groups can be seen in Table 3.8. Representative individual reactive VC responses as well as the responses predicted from the fitting of the monoexponential function can be seen in Figure 3.7. Individual leg vascular conductance kinetic parameters can be seen in Appendix XXIV.

There were no differences for baseline VC (a), amplitude (A) or decay constant between the four groups. In addition, there were no differences between the experimentally measured peak VC and the sum of the predicted baseline and the amplitude (A + a).

Table 3.8. Mean leg reactive vascular conductance kinetic parameters for young and older men and women (mean ± SD).

	Young men n=8	Older men n=8	Young women n=8	Older women n=4
a ($ml.100ml^{-1}.mmHg^{-1}.min^{-1}.10^{-1}$)	2.1±1.0	1.7±0.6	1.6±0.7	2.6±1.4
A ($ml.100ml^{-1}.mmHg^{-1}.min^{-1}.10^{-1}$)	18.1±11.0	23.6±10.0	19.2±8.9	22.8±6.2
Decay constant (s)	0.043±0.026	0.047±0.023	0.032±0.018	0.038±0.014
Peak VC ($ml.100ml^{-1}.mmHg^{-1}.min^{-1}.10^{-1}$)	18.7±11.4	22.9±10.2	19.0±8.9	22.4±6.4
A + a ($ml.100ml^{-1}.mmHg^{-1}.min^{-1}.10^{-1}$)	20.8±10.9	25.3±10.4	20.8±9.0	25.5±7.4

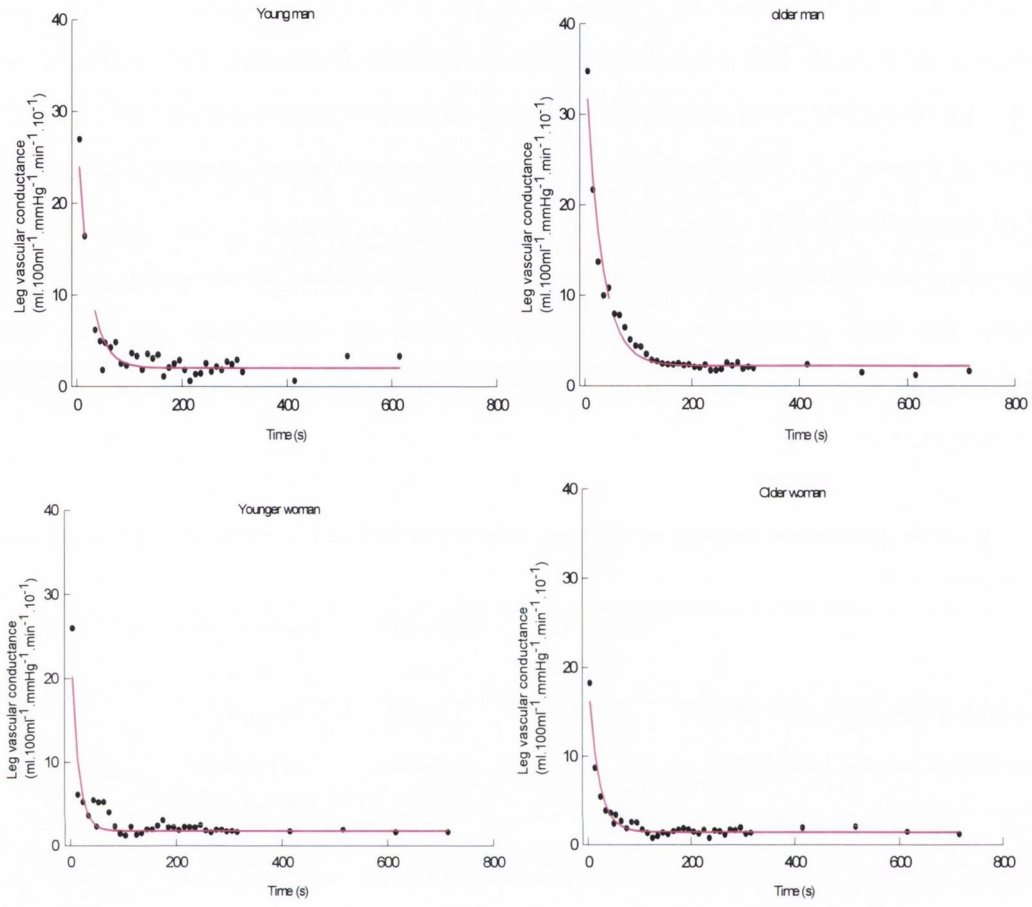


Figure 3.7. Reactive hyperaemic vascular conductance responses (●) and monoexponential curve fit (-) for a representative subject from each group.

Discussion 3.4

The main findings of the present study were the following:

1. The vascular conductance kinetic responses during plantar flexion exercise performed at relative exercise intensities of 30%, 45%, 60% and 70% MVC were slower in older compared with young individuals as evidenced by larger mean response times, primarily due to the larger τ2. However, end exercise amplitudes were not different between the young and older groups at each of the four exercise intensities.
2. The mean forearm and calf vascular conductance reactive hyperaemic responses revealed no differences between the four groups.
3. Absolute resting calf blood flow ($ml.100ml^{-1}.min^{-1}$) and resting vascular conductance ($ml.100ml.min^{-1}.mmHg^{-1}$) responses were higher in older compared with young individuals, but resting vascular conductance ($ml.100ml.min^{-1}.mmHg^{-1}$) responses were similar. When estimates of leg volume were used to calculate blood flow and vascular conductance, older individuals still displayed greater resting blood flow responses but again resting vascular conductance responses were similar between all four groups.
4. Older subjects displayed lower maximum voluntary force compared with young subjects, while men achieved a higher maximum voluntary force compared with women.

3.4.1 VC kinetic responses during calf constant force plantar flexion exercise

To our knowledge this is the first study to demonstrate age and gender related vascular conductance kinetic responses during constant force plantar flexion exercise performed at relative exercise intensities of 30, 45, 60 and 70% MVC.

Leg vascular conductance responses were modelled using a biexponential function on the basis that of the 44 subjects who completed all 4 exercise bouts, 38 of them exhibited a biphasic hyperaemic and vascular conductance response for all exercise intensities (30%, 45%, 60%, 70% MVC), while the remaining eight subjects' response more closely resembled a monophasic function only at the lower intensities (two older men, two young men, one young woman and one older woman at 30% MVC, one young woman and one young man at 45% MVC). This is consistent with

the biphasic response recently demonstrated for diabetic and non-diabetic lean and over-weight women during moderate intensity plantar flexion exercise (MacAnaney *et al.*, 2010) as well as for healthy young men and women during moderate handgrip exercise (Saunders *et al.*, 2005).

The present findings indicated that the time to reach steady state among a range of submaximal intensities was slower for older adults compared with young individuals. This was illustrated by a larger time constant for the second phase ($\tau 2$) and a larger MRT. An elevated MAP response was also evident for the older group of subjects compared with young individuals for most exercise intensities. However, despite the slower age-related kinetic response, the size of increase in blood flow and vascular conductance was similar between the young and older groups, as highlighted by similar end-exercise amplitudes. This indicated that older adults retained the capacity to vasodilate in a similar manner to their young counterparts during steady- state exercise. This is similar to our previous findings observed during the incremental exercise test to failure (chapter 2) where older adults achieved similar submaximal and maximal hyperaemic and vasodilatory responses compared with young adults.

Furthermore, young individuals displayed greater amplitudes for the primary phase (A_1) only at the intensity of 45% MVC, while all other parameters of the phase 1 response were not different between young and older individuals. In relation to gender differences, compared with women, men displayed greater end-exercise amplitudes (A_{360}) and greater secondary phase amplitudes (A_2) at exercise intensities of 45%, 60% and 70% MVC. The gender effects were most likely due to men displaying a greater MVC than women, which resulted in greater overall blood flow and vascular conductance responses during each of the exercise protocols.

Previously, DeLorey *et al.*, (2005) reported slower pulmonary VO_2 kinetics, indicated by a larger time constant (τVO2: older: 49±8 s, young: 29±4 s) and faster muscle deoxygenation kinetics in older (τHHb: 8 ± 2 s) compared with young (τHHb:14 ± 2 s) men, which implied that the kinetics of muscle perfusion may be adjusted at a slower rate in the older compared with young adults at the onset of heavy intensity exercise. The present findings of reduced VC kinetic responses (indicated by a larger $\tau 2$) at all exercise intensities in older adults is in agreement with this notion, and thus,

it is likely that the slower VO_2 kinetic responses observed by DeLorey *et al.,* (2005) in the older group may have been, at least in part, linked to a limitation in convective delivery of oxygen to the working muscle during exercise. Consistent with this, more recently, the kinetics of pulmonary oxygen uptake and femoral artery leg blood flow were shown to be slower, as indicated by greater time constant (τ) values, in older compared with young adults (τVO_{2p} older: 58±21 s, young: 29±11 s; $\tau 2$ blood flow, older: 44±19 s, young: 18±7 s) during the transition to moderate knee-extension exercise performed at absolute (24W) exercise intensities (DuManoir *et al.*, 2010).

3.4.2 Mechanisms underlying slower vascular conductance kinetics in ageing

Since in the present study ageing did not significantly influence parameters describing phase 1 of the biphasic response, it is likely that the control mechanisms contributing to the initial hyperaemic response, including the muscle pump action, are not greatly impacted by the ageing process. Instead, mechanisms contributing to the phase two leg vascular conductance responses are most likely to be involved. These mechanisms may include elevation of adenosine levels, impaired nitric oxide (NO) release from the endothelium due to shear stress, a decreased in both pH & pO_2, and/or an increase in pCO_2 (Saltin *et al.*, 1998; Kingwell, 2000).

Indeed, in relation to ageing and endothelial dysfunction, healthy human adults display a reduction in endothelium-dependent vasodilation in various blood vessels including both brachial (Taddei *et al.*, 1995, 1997b) and coronary arteries (Yasue *et al.*, 1990, Egashira *et al.*, 1993). The exact underlying mechanisms responsible for the age-associated decline in endothelium-dependent vasodilation have not been fully established, but deficit in the L–arginine–nitric–oxide (NO) pathway involving endothelial cell surface receptors and/or membrane bound G-protein signal transduction pathways may be involved. This deficit may result in reduced NO bioavailability (Shimokawa, 1999).

Similarly, a decline in ACh-mediated endothelium dependent vasodilation (Taddei *et al.*, 1995, 1997b, DeSouza *et al.*, 2000) may also contribute to the reduced kinetic response which was observed in the present study. It is thought that increased production of cyclo-oxygenase-dependent endothelium-derived constricting factors

and heightened oxidative stress may be involved in the age-associated reduction in endothelial vasodilation in response to acetylcholine (Taddei *et al.*, 1997a,b). For instance, administration of indomethacin, a cyclooxygenase inhibitor, and vitamin C, a potent antioxidant, potentiated the forearm vasodilator response to ACh in healthy older adults (Taddei *et al.*, 2001). However, additional factors such as a decline in muscarinic receptor number and function (Brodde *et al.*, 1998) are also likely to contribute to this age associated decline in ACh-mediated reduction in endothelial vasodilation.

In addition, endothelial oxidative stress resulting from increased production of reactive oxygen species (ROS) (such as superoxide, hydrogen peroxide) relative to anti-oxidant defences, may be involved in the development of endothelial dysfunction with ageing. Low levels of ROS may function in cell signalling processes but at higher levels they may damage cellular macromolecules (RNA, DNA). Indeed, nitrotyrosine, a cellular marker of oxidative stress, is augmented in endothelial cells of older compared with young individuals (Donato *et al.*, 2007). This increase is significantly related to the age-associated reduction in endothelial-dependent dilation. Also, NAD(P)H oxidase-p47phox, a component of the oxidant-producing enzyme NAD(P)H oxidase, is up-regulated in venous endothelial cells in older men in the absence of up-regulation of xanthine oxidase, an oxidant producing enzyme (Donato *et al.*, 2007). Furthermore, NF-κB p65, a primary component of the radox sensitive, pro-inflammatory NF-$_{\kappa}$B transcription factor complex, is increased in endothelial cells from older men and is positively related to the age-associated increase in endothelial cell nitrotyrosine. Thus, the combination of these findings suggests that oxidative stress develops in endothelial cells with age in healthy men and this may be another factor which ultimately contributes to the reduced vascular conductance kinetic response observed during our exercise protocol.

In relation to the older women who took part in the present study, all were post menopausal not undergoing hormone replacement therapy (HRT) and therefore, were presumably oestrogen deficient. The deficiency in oestrogen may also be a contributing factor in the slower kinetic response observed during all exercise intensities in the present study. It has previously been shown that an absence of

oestrogen is a major factor in the decline in both basal and exercising leg blood flow in older post-menopausal women (Gilligan *et al.*, 1994b). Also, it has been revealed that estradiol exerts its beneficial effects by enhancing the release of nitric oxide, resulting in smooth muscle relaxation (Gilligan *et al.*, 1994a). Indeed, 17β-estradiol acts directly on endothelial smooth muscle, enhancing smooth muscle relaxation (Jiang *et al.*, 1991). Furthermore, physiological levels of 17β-estradiol potentiate endothelium-dependent and independent vasodilation in post-menopausal women (Gilligan *et al.*, 1994b). Thus, there is a strong link between the loss of oestrogen and endothelial dysfunction, which in turn may be linked to the slower phase two kinetic response evident in older women during the present study.

Moreover, age-associated increases in inflammatory agents including cytokines, such as TNF-α, advanced glycation products (AGE's) and matrix metalloproteinases (MMP's) as well as storage cells for inflammatory mediators (mast cells) can disrupt intercellular endothelial gap junction communication which results in a loss of endothelial integrity and an impaired co-ordinated vasodilatory response. Indeed the formation of gaps between adjacent endothelial cells in response to these inflammatory mediators has been well investigated. For instance, a decline in myoendothelial communication in human umbilical vein endothelial and smooth muscle cells in response to TNF-α have been reported (Hu *et al.*, 1997). Studies examining ageing tissue have identified an accumulation of AGE's in various vascular beds in humans lens (Ahmed *et al.*, 1997) and blood vessels (Schleicher *et al.*, 1997) and the increased levels of these inflammatory agents may be considered as a biomarker for advancing age (Payne *et al.*, 2006). Also, ageing has been shown to increase the expression of MMP-2 in the intima of the aorta from rats (Li et al., 1999), humans (McNulty *et al.*, 2005) and non-human primates (Ehringer *et al.*, 2000). Furthermore, both AGE's and MMP-2 can proteolyse vascular endothelial (VE)-cadherin, one of the important regulators of endothelial integrity and thus, inhibit cell-to-cell communication (Lampugnani *et al.*, 1995, Harris *et al.*, 2001,). Indeed, a loss of a co-ordinated vasodilatory response in ageing mouse skeletal muscle compared with that of young mice has been recently observed (Bearden *et al.*, 2004). Overall, with ageing the endothelial cells lose their ability to communicate and

facilitate a co-ordinated vasodilatory response to increase blood flow in order to meet the metabolic demands of the skeletal muscle during exercise (Payne *et al.*, 2006). Thus, it is likely that there may be a link between the slowed leg vascular conductance responses observed in the present study in older men and women and the loss of communication between endothelial cells.

3.4.3 Forearm and calf reactive vascular conductance

Forearm and leg reactive vascular conductance have been used as a measure of endothelial function. An impaired or blunted reactive hyperaemia response is thought to be due to endothelial dysfunction.

Results from the present study did not indicate an age difference in forearm or leg reactive vascular conductance, as indicated by similar peak amplitudes and decay constants of the VC response. This is similar to the findings of Martin *et al.*, (1991) who showed that increasing age was associated with a preservation of maximal calf vasodilatory capacity among sedentary men, assessed using venous occlusion plethysmography. Furthermore, calf vasodilatory capacity was higher by approximately 23% in trained older men compared with untrained older men (Martin *et al.*, 1991). In relation to women, increasing age was associated with lower maximal calf vasodilatory response compared with young women, but similar to men, trained older women displayed a 40% greater calf vasodilatory capacity compared with untrained older women, highlighting the influence of fitness in maintaining vasodilator capacity with advancing age (Martin et al., 1991).

Similarly, Jasperse *et al.*, (1994) observed no difference in active forearm (dominant) peak blood flow or vascular conductance following ischaemic dynamic handgrip exercise between healthy older and young men. In this study, the protocol consisted of 10 minutes of blood flow occlusion, with the subject sustaining forearm muscle contraction at 35% of maximal force for the final two minutes. In relation to subjects, they were matched for fitness levels (they were chronically physically active) and prior to the study displayed similar peak workloads (young: 5.1±0.2 J, older: 4.9±0.2 J) (Jasperse *et al.*, 1994).

On the contrary, Proctor *et al.*, (2005) showed that normally active older men displayed a reduction in peak vascular conductance, assessed using venous occlusion

plethysmography, in both the non-dominant forearm (-6.6% per decade) and calf muscle (-3.4% per decade) after 10 minutes of arterial occlusion. The magnitude of the effect was reduced in the calf relative to the forearm (Proctor *et al.*, 2005). The authors suggested that the decline in peak vascular conductance may be due to a reduction in vasodilation as opposed to an alteration in vascular structure.

The conflicting findings between the aforementioned studies may be due to methodological differences in relation to the limbs being studied (calf versus forearm, dominant versus nondominant limbs), the transducer used to assess blood flow (silastic versus air cuff plethysmography), the fitness levels of the subjects studied (chronically physically active vs. normally active) and the stimulus used to evoke the hyperaemia (cuff occlusion alone vs. cuff occlusion and handgrip exercise). Independently, each of these factors may influence peak limb vascular conductance. Furthermore, differential blood flow responses have been observed for upper and lower limb regions (Moore, 2002, Newcomer *et al.*, 2004).

Additionally, the inconsistencies between the present study and other previous findings (Proctor *et al.*, 2005, Ridout *et al.*, 2005) may be as a result of the small numbers of individuals who were willing to take part in both the leg and arm reactive hyperaemic tests in the present study. Proctor *et al.*, (2005) and Ridout *et al.*, (2005) examined peak vascular conductance responses in a large group of men and women (men: n=68, women n=58), while in the present study only five older women were willing to take part in the leg reactive hyperaemic test.

3.4.4 Basal calf blood flow and vascular conductance

The present study revealed that resting calf blood flow was higher in older individuals compared with young individuals, while resting vascular conductance was similar between both groups, due to a greater pressor response displayed by older adults. This is in direct contrast to findings from previous research studies (Dinenno *et al.*, 1999, 2001, Miyachi *et al.*, 2005, Anton *et al.*, 2006, Donato *et al.*, 2006, Parker *et al.*, 2008) which, reported that older adults displayed lower resting blood flow and vascular conductance responses compared with their younger counterparts.

For instance, Dinneno *et al.*, (2001) measured basal blood flow from the femoral artery in a group of sedentary, physically active and endurance trained men and found

a 18-22% reduction in older men compared with young men across all physical activity levels. Similarly, femoral vascular conductance was 20-30% lower and vascular resistance was 25-38% higher in the older group of men. However, physically active older men displayed significantly higher basal femoral blood flow responses compared with sedentary subjects (283 ± 13 vs. 272 ± 18 $ml.min^{-1}$ respectively), highlighting the influence of aerobic fitness on basal leg blood flow. Thus, it is possible that the older individuals in the present study had higher fitness levels compared with older individuals from previous published studies, and this may have a bearing on the different results obtained among studies. Alternatively, it is also possible that the older participants in the present study had a larger leg skeletal muscle mass than young and thus, greater oxygen and blood flow demand. The fact that in the present study older participants had significantly higher BMI levels than the young might have contributed to this. The different techniques employed to measure blood flow my also contribute to differences between study findings since VOP measures total lower leg blood flow, while DOP measures conduit artery blood flow.

3.4.5 Conclusion

In conclusion, the findings from the present study indicate slower leg vascular conductance kinetic responses in older compared with young adults over a wide range of submaximal exercise intensities as illustrated by a larger phase two time constant and MRT. Although further studies are needed to elucidate the underlying mechanisms, it is likely that the function of, and communication between, endothelial cells might be involved.

Chapter 4

4.1 Introduction

Ageing is associated with unfavourable haemodynamic adaptations including a reduced basal blood flow response and slower blood flow kinetics during exercise. The slowed blood flow kinetic responses are in turn, linked to slowed VO_2 kinetics, which have been previously associated with the early onset of muscle fatigue (Hughson & Tschakovsky, 1999).

However, the vast majority of previous studies indicate an age-associated maintenance or even enhancement of fatigue resistance despite deleterious physiological alterations (Ditor & Hicks, 2000; Bilodeau *et al.*, 2001; Stackhouse *et al.*, 2001; Kent-Braun *et al.*, 2002; Hunter *et al.*, 2004, 2005; Russ *et al.*, 2008). This age-associated maintenance or even enhancement of fatigue resistance has been associated with positive age related compensatory mechanisms including alterations in fibre types to a greater number of fatigue resistant type I fibres, a greater reliance on oxidative phosphorylation and an overall age- associated metabolic economy (Tevald *et al.*).

Although a large number of studies have examined the age-related effects of muscle fatigue (Ditor & Hicks, 2000; Bilodeau *et al.*, 2001; Kent-Braun *et al.*, 2002; Hunter *et al.*, 2004, 2005; Russ *et al.*, 2008), and to a lesser extent the age-associated haemodynamic changes (Proctor *et al.*, 1998; Proctor *et al.*, 2003; Proctor *et al.*, 2004; Donato *et al.*, 2006; Parker *et al.*, 2008), to our knowledge none of the previous studies have investigated the relationship between the dynamic characteristics of leg blood flow and leg muscle fatigue in the same aged individuals following the same exercise protocol. Thus, the aim of our third study was to examine muscle fatigue profiles during constant-force plantar flexion exercise in older and young individuals following the same submaximal exercise intensities (30%, 45%, 60% and 70% MVC) used in our previous study (chapter 3), where blood flow and vascular conductance kinetics were determined in the same participants. The primary hypothesis was that

despite older adults displaying an impaired kinetic response (chapter 3), they would have an enhanced fatigue resistance and greater muscular endurance compared with young adults.

4.2 Materials and methods

4.2.1 Subjects

Eight young men, 8 young women, 8 older men and 10 older women were recruited for the study. All subjects were non-obese (BMI <30 $kg.m^{-2}$), were non-smokers and were classified as normally active.

4.2.1.1 Subject recruitment

See chapter 2 section 2.2.1.1

4.2.1.2 Subject inclusion and exclusion criteria

See chapter 2 section 2.2.1.2

4.2.2 Medical assessment

See chapter 2 section 2.2.2

4.2.2.1 Blood analysis

See chapter 2 section 2.2.2.1

4.2.3 Experimental design

4.2.3.1 Experimental protocol overview

Subjects were required to attend the cardiovascular laboratory in the Department of Physiology on three occasions separated by at least 48 hours. Prior to each visit, subjects were required to refrain from alcohol, caffeine and exercise for 24 hours preceding the exercise test.

On visit one, subjects were familiarised with the constant-force isometric calf exercise test to failure. Subjects then completed five calf maximal voluntary contractions (MVC) (1 min rest periods between maximal efforts) in the upright position (tilt angle of 67°). The greatest MVC produced was used to calculate 30%, 45%, 60% and 70% MVC for subsequent fatigue exercise tests to failure.

On visit two, subjects completed two constant-force isometric plantar flexion exercises to failure at two randomly selected intensities of 30%, 45%, 60% or 70% MVC separated by 45 minutes of passive rest. Following a rest period (supine position) of 45 minutes, the subject completed another constant-force plantar flexion exercise test to failure at a different randomly selected intensity of 30%, 45%, 60% or 70% MVC.

During visit three, subjects again completed two more constant-force plantar flexion exercise tests to failure performed at the remaining two randomly selected intensities of 30%, 45%, 60% or 70% MVC.

4.2.3.2 Visit-1 Familiarisation and MVC

Subjects performed three to four low intensity isometric plantar flexions of the right calf muscle in the horizontal position. The contraction/relaxation ratio was 2s/4s. Then, the exact procedure for performing the constant-force exercise test to failure was explained to each of the subjects. The subjects then practised the light and moderate intensity constant-force exercise contractions, interspersing an MVC every 30 s until they felt comfortable with the procedure. The entire procedure was repeated at an inclination of 67°.

Following a rest period of 15 minutes, subjects exerted the greatest amount of force possible on the force-plate while plantar flexing the right foot (while at a tilt angle of 67°) for determination of their MVC. Each contraction was sustained for approximately 3 s and the procedure was repeated six times with a 1 min rest period between contractions. The highest force produced was recorded as the MVC.

4.2.3.3 Visit-2 Fatigue test (I)

Subjects performed two constant-force plantar flexor exercise tests to failure at two randomly selected intensities of 30%, 45%, 60% or 70% MVC at a contraction/relaxation ratio of 2 s: 4 s while positioned in the upright posture at an inclination of 67°. Both tests were separated by 45 minutes of passive rest. A maximum voluntary contraction was produced at the beginning of each exercise test

and then every fifth contraction (i.e. every 30 s) if exercising at 70% MVC or every tenth contraction (i.e. every min) if exercising at 30%, 45% or 60% MVC (Fig. 4.1). A visual display (Chart^{TM} v 5.5.4, AD Instruments) was used to assist subjects to reach their required intensity. Failure occurred when the force during the submaximal contractions failed to reach the required force during two consecutive efforts, and at this point the test was stopped. The 30%, 45% and 60% MVC fatigue exercise tests were limited to a duration of 20 min, while the 70% MVC exercise test was performed to failure. Brachial blood pressure (BP) and heart rate were measured beat-to-beat at rest and during each of the four exercise tests using either applanation tonometry of the right radial artery (COLIN, CBM7000, Japan) or the volume clamp method (Finometer) (see chapter 2, section 2.2.4.7).

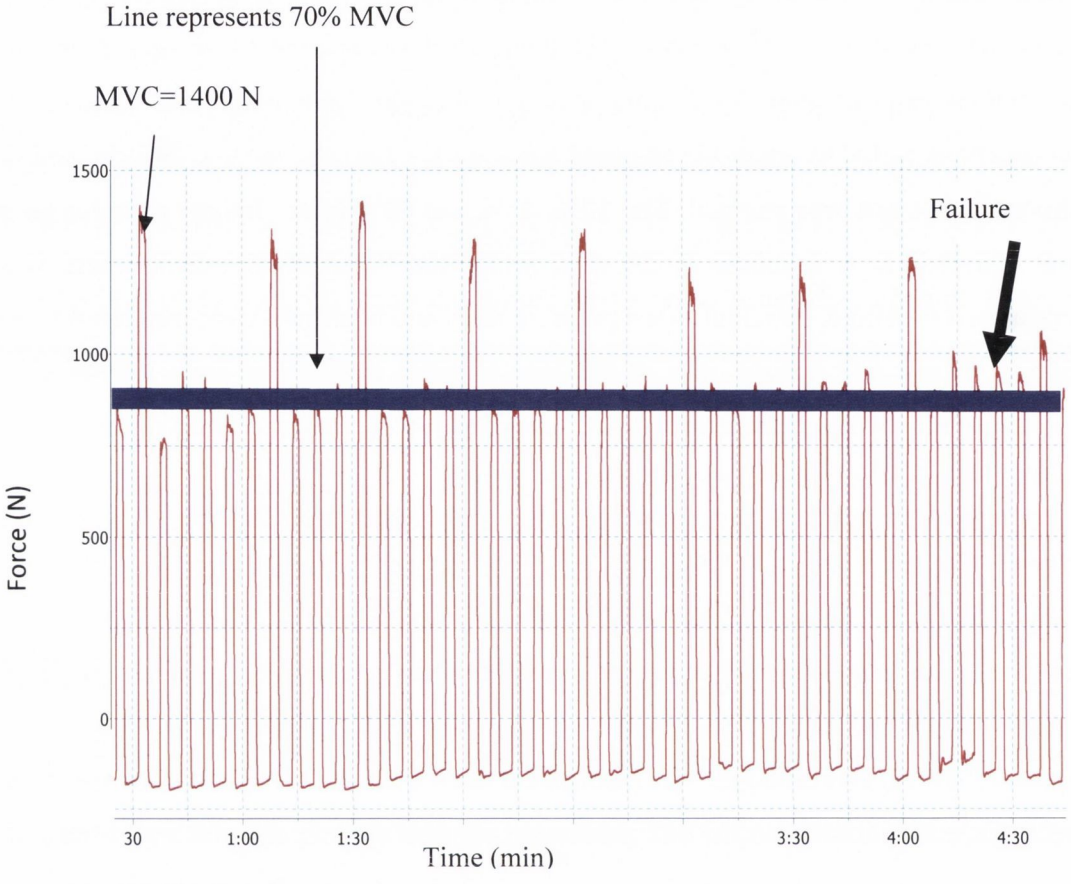


Figure 4.1 *Constant-force exercise test for an individual (young man) performed at 70% MVC. The subject initially produces a MVC (in this example MVC is 765N) at the beginning of the exercise test and every fifth contraction (i.e. every 30 s). As the exercise test progresses, muscle fatigue is apparent as the maximum voluntary contraction declines over time. Failure is reached when the subject is unable to sustain a force of 70% MVC for two consecutive contractions.*

The decline in force during the maximum efforts was expressed using the linear equation:

$$y = bx + a$$

where, y is force, x is time, a represents force at time = 0 (i.e., predicted MVC) and b represents rate of fatigue (slope of the regression).

Most subjects showed a linear decline in the rate of fatigue, which is consistent with previous studies in our lab (Egana & Green, 2005, 2007).

4.2.3.4 Visit-3 Fatigue tests (II)

The remaining two fatiguing constant-force exercises were performed following the same procedures used during visit-2 (see section 4.2.3.3).

4.2.4 Equipment and measurements

4.2.4.1 Calf ergometer and exercise model see chapter 2, section 2.4.1

4.2.4.2 Body mass, height and BMI; see chapter 2, section 2.2.4.3

4.2.4.3 Blood analysis; see chapter 2, section 2.2.4.1

4.2.4.4 Low-level physical activity recall (LOPAR); see chapter 2, section 2.2.4.6

4.2.4.5 Blood pressure; see chapter 2, section 2.2.4.7

4.2.4.6 Heart rate; see chapter 2, section 2.2.4.8

4.2.5 Statistical Analysis

All anthropometrical and haematological data were compared between the young and older men and women using a two-way ANOVA (gender x age). A two-way ANOVA was also used to compare endurance time to failure, rate of fatigue and predicted MVC between groups. Differences were located using a Tukey's *post hoc* test. A three-way repeated measures ANOVA (age x gender x time) was used to compare MAP and HR responses at different time points during the tests. Differences

were located using a Bonferroni *post hoc* test. The level of significance was set at $P<0.05$. All results are given as mean ± standard deviation (SD). The statistical software Sigma Stat, (USA) was used for all two-way, ANOVA statistical analyses, while for three-way, repeated measures ANOVA, data desk® 6.1 was used.

4.3 Results

4.3.1 Subjects

4.3.1.1 Physical characteristics

Physical characteristics and activity levels for the four groups can be seen in Table 4.1. Men were taller and had a greater body mass than women (main effect = gender) and young subjects were taller and had a greater body mass than older subjects (main effect = age) with no interaction between gender and age. Older subjects had a higher BMI than young subjects (main effect = age). No differences in leg volumes or activity levels were detected between the groups. Individual anthropometrical data can be seen in Appendix XXVI.

Table 4.1. Physical characteristics and activity levels for young and older men and women (mean ± SD).

	Young men n=8	Older men n=8	Young women n=8	Older women n=10
Age (yr)	23±2	67±5†	22±1	64±7†
Height (cm)	181±7*	170±7*†	166±3	158±7†
Body mass (kg)	70.3±9.5*	76.0±9.0*†	59.4±4.2	68.6±9.0†
BMI ($kg.m^{-2}$)	21.6±2.9	26.2±2.7†	21.6±1.5	27.7±4.1†
Leg volume (mL)	2686±601	2860±405	2618±484	2760±623
Activity level ($MET\ h.week^{-1}$)	196±24	197±41	210±42	206±44

* Significantly different ($P<0.05$) compared with women of the same age group. † Significantly different ($P<0.05$) compared with young within the same gender.

4.3.1.2 Haematology

Haematological values for the four groups can be seen in Table 4.2. Older individuals displayed higher total cholesterol, low density lipoprotein, triglycerides and total cholesterol/HDL compared with young subjects (main effect = age). Males displayed higher haematocrit, triglycerides and haemoglobin levels but lower fasting glucose levels compared with females (main effect = gender). Individual haematological data can be seen in Appendix XXVII.

Table 4.2. Haematological values for young and older men and women (mean ± SD).

	Young men n=8	Older men n=8	Young women n=8	Older women n=10
Haemoglobin ($g.dL^{-1}$)	15.4±0.8*	15.1±0.8*	13.5±0.8	13.7±1.0
Haematocrit (%)	45.1±2.2*	45.2±1.9*	39.7±1.9	40.2±2.8
Plasma Glucose ($mmol.L^{-1}$)	4.5±0.3*	4.3±0.8*	4.9±0.5	4.8±0.4
Total Cholesterol ($mmol.L^{-1}$)	3.6±0.6	4.6±0.6†	3.9±0.9	4.9±0.5†
High Density Lipoprotein ($mmol.L^{-1}$)	1.1±0.2	1.0±0.3	1.2±0.3	1.1±0.2
Triglycerides ($mmol.L^{-1}$)	1.3±0.1*	2.1±0.7†*	1.3±0.0	1.5±0.4†
Low Density Lipoproteins ($mmol.L^{-1}$)	2.2±0.7	3.3±0.6†	2.4±0.6	3.5±0.6†
Total Cholesterol/High Density Cholesterol ($mmol.L^{-1}$)	3.6±1.4	5.1±1.3†	3.1±0.3	4.6±1.2†

* Significantly different ($P<0.05$) compared with women of the same age group. † Significantly different ($P<0.05$) compared with young within the same gender.

4.3.2 Fatigue tests

4.3.2.1 Time to failure

Only the fatigue tests at intensities relative to 70% MVC were brought to failure. Older men displayed a longer time to failure compared with young men (15.5 ± 5.7 min vs. 10.6 ± 2.4 min). No differences were observed between older and young women (11.9 ± 5.1 min vs. 13.8 ± 4.9 min). Individual time to failure responses at 70% MVC can be seen in Appendix XXVIII.

4.3.2.2 Maximum voluntary force (MVC)

The maximum voluntary force recorded during the MVC session (visit 1) and prior to all four fatigue tests performed at 30%, 45%, 60% and 70% MVC was significantly larger in young compared with older subjects (main effect = age) (Table 4.3). There was also a significant interaction between age and gender so that young men were stronger than young women but no strength differences were observed between older men and women. Importantly, there was no significant difference among the MVC's performed during the MVC session and those performed prior to each of the four fatigue tests within any of the four groups. Individual values for maximum voluntary contractions can be seen in Appendix XXIX.

Table 4.3. Maximum voluntary force (MVC) (N) values for young and older men and women (mean ± SD) during the MVC session (visit 1) and immediately prior to each of the four fatigue tests.

	Young men n=8	Older men n=8	Young women n=8	Older women n=10
MVC SESSION	1146±268*	920±279†	811±117	736±152†
Pre-30%MVC fatigue test	1136±204*	896±291†	820±215	773±165†
Pre-45%MVC fatigue test	1161±222*	904±308†	816±266	762±186†
Pre-60%MVC fatigue test	1174±222*	922±297†	829±208	773±182†
Pre-70%MVC fatigue test	1165±247*	926±275†	872±118	763±181†

* Significantly different ($P<0.05$) compared with women of the same age group. † Significantly different ($P<0.05$) compared with young within the same gender.

4.3.2.3 Rate of fatigue

Mean rate of fatigue responses for the four groups for each exercise intensity can be seen in Figure 4.2, while individual fatigue responses can be seen in Figures 4.3 (young and older men) and Figure 4.4 (young and older women). Young individuals displayed greater fatigue at 60% MVC compared with older subjects (main effect = age). At 70% MVC, there was a significant interaction between age and gender so that young men displayed greater fatigue compared with older men, but there were no differences between young and older women. The rates of fatigue were not different among the four groups at either 30% or 45% MVC. Individual rate of fatigue responses can be seen in Appendix XXX.

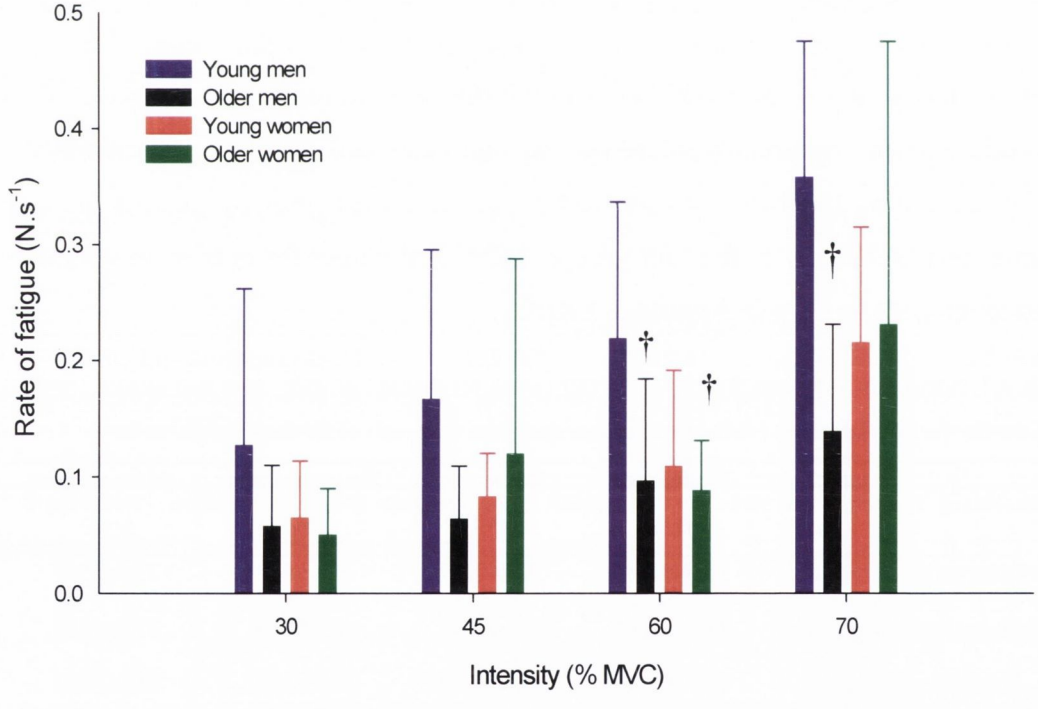


Figure 4.2. Rate of fatigue at intensities of 30%, 45%, 60% and 70% MVC for young and older men and women (mean ± SD). † Significantly different (P<0.05) compared with young within the same gender.

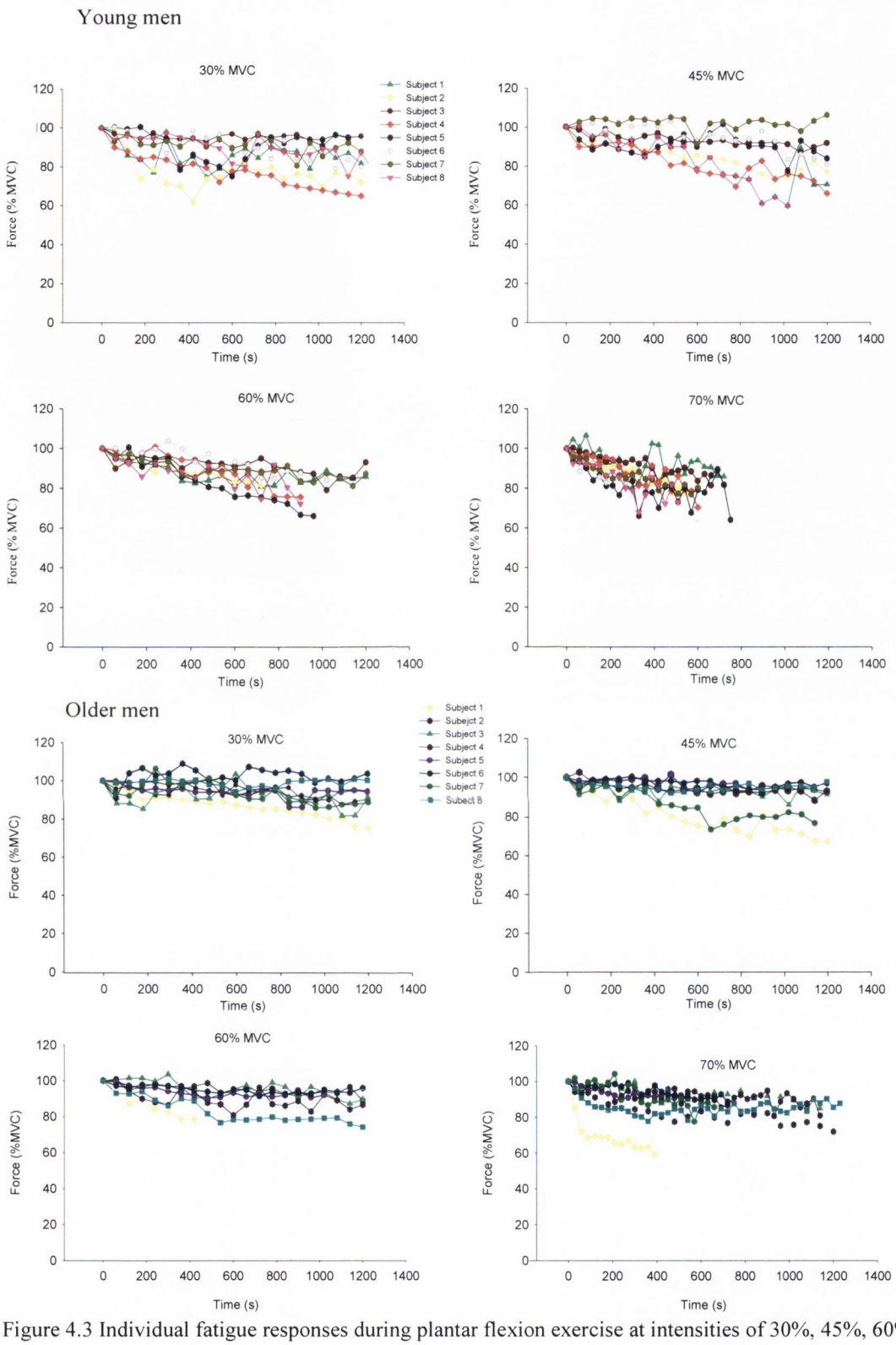


Figure 4.3 Individual fatigue responses during plantar flexion exercise at intensities of 30%, 45%, 60% and 70% MVC for young (top four panels) and older men (lower four panels). The y-axes were converted to percentage of MVC to better detect the differences between groups.

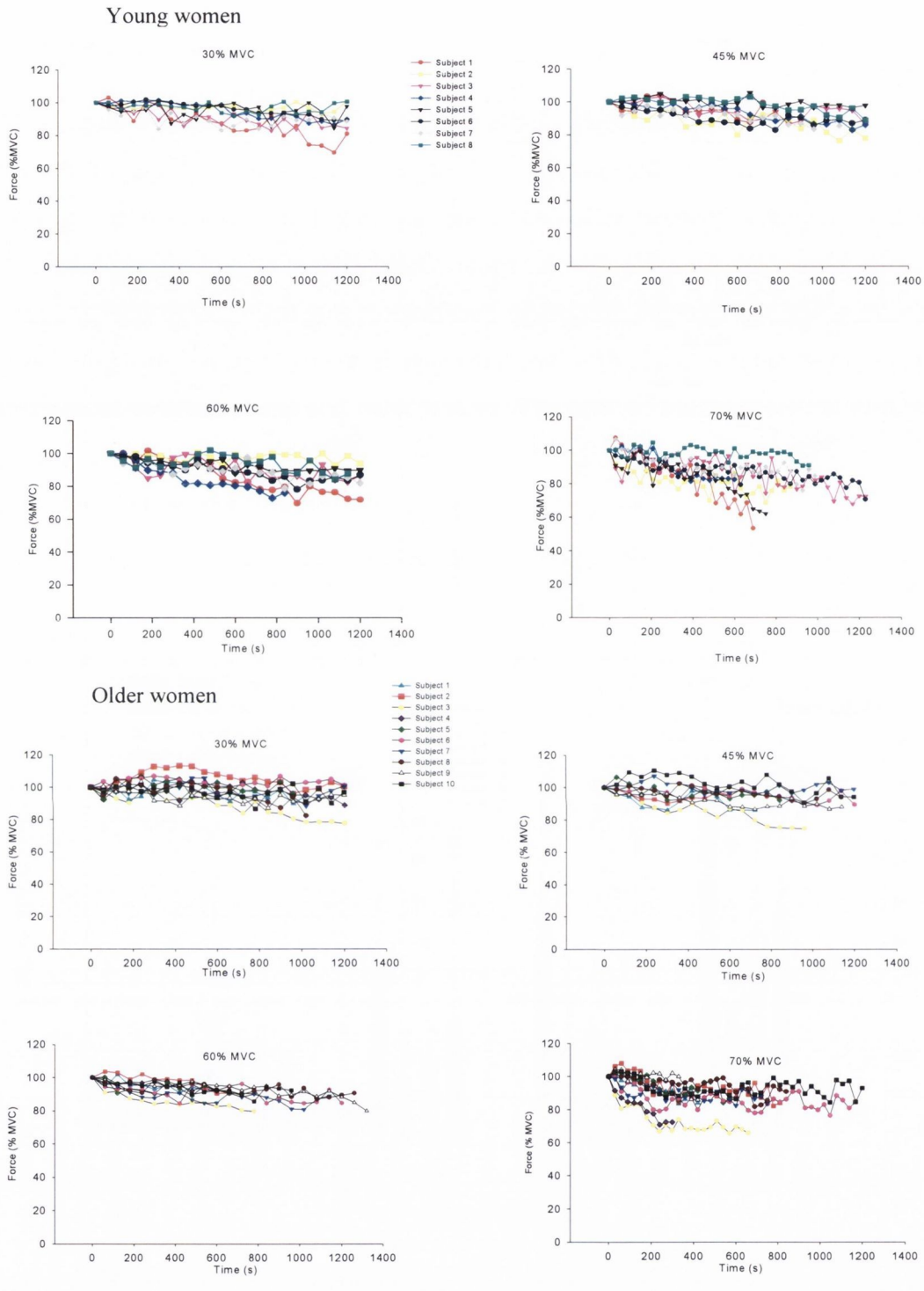


Figure 4.4 Individual fatigue responses during plantar flexion exercise at intensities of 30%, 45%, 60% and 70% MVC for young (top four panels) and older women (lower four panels). The y-axes were converted to percentage of MVC to better detect the differences between the groups.

4.3.2.4 End-exercise force relative to pre-exercise MVC

Mean end-exercise percent (%) forces (relative to pre-exercise MVC) for all groups during the four fatigue tests are shown in Table 4.4. Young individuals displayed a lower end-exercise percent (%) force compared with older individuals at 70% MVC only. No differences were apparent between the four groups at 30%, 45% or 60% MVC. Individual end-exercise percent (%) forces (relative to pre-exercise MVC) can be seen in Appendix XXXI.

Table 4.4. Mean end-exercise percent (%) forces (relative to pre-exercise MVC) for young and older men and women (mean ± SD) during the four fatigue tests.

	Young men n=8	Older men n=8	Young women n=8	Older women n=10
30% MVC	80±16	92±8	91±7	91±7
45% MVC	80±14	89±11	89±7	88±6
60% MVC	80±8	87±8	85±8	88±5
70% MVC	73±6	79±10†	69±11	80±6†

* Significantly different ($P<0.05$) compared with women of the same age group. † Significantly different ($P<0.05$) compared with young within the same gender.

3.3.2.5 Predicted maximum force

Mean predicted maximum forces for all groups during the four fatigue tests are shown in Table 4.5. Young men displayed greater predicted maximum forces compared with young women and older men for all four exercise intensities. No differences were apparent between young and older women. The recorded maximum voluntary contractions and predicted maximum forces at each of the exercise intensities for each of the four groups were similar. In addition, within each group, predicted MVC's were not different prior to each of the four exercise intensities. Individual predicted maximum force values can be seen in Appendix XXXII.

Table 4.5. Predicted maximum voluntary forces (N) for young and older men and women (mean ± SD).

Intensity	Young men n=8	Older men n=8	Young women n=8	Older women n=10
30% MVC	1082±185*	857±316†	822±213	798±211
45% MVC	1096±173*	901±320†	821±234	772±189
60% MVC	1139±191*	900±302†	844±190	779±176
70% MVC	1141±266*	893±330†	834±155	822±189

* Significantly different ($P<0.05$) compared with women of the same age group. † Significantly different ($P<0.05$) compared with young within the same gender.

4.3.2.6 Heart rate

Heart rate responses recorded at rest, exercise onset (~6 s) and at the end of each of the four fatigue tests for the four groups are displayed in Table 4.6. Resting heart rate, heart rate at exercise onset and end-exercise heart rate responses were similar between all four groups for the 30%, 60% and 70% MVC fatiguing protocols. At 45% MVC, young women displayed a higher heart rate response at exercise onset compared with young men, while older women displayed lower heart rate responses compared with young women. Heart rate responses for each of the four exercise intensities for each group can be seen in Appendix XXXIII.

Table 4.6. Heart rate (beats.min^{-1}) responses for young and older men and women (mean ± SD).

		Young men n=8	Older men n=8	Young women n=8	Older women n=10
30% MVC	Resting HR	74±11	78±7	84±13	75±7
	HR at onset	79±13	83±12	90±6	83±7
	End-exercise HR	87±12	82±10	93±11	79±7
45% MVC	Resting HR	74±13	79±14	81±12	76±8
	HR at onset	83±13*	84±15	98±7	82±8†
	End-exercise HR	89±13	85±17	89±12	83±12
60% MVC	Resting HR	73±11	79±13	78±12	75±9
	HR at onset	86±14	85±11	90±10	85±8
	End-exercise HR	90±10	84±14	90±9	85±12
70% MVC	Resting HR	76±9	80±14	79±14	75±7
	HR at onset	86±10	84±12	97±14	80±9
	End-exercise HR	98±17	89±13	100±11	85±16

* Significantly different ($P<0.05$) compared with women of the same age group. † Significantly different ($P<0.05$) compared with young within the same gender.

4.3.2.7 Mean arterial pressure

Mean arterial pressure responses recorded at rest, at the onset (~6 s) and at the end of each of the four fatigue tests for each group are displayed in Table 4.7. There were no differences in resting, exercise onset, or end-exercise MAP responses between the four groups at the four exercise intensities (Table 4.7). Individual MAP responses can be seen in Appendix XXXIII.

Table 4.7. Mean arterial pressure (mmHg) responses for young and older men and women (mean ± SD).

		Young men n=8	Older men n=8	Young women n=8	Older women n=10
30% MVC	Resting MAP	91±18	101±20	82±14	89±11
	Exercise onset MAP	91±15	105±14	81±15	94±14
	End exercise MAP	92±18	105±19	78±14	100±14
45% MVC	Resting MAP	82±14	84±12	75±15	89±13
	Exercise onset MAP	88±16	91±11	76±10	90±17
	End exercise MAP	88±12	106±18	79±15	103±15
60% MVC	Resting MAP	86±13	91± 13	79±12	88±19
	Exercise onset MAP	97±9	94±8	80±14	93±16
	End exercise MAP	99±17	106±14	80±19	103±21
70% MVC	Resting MAP	92±18	101±14	81±14	92±21
	Exercise onset MAP	94±22	107±13	81±12	100±15
	End exercise MAP	101±20	116±15	85±17	109±17

4.4 Discussion

The main findings of the present study were the following:

1. Older men displayed greater muscular endurance, as indicated by a longer time to failure during the 70% MVC plantar flexion exercise compared with young men, while no differences in time to task failure were observed between older and young women at this intensity.

2. Both older men and women fatigued relatively less at 60% MVC, as indicated by a lower rate of fatigue, compared with young individuals, while at 70% MVC older men displayed enhanced fatigue resistance compared with young men. No differences in the rate of fatigue were observed between young and older women at 70% MVC. There were no differences in fatigue among the four groups at the lower intensities of 30% and 45% MVC.

3. In addition, fatigue resistance, assessed using percent force decrements from the start of the 70% MVC exercise was greater in older compared with young adults. There were no differences in percent force decrements between the four groups for the three other exercise intensities of 30%, 45% or 60% MVC.

4.4.1 Muscle fatigue and endurance among men

The findings from the present study are in agreement with previous research indicating an age-related enhancement of fatigue resistance and muscular endurance during intermittent (duty cycle; 2 s contraction, 3 s relaxation) maximal isometric plantar flexion and dorsiflexion exercises (Bemben *et al.*, 1996). Fatigue resistance, assessed using percent force decrements (percent decline in force from the start of the exercise compared with the end of the exercise), was greater in older compared with young adults for both muscle groups (Bemben *et al.*, 1996). This is similar to the findings of the present study where older adults displayed lower percent force decrements compared with young adults at 70% MVC. This is also consistent with findings of Ditor & Hicks, (2000) who showed that older men were significantly less fatigable than young men as indicated by a lower voluntary fatigue index (FI)

(percent of force reduction from baseline) (FI young: 40.2±12.6% vs. FI older: 25.2±12.3%) during a 3 minute intermittent (duty cycle; 5 s, 2 s) maximal adductor pollicis exercise.

Similarly, fatigue resistance (assessed using the ratio of post-exercise MVC to pre-exercise MVC) was significantly enhanced in older men (0.80±0.04) compared with young men (0.72±0.02) during a 16 minute incremental (starting at 10% MVC with increments of 10% every 2 minutes) intermittent (duty cycle: 4 s, 6 s) ankle dorsiflexor exercise (Kent-Braun *et al.*, 2002). The age-related enhancement of fatigue resistance, defined as the ratio of post-exercise to pre-exercise MVC, has also been reported during ankle dorsiflexion exercise for both repeated maximal dynamic (90 repetitions at 90°/s) (older: 0.45 ± 0.07, young: 0.27 ± 0.02) and maximal isometric intermittent (5 s contraction, 5 s relaxation) contractions, performed for a total of 3 minutes (older: 0.77 ± 0.07, young: 0.66 ± 0.02) (Lanza *et al.*, 2004). On the other hand, Hunter *et al.*, (2005a) showed that during sustained submaximal (20% MVC) isometric elbow flexion exercise, older men displayed greater muscular endurance compared with young men (time to task failure: older: 22.6 ± 7.4 min, young: 13.0 ± 5.2 min). Young men were only able to sustain the task for 42% of the duration achieved by the strength-matched older men (Hunter *et al.*, 2005a).

On the contrary, Bilodeau *et al.*, (2001) reported similar fatigue profiles (assessed using the change in MVC torque over time) in older and young men following sustained submaximal (35% MVC) elbow flexion exercise. This is consistent with the findings from the present study during the 30% and 45% MVC exercise protocols, where both older and young individuals displayed similar rates of fatigue. In the present study, muscle endurance was not measured during the 30% and 45% MVC exercise protocol (exercise bouts were limited to 20 min), but Bilodeau *et al.*, (2001) found that older men exhibited longer times to failure at 35% MVC compared with the young group of men (older: 471.7 ± 260.8 s, younger: 179.6 ± 51.3 s), highlighting the age-associated advantage in terms of muscular endurance even at lower exercise intensities.

4.4.2 Muscle fatigue and endurance among women

Older women displayed enhanced fatigue resistance during the 60% MVC exercise compared with the young women (rate of fatigue, older: -0.09 ± 0.04, young: -0.11 ± 0.08 $N.s^{-1}$). However, at the higher exercise intensity of 70% MVC, there were no differences in fatigue profiles (rate of fatigue, older: -0.321 ± 0.244, young: -0.206 ± 0.09 $N.s^{-1}$) or muscular endurance (time to failure older: 11.9 ± 5.1 min, young: 13.8 ± 4.9 min) between young and older women. Therefore, the age-related effect was less pronounced in women than in men at the higher intensity of 70% MVC.

In agreement with the findings from the present study, similar fatigue resistance responses were observed in older compared with young women (percent of force reduction from baseline voluntary fatigue index young: 37.8 ± 14.1% vs. older: 26.3 ± 14.5%, $P>0.05$) during 3 minutes of intermittent (5 s contraction, 2 s relaxation) maximal voluntary adductor pollicis exercise (Ditor & Hicks, 2000). Ditor & Hicks, (2000) also showed that muscular endurance was more pronounced in older men than in older women, which is in agreement with the findings from the present study. This finding is also consistent with research by Stackhouse *et al.*, (2001) indicating similar muscle fatigue (as indicated by the decline in peak force over time) in older and young women during a fatiguing leg kicking exercise consisting of a total of 25 isometric knee extensor MVC's performed intermittently (5 s contraction, 2 s relaxation) using the quadriceps muscles.

Furthermore, during 100 repeated maximal dynamic contractions at $90°.s^{-1}$ using the knee extensor muscles, older women displayed similar fatigue rates (rate of reduction in muscle force) (older: 39 ± 23, young: 42 ± 10) and fatigue indices (percent loss of muscle strength over time) (older: 48 ± 9%, young: 55 ± 9%) compared with young women (Lindstrom *et al.*, 1997). However, unlike the findings from the present study, older women displayed significantly lower muscle endurance compared with young women, although in this study by Lindstrom *et al.*, (1997) endurance was defined in

terms of muscle force (Nm), while in the present study muscle endurance was assessed using time to task failure (min).

Overall, these present findings demonstrate the age-associated advantage in terms of both fatigue resistance and muscular endurance. Furthermore, the ageing advantage was more pronounced in men compared with women, highlighting the influence of gender effects in maintaining performance during this plantar flexion exercise model.

4.4.3 Potential reasons for discrepancies between study findings

Methodological differences including contraction mode (dynamic vs. isometric), experimental protocol, subject characteristics (age and/or the activity status) and/or the duty cycle employed may contribute to the discrepancies between findings from studies using men only or studies employing both men and women. For instance, interestingly, exercise protocols using duty cycles of 50% or less (shorter work periods than recovery periods) have overall found enhanced fatigue resistance in older compared with young men, which is consistent with our present findings. Bemben *et al.*, (1996) showed that during finger flexor, thumb abductor, calf dorsiflexor and plantar flexor exercise where a 40% duty cycle (2 s contraction, 3 s relaxation) was employed, older men displayed enhanced fatigue resistance compared with young men. This enhanced fatigue resistance was also evident for both men and women during a 16 minute incremental calf dorsiflexor exercise, again where a 40% duty cycle was employed (4 s contraction, 6 s relaxation) (Kent-Braun *et al.*, 2002).

In contrast, many protocols involving duty cycles of more than 50% have generally found no effect of age on the development of muscle fatigue in either men or women. This was evident for both men and women during an intermittent maximal knee extension (25 in total) exercise, which employed a duty cycle of 75% (5 s contraction, 2 s relaxation) (Stackhouse *et al.*, 2001). Also, both men and women displayed similar fatigability (assessed using muscle compound action potential or M wave activity which reflects muscle membrane excitability) during maximal isometric contractions for the brachioradialis, tibialis anterior and thenar muscles performed for

a total duration of 2 minutes using a 60% duty cycle (3 s contraction, 2 s relaxation) (Hicks & McCartney, 1996). However, more recently, Russ *et al.*, (2008) reported greater fatigue resistance among older compared with young men during maximal isometric dorsiflexion exercise where a 70% duty cycle (7 s contraction, 3 s relaxation) was employed (Russ *et al.*, 2008). Thus, other factors apart from the duty cycle employed may impact the different outcomes reported between studies.

4.4.4 Mechanisms for enhanced fatigue resistance in ageing

Enhanced fatigue resistance displayed by older adults during various sustained and intermittent isometric and/or dynamic exercise protocols is due to a combination of significant changes occurring within the aged neuromuscular system. These include the loss of muscle mass (sarcopenia) (Kent-Braun & Ng, 1999), fibre type alterations (Jakobsson *et al.*, 1990) and age-associated changes in metabolic capacity (Kent-Braun *et al.*, 2002; Lanza *et al.*, 2004; 2005) supporting the idea of an age-associated enhanced metabolic economy (Tevald *et al.*, 2010; Russ *et al.*, 2008; Wust *et al.*, 2008).

It is well established that older adults display a significant loss in force production primarily due to the age-associated loss of muscle mass. Differences in muscle mass may account for some of the differences in fatigue profiles observed across gender and age via the impact of intramuscular pressure on muscle perfusion during contractions. Lower muscle mass and thus, strength may reduce intramuscular pressure and blood flow occlusion during muscle contractions in older compared with young muscles, which results in a greater delivery of oxygen to the working muscle and a greater clearance of exercise induced metabolic by-products (Kent-Braun *et al.*, 2002). Although muscle mass was not measured in the present study, older adults displayed a significantly lower maximum voluntary contraction (MVC) compared with the young adults, presumably due to a smaller muscle mass and this may have in part contributed to the enhanced fatigue resistance and muscle endurance displayed by the older men during both the 60% and 70% MVC exercise protocols. Similar to findings for older men, older women displayed enhanced fatigue resistance during the

60% MVC fatiguing exercise protocol and although muscular endurance was not enhanced during the 70% MVC exercise protocol, older women performed as well as young women during this task. This highlights the fact that alterations within the ageing neuromuscular system confer positive advantages to both men and women during fatiguing exercise protocols.

Improved fatigue resistance with age is also thought to arise as a consequence of the age-related motor unit (MU) remodelling theory. This theory suggests a progressive loss of motor units with age beginning with the death of the motor units that innervate fast twitch muscle fibres. Whether fast twitch muscle fibres are lost or reinnervated by a viable motor neuron responsible for slow twitch fibres, therefore adapting a more fatigue resistant profile, the overall result is that a greater percentage of the remaining muscle fibres exhibit slow twitch properties. As a result, MU remodelling may contribute to the age-associated slowing of contractile properties both under voluntary isotonic and isometric conditions (Lexell *et al.*, 1988; Jakobsson *et al.*, 1990). The economy of force production is 3-4 fold higher in type I fibres than type II fibres due to both the myosin and the cost of calcium handling (Stienen *et al.*, 1996; Szentesi *et al.*, 2001). Therefore the age-related shift in fibre type composition could reduce contractile costs in the older muscle. In addition, the age-related shift in fibre type composition will contribute to the slowing of muscle contractile properties (Vandervoort & McComas, 1986; Connelly *et al.*, 1999).

With the greater shift towards type I muscle fibres with ageing, there is a greater reliance on oxidative metabolism to provide the energy needs for exercise contractions, which may confer an age-associated advantage. This was highlighted in a study reporting that older adults fatigued less compared with young adults during incremental (starting at 10% MVC, with increments of 10% every 2 minutes) intermittent (4 s contraction, 6 s relaxation) exercise (16 min in duration). Enhanced fatigue resistance correlated with a lower accumulation of metabolic by-products, Pi and $H_2PO_4^-$, and a smaller decrease in pH (Kent-Braun *et al.*, 2002). Furthermore, pre-exercise MVC, which is indicative of strength, was associated with a greater

production of H^+, $H_2PO_4^-$ and Pi as well as lower pH. In both groups, the decline in MVC was significantly related to an increased concentration of $H_2PO_4^-$. The smaller increase in H^+ concentration in the older group reflected their reduced capacity for glycolytic metabolism compared with young adults (Kent-Braun *et al.*, 2002).

This was further investigated using phosphorus magnetic resonance spectroscopy, employed to measure high-energy phosphate metabolite concentrations (PCr, Pi, phosphomonoesters and ATP) during sustained (60 s) maximal dorsiflexion exercise (Lanza *et al.*, 2005). The concentrations of these metabolites along with the change in pH during contraction and recovery were used to assess oxidative capacity and the rates of ATP synthesis by the creatine kinase (CR) reaction, anaerobic glycolysis and oxidative phosphorylation. Older men derived their ATP for exercise by oxidative phosphorylation and to a lesser extent by glycolysis compared with young individuals; and as a result, glycolytic flux was higher in young (1.41 ± 0.15 mM ATP/s^{-1}) compared with older adults (0.84 ± 0.17 mM ATP/s^{-1}).

Indeed, more recent research indicates that older adults may have a preference for, rather than a reliance on, oxidative metabolism. This hypothesis was investigated during intermittent (12 s/12 s) maximal (6 MVC's in total) exercise under both free flow and ischaemic conditions (Lanza *et al.*, 2007). By using ischaemic conditions, the ability of the muscle to generate ATP oxidatively was removed and its ability to generate sufficient ATP via anaerobic glycolysis was determined. Interestingly, older adults fatigued less (assessed using central activation ratio) than young adults under both free-flow and ischaemic conditions. While during free-flow conditions the young group generated more ATP via glycolysis, there was no difference in glycolytic flux during ischaemia, implying that glycolytic flux was unimpaired in the older group. This suggests that older adults show a preference for, rather than a reliance on, oxidative metabolism. Furthermore, consistent with previous studies (Kent-Braun *et al.*, 2002; Lanza *et al.*, 2005) a significant correlation existed between the decline in force production during the fatiguing protocols and the accumulation of the metabolite $H_2PO_4^-$ (Lanza *et al.*, 2007). Also, upon analysing a subgroup of individuals, older adults were more energy efficient (greater metabolic economy)

during the unoccluded exercise protocol compared with the young group (Lanza *et al.*, 2007).

Consistent with this notion, the energy cost of an evoked twitch was found to be lower (27%) in older compared with young adults during ankle dorsiflexion exercise (Tevald *et al.*, 2010). The energy cost of an evoked twitch correlated strongly with the maximum rate of force relaxation, suggesting that the lower energy cost may be related to age-related changes in fibre type composition, which reduces contractile costs in older men (Tevald *et al.*, 2010). Overall, the age-related reduced energy cost of a contraction may be due to both the increase in the proportion of type I fibres, which have been shown to be more economical than type II fibres, resulting in slower contractile properties, lower motor unit discharge rates and a leftward shift in the force frequency curve. This would also result in a greater reliance on oxidative metabolism and therefore less accumulation of by-products as a result of glycolysis. Thus, changes within the ageing neuromuscular system may infer an economical advantage during fatiguing exercise protocols.

In the present study, the age-associated enhancement of fatigue resistance appeared to be more pronounced in older men compared with older women at the higher exercise intensity of 70% MVC. It is difficult to specify the reason for this gender difference as there are several age-related alterations that occur within the human skeletal muscle system. It may be that as women age, they experience the concomitant effects of loss of oestrogen, which may hinder fatigue resistance and muscle endurance. However, an increase in the proportion of type I fibres would enhance fatigue resistance among women but to a lesser extent than among men. On the other hand, as men age, their muscular endurance may largely reflect histological changes alone.

4.4.5 Conclusion

In conclusion, in the present study older men displayed enhanced fatigue resistance and muscular endurance during plantar flexion intermittent exercise compared with young individuals, while older women displayed similar fatigue profiles compared with young women. Both older groups of individuals displayed lower MVC's than

their respective young groups. These results indicate that ageing is associated with positive neuromuscular alterations, so that older individuals can maintain a similar level of muscular endurance as young individuals in order to preserve physical functioning in the face of declining muscular strength and slowing of haemodynamic adaptations.

Chapter 5 General discussion

Population ageing has become one of the most critical issues facing modern society, due to the rapid increase in the number of ageing individuals. In Ireland, the proportion of the population aged 65 years and over is projected to double in the next 20 years, while the over 80's will be the fastest growing segment of the population, with numbers expected to treble by 2036 (Central statistics office, 2004). This change in population demographics will pose a major challenge in sustaining an independent standard of living for older people.

Physiological ageing is an unavoidable multifaceted process, which involves a dramatic reduction in neuromuscular function and exercise performance (Doherty, 2003). This usually results in a progressive decline in functional capacity and an ultimate loss of independence. Much of the previous research examining the physiological basis of age-related reductions in exercise performance have focused on quantifying the reductions in muscular strength and muscle mass (Porter *et al.*, 1995) which result as a consequence of a loss of muscle fibres, which are linked to the overall age-associated decline in maximal power output. However, an age-related reduction in leg blood flow to the active skeletal muscle has been implicated as one of the underlying factors contributing to a decline in physical activity and exercise performance evident with the ageing process. Despite these findings, there have only been a few investigations that have directly examined the influence of age and gender related haemodynamic responses and exercise performance (Martin *et al.*, 1991; Proctor *et al.*, 1998; Proctor *et al.*, 2003b; Proctor *et al.*, 2004; Donato *et al.*, 2006; Parker *et al.*, 2008) and the results are still inconclusive. Methodological differences (mode/modality) or variations in subject's characteristics (age, fitness levels) may account for the contrasting findings. For instance, some studies using older males have reported a preservation in leg blood flow and vascular conductance during graded single knee extension exercise performed to maximal exertion (Parker *et al.*, 2008) and during both graded and constant-force leg cycling exercise (Proctor *et al.*, 2003b). In contrast, other investigations have indicated that older men display an attenuated leg blood flow response when performing similar dynamic single knee

extensor exercise (Donato *et al.*, 2006). The limited number of studies which have examined exercising leg blood flow and vascular conductance responses in older women, report attenuated responses for single leg graded knee extension exercise (Parker *et al.*, 2008) and for graded and constant-force dynamic leg cycling exercises (Proctor *et al.*, 2003a; Proctor *et al.*, 2004).

Thus, there are inconsistencies in the literature as to whether older adults have a preserved vasodilatory capacity during lower limb exercise, which is functionally very relevant given the fact that most physically demanding activities of daily living involve leg exercise. Furthermore, it is vital to ascertain whether healthy older adults have the ability to submaximally and maximally augment blood flow in response to functionally demanding tasks requiring both submaximal and maximal muscle force generation, which may ultimately determine their ability to live and function independently. In addition, the majority of previous studies examining age-related peak and exercising leg blood flow and vascular conductance responses have primarily involved cycling or knee extension exercise whereas plantar flexor exercise, which is fundamental for walking and maintaining postural stability, has not been investigated. Hence, our first study examined peak and exercising calf blood flow and vascular conductance responses during a graded plantar flexion exercise to maximal exertion in older compared with young men and women.

In this initial study, older men and older women exhibited similar peak hyperaemic and peak vascular conductance responses to graded exercise relative to their young counterparts. In addition, the slopes of the hyperaemic and vasodilatory responses (expressed relative to each persons maximal force) did not reveal any age or gender differences. Thus, although performance, expressed as the peak force achieved at the end of the incremental test, was significantly lower, older adults retained the ability to augment blood flow and vascular conductance in a similar manner to young adults. As discussed earlier, these findings are in agreement with some previous studies (Proctor *et al.*, 2003b; Parker *et al.*, 2008) but at odds with others (Proctor *et al.*, 1998; Donato *et al.*, 2006) that have investigated age-related differences in blood flow and vascular conductance responses in men. However, the preserved blood flow

and vascular conductance responses among older women observed in the present study are in contrast with previous findings in older women during cycling (Proctor *et al.*, 2003a; Proctor *et al.*, 2004) and leg kicking exercise (Parker *et al.*, 2008). This may be possibly due again to methodological differences and/or our female subjects displaying higher fitness levels compared with subjects from previous studies (Parker *et al.*, 2008). Overall, the initial study indicates that the reduction in calf muscle performance exhibited by the older group of adults was not due to attenuated vascular conductance responses since peak haemodynamic parameters were not different beween young and older adults, instead it may have been related to a slower rate of increase in blood flow (i.e. blood flow kinetics) and thus oxygen, to the exercising muscles.

To our knowledge the kinetics of blood flow in response to exercise has only been investigated in one study (DuManoir *et al.*, 2010). DuManoir *et al.*, (2010) showed slower blood flow kinetics in older adults at absolute intensities (24 W) but not intensities relative to lactate threshold (80% LT) during knee extension exercise. Also, DuManoir *et al.*, (2010) only employed moderate intensity exercise and thus, it was not clear if blood flow kinetics were affected by age at low and high exercise intensities.

Thus my second experiment examined the vascular conductance (VC) kinetic response during constant-force plantar flexion exercise performed at a wide range of relative exercise intensities (30%, 45%, 60% and 70% MVC) in older men and women compared with young men and women. These exercise intensities were chosen since every day functional tasks involve performing muscle contractions at both low and high intensities. The vascular conductance kinetic responses were fitted using a biexponential model because this model was a better fit than either the monoexponential or triexponential models (higher r^2 values).

The results from this study revealed no age differences in the parameters defining phase 1 of the hyperaemic response at any intensity. However, phase 2 time constant (τ_2) and the mean response time responses were larger in older compared with young

individuals during plantar flexion exercise performed at all four relative exercise intensities of 30%, 45%, 60% and 70% MVC. Despite older individuals showing slower vascular conductance kinetics at all intensities, the size of increase in vascular conductance was not different to the young group as evidenced by similar end-exercise amplitudes. This implied that older adults retained the capacity to vasodilate in a similar manner to their young counterparts during steady state exercise despite a slower adjustment in the vascular conductance kinetics at the onset of exercise.

Since ageing did not significantly influence phase 1 of the biphasic vascular conductance response, it is likely that the control mechanisms contributing to the initial hyperaemic response during exercise, including the muscle pump action and /or vasodilation, are not greatly impacted by the ageing process. Instead, mechanisms contributing to phase two of the leg vascular conductance response are most likely to be involved in the impaired vascular conductance kinetic response including an attenuation in the release of nitric oxide from the endothelium during exercise, a decline in ACh-mediated endothelium dependent vasodilation (Taddei *et al.*, 1995; Taddei, 1997; DeSouza *et al.*, 2000) and the age-associated increase in inflammatory agents. Thus, in order to further examine the role that the endothelium may play in blood flow and vascular responses in ageing individuals, both forearm and calf vascular conductance (VC) reactive hyperaemic response were determined. However, there were no age-detected differences in peak VC, amplitude (A) or decay constant between the groups. An explanation for this may be the small number of individuals willing to take part in both the arm and leg reactive hyperaemic tests in the present study.

The second experiment also revealed that resting calf blood flow was higher in older individuals compared with young individuals, while resting vascular conductance was similar between both groups, due to a greater pressor response displayed by older adults. This is in direct contrast to findings from previous research studies (Dinenno *et al.*, 1999; 2001; Miyachi *et al.*, 2005; Anton *et al.*, 2006; Donato *et al.*, 2006; Parker *et al.*, 2008), which found that older adults displayed lower resting blood flow and vascular conductance compared with their young counterparts. One plausible

explanation for the differences between findings may be that older individuals in the present study were more physically active compared to individuals, which took part in previous studies. Alternatively, these contrasting findings might be due to our older groups displaying larger skeletal muscle mass and thus a greater leg oxygen requirement. Also significantly larger BMI scores in the older compared with the young groups might contribute to this. However, due to experimental limitations, leg muscle mass was not measured in the present study and future studies are required to elucidate this.

The third study examined the relationship between haemodynamic alterations and muscle fatigue responses within the same exercise model in the same older and young participants. To our knowledge this has not been previously investigated. It is necessary to understand how the development of muscle fatigue may be impacted by the delivery of blood flow and hence oxygen, to the exercising muscle in ageing individuals. Furthermore, the ability of older adults to perform repeated muscle contractions at a given intensity while avoiding muscle fatigue is critical to activities of daily living. Hence, the third study examined fatigue profiles performed at the same relative exercise intensities (30%, 45%, 60% and 70% MVC) that were previously employed during the constant-force plantar flexion exercise where blood flow and vascular conductance were assessed (chapter 2).

In this third expeiment, muscle fatigue was determined by the degree to which maximum force is reduced over time (rate of fatigue), while muscle endurance was assessed by the endurance time to task failure only at the highest intensity of 70% MVC. Fatigue resistance, as evidenced by percent force decrements was greater in older adults compared with young adults only at 70% MVC. During the 60% MVC plantar flexion exercise, both older men and women displayed enhanced fatigue resistance compared with young men and women, as evidenced by a lower rate of fatigue. Muscular endurance was greater for older compared with young men during the 70% MVC plantar flexion exercise, while in addition, older men displayed greater fatigue resistance at this intensity compared with young men. However, at the higher exercise intensity of 70% MVC, women displayed similar fatigue profiles and

muscular endurance compared with young women suggesting that the age-related effect on fatigue was less pronounced among women. This was possibly due to the fact that all older women were post-menopausal and thus lacked the positive effects of oestrogen. At the lower intensities of 30% and 45% MVC, there were no differences in fatigue profiles as evidenced by similar rates of fatigue among the four groups.

These results indicate that ageing is associated with positive neuromuscular alterations, allowing older individuals to maintain similar or even higher levels of muscular endurance as young individuals so as to preserve physical functioning despite declining muscular strength. Thus, even though leg vascular conductance kinetics were slower in older adults, performance and fatigue were not impaired and were in fact enhanced in older men compared wtih young mwn. This is consistent with the majority of previous research investigations examining fatigue in older compared with young individuals during knee extension (Rawson, 2009), elbow flexion (Bilodeau *et al.*, 2001), and dorsiflexion (Kent-Braun *et al.*, 2002, Russ *et al.*, 2008) exercise.

5.1 Possible mechanisms underlying fatigue resistance despite blunted kinetic response

The possible mechanisms responsible for the age-associated fatigue resistance despite blunted kinetic response are depicted in figure 5.1. It seems that ageing is associated with positive mechanisms, which compensate for the reduction in VC kinetic response observed during exercise. Firstly, a decline in strength as the result of lower muscle mass reduces intramuscular pressure and blood flow occlusion. This facilitates a greater delivery of oxygen to the working muscle and a greater clearance of exercise induced metabolic by-products, which results in enhanced fatigue resistance and muscular endurance. Furthermore, older adults have a greater reliance on oxygen extraction to accommodate oxidative requirements of the muscle during the transition to steady-state exercise. This coupled with the age-related

morphological shift in fibre type to a more fatigue resistance type I fibre and a relatively greater reliance on oxidative metabolism, may contribute to the enhancement of fatigue resistance displayed by older adults compared with young adults during this type of exercise. Thus, despite an age-associated slowing in vascular conductance kinetics observed at relative exercise intensities, age-associated neuromuscular changes confer a positive effect on performance during fatiguing exercise in order to maintain a minimum level of functional capacity with ageing in the face of declining muscular strength and haemodynamic alterations.

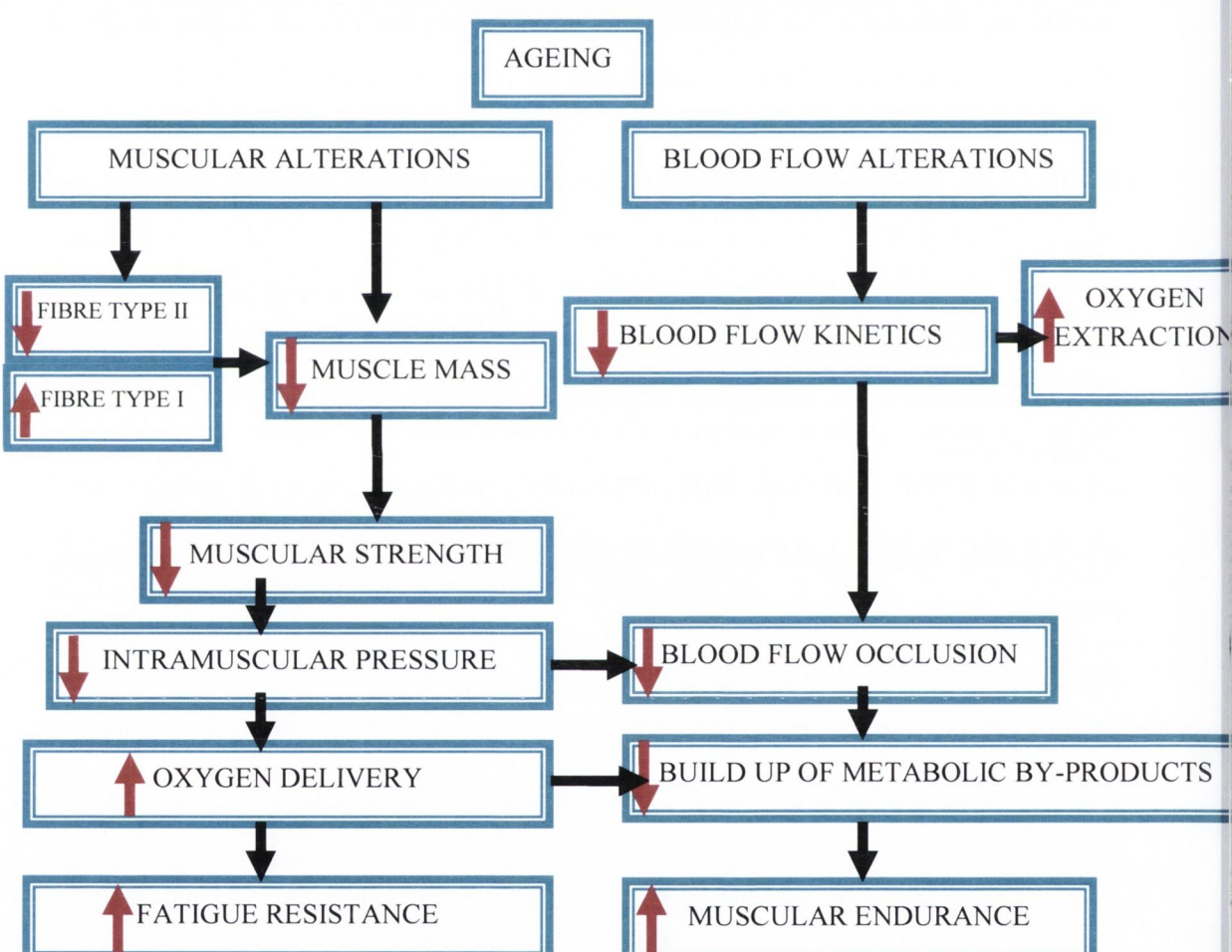


Figure 5.1. Some possible mechanisms underlying enhanced fatigue resistance and muscular endurance despite blunted kinetic response in older compared with young adults.

5.2 Important functional implications of thesis findings

There are several functional implications of these findings. The slower leg blood flow kinetic responses evident in older individuals but yet similar peak heaemodynamic responses suggests that older adults may be compensating for the slower blood flow kinetics with augmented oxygen extraction. This together with age-related morphological and muscle metabolism alterations may also offset the negative haemodynamics apparent during plantar flexion exercise allowing older adults to continue an activity despite lower muscular strength .

In addition, given the decline in muscle strength that accompanies ageing, any advantage in terms of the capacity to resist fatigue has major implications for ageing adults that are facing mobility impairments. For instance, an ability to prevent the fall in force during repetitive, muscle contractions could in some way counteract the decline in baseline strength and allow an older individual to remain above the minimum level of force needed to continue an activity of daily living. Thus, fatigue resistance would have a significant impact on functional mobility.

5.3 Limitations

There are a number of experimental limitations in the present study. Firstly, we did not perform measures of deoxyhaemoglobin (to estimate extraction of O_2), leg muscle mass and/or accelerometry or $VO_{2\ max}$ (to objectively quantify activity/fitness levels). This was due to a lack of access to equipment to measure the above parameters.
Secondly, blood flow responses were not measured using Doppler ultrasound, which would also have allowed the measurement of blood velocity and cross sectional area of blood vessels. However, a recent study (Green *et al.*, 2011) indicates similar blood flow responses between Doppler ultrasound and venous occlusion plethysmography during a graded calf plantar flexion exercise, although more reliability studies are needed to demonstrate that blood flow kinetic responses obtained using VOP are well correlated to those obtained using Doppler ultrasound. It would also have been

beneficial to determine blood biomarkers of endothelial function (iCAM and vCAM) to detect a potential endothelial dysfunction among the older participants. However, funding limitations precluded us from performing these measurements.

The main conclusions from the present thesis are depicted in figure 5.2. Older adults demonstrated a preservation in maximal vascular conductance during incremental plantar flexion exercise performed to maximal capacity but slower leg vascular conductance kinetic responses during constant-force exercise performed at high and low relative exercise intensities. However, despite deleterious haemodynamic effects, performance and fatigue were not impaired and were in fact enhanced in older adults. This highlights the important physiological alterations that occur with ageing so that older adults retain the ability to perform activities of daily living, which include performing tasks repeatedly or for long periods of time, which requires the capacity to resist the development of muscle fatigue.

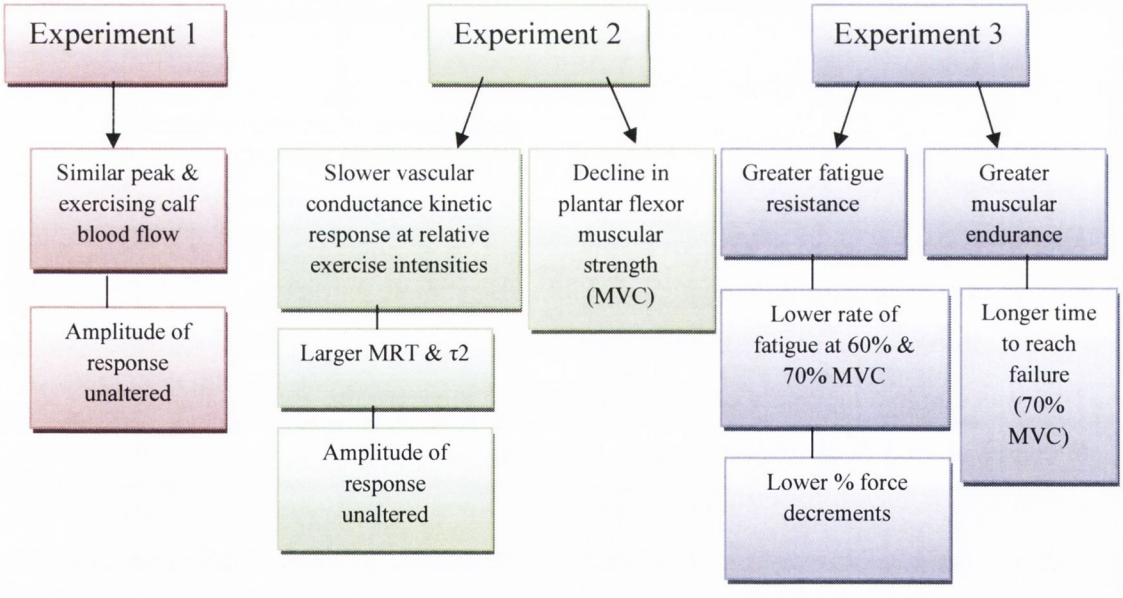


Figure 5.2. Main conclusions from the present thesis

Appendix I: Participant information and consent form

PARTICIPANT INFORMATION AND CONSENT FORM

1. Title of study: Leg blood flow responses in young and older healthy non-diabetic men and women.

2. Introduction: The main objective is to quantify peak muscle performance and blood flow responses during a novel incremental calf-exercise in healthy young versus old men and women.

3. Procedures: You will be one of the 50 volunteers recruited for this study. Your age will be over the age of 55 years (Group 2, OLDER). Before the commencement of the project, you will undergo a full medical examination by a General Practitioner, where a blood sample will be taken. If found able to participate in this study, you will be required to visit the Cardiovascular Health Unit in the Department of Physiology in Trinity College Dublin on 2 occasions separated at least by 72 hours.

- Visits to the laboratory (TCD):

Visit 1: Familiarisation session and calf maximum voluntary contractions.

You will be familiarised with the calf ergometer and the protocol to be completed on visit 2. You will then try and complete six calf maximal voluntary contractions (MVC) (1 min rest periods between maximal efforts) in the upright position (tilt angle of 67°).

Visit 2: Incremental calf muscle exercise test (session will last up to 90 min).

You will try and complete an incremental plantar flexion exercise to the point of failure in the inclined position. During the exercise test you will be required to increase your level of effort in a stepwise manner. The test will be terminated when you are unable to sustain the required contraction force for 2 consecutive contractions.

4. Benefits:

There are no direct benefits to you in this study. However, you will gain an understanding into the changes that occur in blood flow with age.

5. Risks:

There are very few risks associated with the exercise procedures used during this study:

Calf exercise: Performance of plantar flexion exercise may result in muscle tightness, soreness, fatigue and rarely a pulled muscle.

Blood sampling may make some volunteers feel uneasy, or prove painful to some. Some may experience slight bruising or discomfort around the sampling area.

6. Exclusion from participation:

You will be excluded from the study if you are taking any medications that could affect your peripheral circulation, have an abnormal HbA_{1c}, and have any active medical problems.

7. Confidentiality:

Your identity will remain confidential. Your name will not be published and will not be disclosed to anyone outside the study group. The data or material will be retained after the study is completed. This material will not be used in future unrelated studies without further specific permission being obtained.

8. Compensation:

This study is covered by standard institutional indemnity insurance. Nothing in this document restricts or curtails your rights.

9. Voluntary Participation:

You have volunteered to participate in this study. You may quit at any time. If you decide not to participate, or if you quit, you will not be penalised and will not give up any benefits that you had before entering the study.

10. Stopping the study:

You understand that the investigators may stop your participation in the study at any time without your consent.

11. Permission: This trial has Research Ethics Committee approval from Trinity College Dublin.

12. Further information:

You can get more information or answers to your questions about the study, your participation in the study, and your rights, from Ms Heather Reilly (086-3273872/ heathernreilly@hotmail.com)) or Dr Mikel Egaña, (01-8963728 / megana@tcd.ie). If the study team learns of important new information that might affect your desire to remain in the study, you will be informed at once.

DECLARATION:

I have read, or had read to me, this consent form. 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. 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.

I understand I may withdraw from the study at any time.

(Name of sponsor:)

……

PARTICIPANT'S NAME:……

CONTACT DETAILS:……..

PARTICIPANT'S SIGNATURE:……

Date:……..

NAME OF CONSENTER, PARENT or GUARDIAN……..

(if under 18 years old)

SIGNATURE:……

RELATION TO PARTICIPANT:……..

Statement of investigator's responsibility: I have explained the nature and purpose of this research study, the procedures to be undertaken and any risks that may be involved. 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.

INVESTIGATOR'S SIGNATURE:…… **Date:**……

Appendix II: Medical history questionnaire

DEPARTMENT OF PHYSIOLOGY, TRINITY COLLEGE, DUBLIN.

MEDICAL QUESTIONNAIRE

Project Title:_____

Supervising Researcher:_____

Principal Investigator:_____

Medical Personnel/Physician:_____

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 (eg: anabolics, steroids, β-blockers...)? YES NO

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

_____

_____

15. Are you currently taking any other dietary supplements (eg: 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:_____

Signature of Supervising Researcher:_____Date:_____

Signature of Principal Investigator:_____Date:_____

Following completion of this survey and a physical assessment of he above listed volunteer/subject, I concluded that there are no evident contraindications to participation in the study entitled above and according to the study proposal which has received appropriate ethical approval from the School of Medicine, Faculty Research Ethics Committee.

Signature of Physician:_____Date:_____

Appendix III: Low level physical activity recall (LOPAR) questionnaire

LOW-LEVEL PHYSICAL ACTIVITY RECALL (LO-PAR)

Instructions for Administration: Determine for each major category (sleep, work, house or yard, recreation or leisure) the estimated number of hours/week spent within that category during the **preceding** week. Then using the cards as prompts, ask about specific activities within each intensity of activity (heavy to very light). It is not expected that every hour of the week can be accounted for. However, asking the subject to estimate their total sleep hours, and the total expected hours within each major category of activity (168 hours/week), as compared to the break-down of activities within each major category of activity, helps the subject more reliably remember their activities. Instructions for question 2 pertain to all three major categories of activity.

Scoring: For each activity (heavy to very light), calculate the number of hours/week spent in that activity (days/week x hours/day). Sum hours/week in each category to determine total hours per week. The amount of energy expenditure for each activity is expressed as metabolic equivalents (METs). One MET equals 3.5 ml/kg/min or oxygen consumption. Activities are classified according to the following scale: very light (0.9-2.0 METs), light (2.1-3.0 METs), moderate (3.1-5.0 METs) and heavy (5.1-7.0 METs). Data are reported in MET hours/week (hours/week x the MET value of the activity).

1. How many hours do you sleep a night, on average? _____ hours x 7 **Sleep hours/week = _____**
2. Explain to subject that you are going to ask about typical **WORK** activities performed during the past week (includes work for pay or regular volunteer activities). If subject not employed, go to question #3.
How many total hours did you work per week on average? **Work hours/week= _____**

Here is a listing of typical work activities (**Show participant Card A**). Activities are classified as heavy, moderate, light and very light depending on their average energy demands. With your job, time may be spent in more than one category of activity. Let's start with heavy activities and then go on to moderate, light, and then very light activities. a) Please tell me the average number of days during the last week you performed heavy activities at work. b) Please tell me the average length of time you performed heavy activities in a day. Then, repeat above directions for all intensities of activity.

INTENSITY OF ACTIVITY	DAYS/WEEK (0.5 to 7.0)	HOURS/DAY (nearest 0.5 hr)	HOURS/WEEK	MET HOURS PER WEEK
HEAVY (5.1-7.0 METs)				
MODERATE (3.1-5.0 METs)				
LIGHT (2.1-3.0 METs)				
VERY LIGHT (0.9-2.0 METs)				
TOTAL				

3. Did you perform **HOUSEHOLD CHORES OR YARD WORK** around the home during the past week (**Follow instructions given above, except refer to Card B**)? If yes, how many total hours did you spend in household chores? Household or yard hours/week = _____

INTENSITY OF ACTIVITY	DAYS/WEEK (0.5 to 7.0)	HOURS/DAY (nearest 0.5 hr)	HOURS/WEEK	MET HOURS PER WEEK
HEAVY (5.1-7.0 METs)				
MODERATE (3.1-5.0 METs)				
LIGHT (2.1-3.0 METs)				
VERY LIGHT (0.9-2.0 METs)				
TOTAL				

4. Did you perform **RECREATIONAL OR LEISURE-TIME ACTIVITIES** during the past week (**refer to Card C**)? If yes, how many total hours did you spend in leisure activities?

Recreation or leisure hours/week = _____

INTENSITY OF ACTIVITY	DAYS/WEEK (0.5 to 7.0)	HOURS/DAY (nearest 0.5 hr)	HOURS/WEEK	MET HOURS PER WEEK
HEAVY (5.1-7.0 METs)				
MODERATE (3.1-5.0 METs)				
LIGHT (2.1-3.0 METs)				
VERY LIGHT (0.9-2.0 METs)				
TOTAL				

SELECTED LIST OF ACTIVITIES WITH MET VALUES (IN PARENTHESES)*

CARD A **PHYSICAL ACTIVITIES AT WORK**

HEAVY	MODERATE	LIGHT	VERY LIGHT
Heavy power tools (6.0)	Locksmith (3.5)	Cashier (2.5)	Sitting (1.5)
Coal mining (7.0)	Carrying <20 lbs. (5.0)	Light assembly (2.5)	Standing (2.0)
Loading truck (6.5)	Farming (4.5)	Physician (2.5)	Typing (1.5)
Shovelling (7.0)	Machine tooling (4.0)	Teacher (2.5)	Computer work (1.5)
Heavy carpentry (7.0)	Forestry, chain saw (4.5)	Tailoring, machine (2.5)	Receptionist (1.5)

CARD B **HOUSEHOLD CHORES AND YARD WORK**

HEAVY	MODERATE	LIGHT	VERY LIGHT
Roofing (6.0)	Food shopping (3.5)	Preparing meals (2.5)	Sitting (1.5)
Digging (5.0)	Heavy cleaning (4.5)	Sweeping (2.5)	Standing/laundry (1.5)
Chopping wood (6.0)	Laying carpet (4.5)	Making bed (2.5)	Fold, hang clothes (1.5)
Shovelling snow (6.0)	Weeding (4.5)	Fertilizing (2.5)	Sewing (1.5)
Manual lawn mowing (6.0)	Power lawn mowing (4.5)	Ironing (2.3)	

CARD C **RECREATIONAL ACTIVITIES**

HEAVY	MODERATE	LIGHT	VERY LIGHT
Walking/hiking uphill (6.0)	Locksmith (3.5)	Cashier (2.5)	Sitting (1.5)
Moderate canoeing (7.0)	Carrying <20 lbs. (5.0)	Light assembly (2.5)	Standing (2.0)
Bicycling 10-12 mph (6.0)	Farming (4.5)	Physician (2.5)	Typing (1.5)
Light stationary cycle (5.5)	Machine tooling (4.0)	Teacher (2.5)	Computer work (1.5)
Aerobic dance (7.0)	Forestry, chain saw (4.5)	Tailoring, machine (2.5)	Receptionist (1.5)
Leisurely swimming (6.0)			

*MET values for many activities can be obtained from Ainsworth BE., *et al.* 1993 Compendium of physical activities: classification of energy costs of human physical activities. Med Sci Sport Exerc 1993;25:71-80.

Appendix IV: Medical examination

UNIVERSITY OF DUBLIN

Department of Physiology

MEDICAL EXAMINATION FORM

PERSONAL DETAILS

Name	e-mail
Date of Birth	Telephone
Address:	Do you smoke?
Do you perform regular exercise?	

MEDICAL HISTORY

Have you ever had any of the following?

	Yes	No		Yes	No
Angina			Heart Attack		
Rheumatic Fever			Irregular heart beat (Arrhythmia)		
Heart Murmur			Diabetes		
Stroke			Mini-Stroke (TIA)		
Epilepsy			Asthma		
Tuberculosis(TB)			Cancer		
Jaundice			Arthritis		
Anaemia			Sporting Injury		
Are you on any medicines?			Are you allergic to anything?		

Surgical History

Have you ever had any operations?

If yes, what did you have done and when?

Operation	Year

Were there any complications? _____

Systems Review

Do you suffer from any of the following?

	Yes	No		Yes	No
Headaches			Sore Throat		
Loss of Vision			Abdominal Pain		
Loss of power to the legs or arms			Constipation		
Numbness or unusual feeling in legs or arms			Diarrhoea		
Chest Pain			Nausea		
Shortness of Breath			Vomiting		
Palpitations			Unusual Bleeding or bruising		
Cough			Kidney or Bladder problems		

Examination Findings

Blood Pressure	Pulse		
Respiratory	Cardiovascular		
Other findings	Chol	Height	Weight
ECG	Signed		Date

Appendix V: Calf ergometer calibration for 162 test days. Corresponding mV for 20 kg and 80 kg are shown. Coefficient of variation (CV) is also shown.

Test day	20kg mV	80 kg mV	Test day	20kg mV	80 kg mV	Test day	20kg mV	80 kg mV	Test day	20kg mV	80 kg mV
1	731.5	2801.5	41	731.9	2807.9	82	767.2	2906.2	122	719.6	2840.6
2	753.6	2800.0	42	757.8	2911.8	83	778.0	2672.2	123	781.5	3049.5
3	763.9	2896.9	43	753.4	2862.4	84	793.7	3115.7	124	783.8	3030.8
4	838.7	3298.7	44	712.6	2764.6	85	764.1	2972.1	125	751.1	2842.1
5	800.0	3235.6	45	731.7	2774.7	86	737.1	2912.1	126	752.0	2593.6
6	784.3	2983.3	46	750.0	2814.9	87	752.5	2858.5	127	753.0	2652.2
7	802.5	3094.5	47	801.0	2865.0	88	771.2	2895.2	128	750.9	2904.9
8	762.7	2898.7	48	673.6	2587.6	89	694.2	2659.2	129	780.6	2970.6
9	735.5	2847.5	49	639.2	2496.2	90	765.7	2988.7	130	696.1	2637.1
10	837.0	2800.0	50	822.7	3048.7	91	794.7	2927.7	131	706.9	2647.9
11	867.1	2700.0	51	750.0	2592.9	92	764.1	2897.1	132	725.3	2789.3
12	753.3	2982.3	52	780.5	2988.4	93	721.2	2860.2	133	717.5	2754.5
13	836.8	3196.5	53	711.7	2724.7	94	741.5	2838.5	134	734.1	2843.1
14	750.0	2832.9	54	716.8	2837.9	95	771.2	2895.2	135	741.2	2832.2
15	762.0	2943.0	55	782.9	2963.9	96	768.2	2940.2	136	769.4	2970.4
16	745.5	2800.5	56	711.7	2724.7	97	718.1	2830.1	137	765.2	2919.2
17	700.8	2737.8	57	716.5	2774.5	98	764.1	2897.1	138	752.1	2888.1
18	728.9	2849.9	58	709.2	2752.2	99	742.3	2860.3	139	706.9	2647.8
19	708.1	2769.2	59	734.1	2843.1	100	721.2	2860.2	140	742.9	2833.9
20	693.9	2772.9	60	734.3	2825.3	101	782.7	2852.7	141	728.9	2849.9
21	719.7	2804.7	61	714.6	2805.6	102	798.7	3114.7	142	696.9	2757.9
22	719.5	2774.5	62	708.2	2769.2	103	782.7	2852.7	143	724.5	2752.5
23	723.4	2778.4	63	707.1	2708.1	104	742.3	2860.3	144	729.7	2766.7
24	739.9	2956.9	64	766.8	2932.8	105	748.3	2848.3	145	786.7	2747.0
25	710.7	2738.7	65	735.9	2817.9	106	750.1	2895.1	146	734.1	2843.1
26	738.2	2832.2	66	732.9	2742.9	107	724.5	2773.5	147	722.6	2714.6
27	739.3	2875.3	67	780.5	2988.5	108	763.5	2833.5	148	755.0	2876
28	797.9	3188.9	68	795.4	3036.4	109	745.4	2839.4	149	734.3	2825.3
29	739.9	2956.9	69	742.9	2833.9	110	636.8	2424.9	150	741.2	2832.2
30	710.7	2738.7	71	700.8	2737.8	111	757.9	2854.9	151	749.1	2825.1
31	731.9	2777.9	72	763.2	2935.2	112	745.4	2839.4	152	734.3	2825.3
32	698.8	2660.8	73	732.9	2742.9	113	703.7	2749.7	153	807.9	2714
33	700.7	2653.7	74	735.9	2817.9	114	764.1	2972.1	154	822.7	2610
34	693.5	2655.5	75	782.9	2963.9	115	765.7	2988.7	155	775.0	2593.6
35	749.6	2843.6	76	723.4	2778.4	116	765.7	2988.7	156	773.0	2933
36	720.3	2808.3	77	768.2	2940.2	117	764.1	2972.1	157	775.0	2801
37	731.9	2777.9	78	822.7	3048.7	118	788.6	2981.6	158	780.0	2876
38	735.5	2781.5	79	744.7	2874.7	119	771.0	3009	159	714.5	2766.5
39	720.4	2736.4	80	762.2	2937.2	120	761.5	2942.5	160	711.8	2793.8
40	743.9	2894.9	81	766.8	2932.8	121	727.7	2872.7	161	745.8	2869.8
									162	751.1	2842.1
									Mean	747.7	2845.3
									SD	35.3	130.8
									% CV	4.7	4.6

Appendix VI: Comparison between volume clamp method (Finometer) and applanation tomography (COLIN). Blood pressure responses for five subjects during an incremental test are shown. Coefficient of variation (CV) is also shown.

Subject	Time	Colin	Finopres	C-F	$(C-F)^2$	Subject	Time	Colin	Finopres	C-F	$(C-F)^2$
1	REST	83	79	5	21.1	3	Min 9	107	103	4	20.0
1	Onset	83	82	1	0.9	3	Min 10	105	100	5	26.8
1	Min 1	91	88	2	4.2	3	Min 11	105	111	-7	45.5
1	Min 2	92	89	3	7.0	3	Min 12	106	110	-4	16.9
1	Min 3	97	95	2	5.2	4	REST	86	83	3	8.7
1	Min 4	79	81	-1	2.2	4	Onset	90	92	-2	5.4
1	Min 5	68	69	-1	2.1	4	Min 1	89	91	-2	4.0
1	Min 6	90	85	5	25.8	4	Min 2	92	91	0	0.1
1	Min 7	94	93	0	0.0	4	Min 3	87	92	-6	30.9
1	Min 8	88	89	-1	0.3	4	Min 4	96	90	5	28.5
1	Min 9	98	101	-3	7.3	4	Min 5	98	98	0	0.0
1	Min 10	101	102	-2	3.2	4	Min 6	96	99	-3	9.0
1	Min 11	97	91	5	30.0	4	Min 7	95	96	-1	1.0
1	Min 12	98	94	4	19.3	4	Min 8	96	98	-2	2.8
2	REST	74	77	-3	9.1	4	Min 9	99	98	2	2.8
2	Onset	100	96	4	13.6	4	Min 10	107	105	2	5.4
2	Min 1	95	91	4	16.6	4	Min 11	108	109	-1	0.4
2	Min 2	98	96	2	4.7	4	Min 12	111	113	-2	2.8
2	Min 3	101	97	4	15.5	5	REST	83	85	-3	6.3
2	Min 4	104	100	4	18.7	5	Onset	99	97	2	3.3
2	Min 5	95	93	1	2.0	5	Min 1	109	96	13	181.2
2	Min 6	100	95	6	30.6	5	Min 2	104	88	16	258.1
2	Min 7	100	96	4	17.5	5	Min 3	112	104	8	69.7
2	Min 8	100	96	4	19.4	5	Min 4	101	100	1	0.3
2	Min 9	100	96	4	12.6	5	Min 5	113	99	13	180.1
2	Min 10	99	99	0	0.1	5	Min 6	105	104	1	1.8
2	Min 11	100	99	1	1.3	5	Min 7	97	96	1	1.4
2	Min 12	101	100	1	0.3	5	Min 8	105	102	3	11.3
3	REST	83	85	-3	6.3	5	Min 9	107	106	1	1.7
3	Onset	99	97	2	3.3	5	Min 10	105	100	5	26.8
3	Min 1	109	96	13	181.2	5	Min 11	112	111	1	0.4
3	Min 2	104	88	16	258.1	5	Min 12	106	110	-4	16.9
3	Min 3	112	104	8	69.7		MEAN	97	93	4	1264.6
3	Min 4	109	100	8	68.9		SD	10	8	5	10.5
3	Min 5	113	99	13	180.1						
3	Min 6	105	95	10	103.5						
3	Min 7	110	104	6	40.8						
3	Min 8	105	98	8	61.6						

Inter class correlation co-efficient = 0.8

% TEM = 3.4%

95% LOA= -5±13 mmHg

Appendix VII: Anthropometrical and physical activity data for subjects who completed the incremental exercise test to maximal exertion

Young men

	Age	Height	Weight	BMI	Leg volume	Activity
	yr	cm	Kg	$Kg.m^{-2}$	mL	$MET.h.week^{-1}$
1	24	171	63.0	21.5	2119	142
2	23	192	79.0	21.4	3219	162
3	27	192	87.8	23.8	4470	170
4	24	172	83.8	28.3	2104	140
5	21	180	87.0	26.9	3263	171
6	23	188	76.5	21.6	3388	193
7	23	180	97.2	30.0	4207	133
8	23	166	55.1	20.1	2628	207
9	21	183	62.5	18.6	2021	227
10	24	178	65.4	20.7	1900	212
11	28	173	68.6	22.9	3029	186
12	27	175	65.2	21.2	2309	186
13	24	176	86.5	28.0	3234	169
14	23	180	66.5	20.5	2192	193
15	21	175	57.9	18.9	2508	225
Mean	24	179	73.5	23.0	2839	181
SD	2	7	12.3	3.5	766	29

Older men

	Age	Height	Weight	BMI	Leg volume	Activity
	yr	cm	Kg	$Kg.m^{-2}$	mL	$MET.h.week^{-1}$
1	72	173	77.2	25.8	2967	250.0
2	68	173	65.5	21.8	2837	177.5
3	68	173	71.6	24.0	2877	195.5
4	70	165	71.1	26.0	2827	179.2
5	72	160	70.0	27.3	3009	233.0
6	73	160	68.6	26.9	2518	162.5
7	74	181	90.8	27.7	3818	157.6
8	70	183	90.3	27.0	3678	173.5
9	67	173	70.1	23.3	2506	269.7
10	60	165	85.7	31.4	2976	160.8
11	89	165	71.2	26.3	2079	150.0
12	60	171	80.5	27.5	2951	189.3
13	69	165	64.2	23.6	2406	173.4
Mean	70	170	75.1	26.1	2881	190
SD	7	7	9.0	2.0	474	38

Young women

	Age	Height	Weight	BMI	Leg volume	Activity
	yr	cm	Kg	$Kg.m^{-2}$	mL	$MET.h.week^{-1}$
1	22	160	56.0	21.9	2321	155
2	22	174	60.8	20.0	2347	174
3	22	165	60.3	22.3	2482	234
4	23	165	53.0	19.6	2087	225
5	23	170	56.0	19.5	2024	199
6	23	166	54.8	19.8	2264	286
7	23	167	62.0	22.3	2712	231
8	23	168	63.6	22.5	2590	178
Mean	23	167	58.4	21.6	2353	210
SD	1	4	4.2	1.5	236	42

Older women

	Age	Height	Weight	BMI	Leg volume	Activity
	yr	cm	kg	$kg.m^{-2}$	mL	$MET.h.week^{-1}$
1	60	164	76.1	28.5	3572	153
2	71	160	59.2	23.3	2339	220
3	70	150	55.0	24.4	1484	131
4	66	153	81.7	34.7	3209	234
5	55	148	65.5	29.8	2450	241
6	64	154	68.6	28.8	2691	212
7	63	168	73.6	26.1	3300	240
8	55	162	57.4	21.8	2699	271
9	67	152	75.6	32.7	2494	183
10	72	166	73.2	26.6	3365	179
Mean	64	158	68.6	27.7	2760	206
SD	7	7	9.0	4.1	623	44

Appendix VIII: Haematological data for subjects who completed the incremental exercise test to maximal exertion

Young men

	Hg	Hct	Plasma Glucose	Total Cholesterol	HDL	Triglycerides	LDL	Total Cholesterol/HDL
	$g.dL^{-1}$	%	*	*	*	*	*	*
1	16.0	47.4	4.7	3.5	1.4	1.3	1.8	2.5
2	15.0	44.2	4.4	4.0	0.6	1.3	3.1	6.7
3	13.7	43.5	5.4	4.5	1.1	1.3	3.1	4.1
4	16.3	48.2	3.8	4.0	1.4	1.3	2.3	2.8
5	15.9	46.2	5.8	3.2	1.4	1.3	1.6	2.3
6	15.6	46.1	5.1	3.2	1.0	1.3	1.9	3.1
7	14.5	43.7	5.1	4.0	1.4	1.3	2.3	2.8
8	16.2	49.6	6.5	4.7	0.7	1.3	3.7	7.0
9	16.3	46.6	4.2	4.4	1.1	1.3	3.0	3.9
10	15.1	45.5	4.2	4.0	1.4	1.3	2.3	2.8
11	15.2	46.3	4.2	2.6	1.1	1.3	1.2	2.4
12	16.3	46.9	4.4	3.1	1.2	1.6	1.5	2.5
13	15.0	44.8	3.7	3.0	0.9	1.3	1.8	3.3
14	13.9	40.0	4.7	3.3	1.2	1.3	1.8	3.5
15	15.3	45.0	4.7	4.1	1.1	1.3	2.7	3.7
Mean	15.4	45.6	4.7	3.7	1.1	1.3	2.3	3.6
SD	0.9	2.3	0.8	0.6	0.3	0.1	0.7	1.5

*units=$mmol.L^{-1}$

Older men

	Hg	Hct	Plasma Glucose	Total Cholesterol	HDL	Triglycerides	LDL	Total Cholesterol/HDL
	$g.dL^{-1}$	%	*	*	*	*	*	*
1	15.0	46.0	3.7	5.2	1.0	2.0	3.8	5.5
2	12.8	39.7	6.1	5.2	1.0	1.4	3.7	4.3
3	13.9	43.1	5.0	4.7	1.0	1.3	3.6	5.4
4	14.3	45.7	4.4	3.2	1.0	1.3	1.7	2.2
5	15.3	46.8	6.8	5.1	1.0	2.6	3.9	7.3
6	14.2	44.8	5.7	3.8	1.0	1.3	2.6	3.8
7	14.1	43.2	4.7	4.1	1.0	2.8	2.8	5.8
8	15.5	48.0	4.8	4.0	1.0	2.1	2.9	6.7
9	14.7	43.0	3.6	5.2	2.0	1.3	3.2	3.1
10	16.2	47.2	3.7	4.1	1.0	2.9	2.6	4.3
11	13.9	41.4	3.3	6.0	1.0	2.7	4.2	4.8
12	14.9	43.5	3.6	5.4	1.0	2.9	3.9	6.1
13	16	45.6	3.9	4.7	1.0	2.9	3.3	3.2
Mean	14.7	44.5	4.6	4.7	1.1	2.1	3.2	4.8
SD	1.0	2.4	1.1	0.8	0.3	0.7	0.7	1.5

*units=$mmol.L^{-1}$

Young women

	Hb	HCt	Plasma Glucose	Total Cholesterol	HDL	Triglycerides	LDL	Total Cholesterol/HDL
	$g.dL^{-1}$	%	*	*	*	*	*	*
1	13.9	41.5	4.8	4.3	1.1	1.3	2.9	3.8
2	13.7	41.0	5.5	4.9	1.7	1.3	2.9	2.9
3	13.5	38.1	5.2	3.0	1.0	1.3	1.7	3.0
4	14.2	40.7	5.6	5.2	1.7	1.3	3.2	3.0
5	12.1	37.1	4.6	3.0	1.0	1.3	1.7	2.9
6	14.5	41.8	4.3	3.5	1.2	1.3	2.1	3.0
7	13.5	39.7	4.9	4.1	1.2	1.3	2.6	3.5
8	12.6	37.6	4.6	3.0	1.0	1.3	1.7	3.0
Mean	13.5	39.7	4.9	3.9	1.2	1.3	2.4	3.1
SD	0.8	1.9	0.5	0.9	0.3	0.0	0.6	0.3

*units=$mmol.L^{-1}$

Older women

	Hg	HCt	Plasma Glucose	Total Cholesterol	HDL	Triglycerides	LDL	Total Cholesterol/HDL
	$g.dL^{-1}$	%	*	*	*	*	*	*
1	13.7	40.2	4.8	4.7	1.0	1.3	3.6	4.4
2	14.8	43.8	4.8	4.2	0.9	1.3	3.0	4.4
3	14.4	41.0	5.6	5.2	0.8	1.8	4.0	6.2
4	13.4	39.7	4.0	5.4	1.4	1.3	3.7	4.2
5	13.7	40.2	4.8	4.8	1.1	1.3	3.4	4.4
6	12.3	35.9	4.5	4.8	1.1	1.3	3.4	4.4
7	15.1	44.0	4.5	5.0	1.3	1.3	3.5	4.0
8	12.2	36.0	5.0	3.9	1.6	1.3	2.0	2.4
9	12.8	38.5	4.7	5.5	0.8	2.5	4.1	6.8
10	14.2	42.5	5.0	5.2	1.1	1.3	3.8	4.8
Mean	13.7	40.2	4.8	4.9	1.1	1.5	3.5	4.6
SD	1.0	2.8	0.4	0.5	0.2	0.4	0.6	1.2

*units are $mmol.L^{-1}$

Appendix IX: Time to failure data and number of work rates for subjects who completed the incremental exercise test to maximal exertion

	Young men	
	No. of increments	Time to failure
		min
1	7.0	13.8
2	5.0	9.8
3	6.0	12.0
4	5.0	10.0
5	5.0	10.0
6	5.0	10.0
7	7.0	14.0
8	5.0	10.0
9	5.0	10.0
10	4.0	7.2
11	4.0	8.0
12	4.0	7.1
13	6.0	12.0
14	5.0	10.0
15	4.0	8.0
Mean	5.1	10.1
SD	1.0	2.1

	Older men	
	No. of increments	Time to failure
		min
1	5.0	9.9
2	4.0	6.7
3	4.0	8.0
4	3.0	6.0
5	6.0	12.0
6	3.0	6.0
7	6.0	12.0
8	6.0	12.0
9	5.0	8.9
10	8.0	14.9
11	4.0	7.0
12	5.0	11.0
13	6.0	11.7
Mean	5.0	9.7
SD	1.4	2.8

	Young women	
	No. of increments	Time to failure
		min
1	3.0	5.8
2	4.0	7.0
3	4.0	7.9
4	5.0	9.7
5	6.0	10.2
6	6.0	12.2
7	6.0	10.9
8	5.0	8.9
Mean	4.9	9.1
SD	1.1	2.1

	Older women	
	No. of increments	Time to failure
		min
1	7.0	13.7
2	6.0	11.7
3	5.0	9.0
4	7.0	11.8
5	4.0	7.0
6	6.0	11.1
7	7.0	14.0
8	5.0	10.0
9	7.0	14.0
10	7.0	13.3
Mean	6.1	11.6
SD	1.1	2.3

Appendix X: Peak force data and peak force as percent MVC for subjects who completed the incremental exercise test to maximal exertion

	Young men	
	Peak force	Peak force % MVC
	N	%
1	1300	93
2	900	81
3	1100	102
4	900	69
5	900	93
6	900	63
7	1300	110
8	900	61
9	900	88
10	700	59
11	700	111
12	700	63
13	1100	80
14	900	72
15	700	82
Mean	927	82
SD	198	18

	Older men	
	Peak force	Peak force % MVC
	N	%
1	700	78
2	550	115
3	550	79
4	400	82
5	850	79
6	400	73
7	850	82
8	850	96
9	700	62
10	1150	90
11	550	88
12	700	81
13	850	85
Mean	700	84
SD	212	13

	Young women	
	Peak force	Peak force % MVC
	N	%
1	400	114
2	550	131
3	550	76
4	700	81
5	850	102
6	850	68
7	850	107
8	700	52
Mean	681	91
SD	169	26

	Older women	
	Peak force	Peak force % MVC
	N	%
1	700	75
2	600	67
3	500	85
4	700	113
5	400	73
6	600	97
7	700	70
8	500	56
9	700	100
10	700	97
Mean	610	83
SD	110	18

Appendix XI: Peak blood flow and peak vascular conductance data for subjects who completed the incremental exercise test to maximal exertion

Young men

	Peak blood flow	Peak blood flow	Peak vascular conductance	Peak vascular conductance
	($ml.min^{-1}.100ml^{-1}$)	($ml.min^{-1}$)	($ml.min^{-1}.100ml^{-1}.mmHg^{-1}.10$)	($ml.min^{-1}.mmHg^{-1}.10$)
1	40.5	1372	2.5	86.3
2	20.9	422	1.3	29.6
3	34.9	1560	2.0	89.1
4	24.6	551	1.5	31.4
5	40.2	1057	2.2	57.1
6	26.1	1098	1.6	66.5
7	42.5	1368	2.5	79.6
8	20.9	682	1.3	42.4
9	41.4	877	2.5	52.8
10	28.6	866	1.8	35.0
11	41.5	788	2.6	48.9
12	35.7	783	2.5	55.3
13	34.2	1106	2.0	65.1
14	32.3	746	2.8	65.7
15	51.4	1289	2.8	71.1
Mean	34.4	971	2.1	58.4
SD	8.8	328	0.5	18.9

Older men

	Peak blood flow	Peak blood flow	Peak vascular conductance	Peak vascular conductance
	($ml.min^{-1}.100ml^{-1}$)	($ml.min^{-1}$)	($ml.min^{-1}.100ml^{-1}.mmHg^{-1}.10$)	($ml.min^{-1}.mmHg^{-1}.10$)
1	60.8	1749	3.5	101.1
2	16.8	499	0.9	26.4
3	32.0	908	1.6	44.1
4	6.2	187	0.4	11.4
5	28.2	798	1.5	41.5
6	36.9	929	2.4	60.3
7	54.3	1997	2.8	104.0
8	21.5	821	1.2	44.9
9	28.0	833	1.5	46.0
10	38.0	952	2.2	54.4
11	35.7	1054	1.9	30.5
12	26.7	555	1.5	57.1
13	63.1	1433	3.7	83.8
Mean	34.5	978	1.9	54.3
SD	16.7	497	1.0	27.7

Young women

	Peak blood flow	Peak blood flow	Peak vascular conductance	Peak vascular conductance
	($ml.min^{-1}.100ml^{-1}$)	($ml.min^{-1}$)	($ml.min^{-1}.100ml^{-1}.mmHg^{-1}.10$)	($ml.min^{-1}.mmHg^{-1}.10$)
1	23.7	480	1.7	33.7
2	20.7	469	1.4	32.6
3	38.7	968	2.2	54.6
4	21.7	515	1.4	33.2
5	43.1	907	2.9	60.7
6	35.7	1216	2.4	81.8
7	52.7	1290	2.9	69.7
8	21.8	682	1.3	40.9
Mean	32.3	816	2.0	50.9
SD	12.1	329	0.7	18.8

Older women

	Peak blood flow	Peak blood flow	Peak vascular conductance	Peak vascular conductance
	($ml.min^{-1}.100ml^{-1}$)	($ml.min^{-1}$)	($ml.min^{-1}.100ml^{-1}.mmHg^{-1}.10$)	($ml.min^{-1}.mmHg^{-1}.10$)
1	46.1	1521	2.5	90.6
2	37.6	879	1.8	42.9
3	51.1	759	3.1	45.4
4	20.2	648	1.2	37.8
5	32.7	801	2.1	51.0
6	31.8	856	1.7	45.4
7	23.3	769	1.3	44.4
8	38.4	1036	2.4	64.9
9	47.1	1050	2.9	60.7
10	31.1	1047	1.7	56.3
Mean	35.9	937	2.1	53.9
SD	10.1	247	0.6	15.4

Appendix XII: Individual slopes for calf blood flow and vascular conductance responses versus force (% max).

Blood flow ($ml.min^{-1}$) relative to % force max

	Young men	Older men	Young women	Older women
1	14.0	17.1	5.4	13.5
2	3.6	4.2	4.8	10.5
3	4.6	0.9	5.0	9.7
4	7.9	7.8	11.4	8.5
5	14.1	8.5	11.6	9.9
6	8.9	6.3	9.3	8.4
7	14.3	16.7	6.2	5.7
8	5.4	6.8	14.6	12.2
9	8.2	8.2		9.6
10	6.2	7.4		8.4
11	7.4	10.7		
12	10.1	4.5		
13	10.1	14.3		
14	6.1			
15	13.6			
Mean	9.0	8.7	8.5	9.6
SD	3.6	4.8	3.7	2.2

Vascular conductance ($ml.min^{-1}.mmHg^{-1}.10$) relative to % force max

	Young men	Older men	Young women	Older women
1	0.8854	0.9617	0.3744	0.7730
2	0.2285	0.2198	0.3446	0.4601
3	0.5970	0.2923	0.2962	0.3163
4	0.6053	0.3646	0.6110	0.5328
5	0.4167	0.0576	0.6496	1.1230
6	0.2683	0.8753	0.7914	0.4728
7	0.8368	0.4028	0.7500	0.4400
8	0.3778	0.4480	0.3676	0.6512
9	0.3287	0.4878		0.7311
10	0.5433	0.5643		0.5291
11	0.5031	0.5144		
12	0.3013	0.2375		
13	0.5546	0.8218		
14	0.5901			
15	0.7425			
Mean	0.52	0.48	0.52	0.60
SD	0.2	0.3	0.2	0.2

Blood flow ($ml.100ml^{-1}.min^{-1}$.) relative to % force

	Young men	Older men	Young women	Older women
1	0.41	0.59	0.27	0.41
2	0.18	0.14	0.22	0.41
3	0.32	0.27	0.44	0.67
4	0.22	0.45	0.33	0.18
5	0.17	0.21	0.54	0.29
6	0.48	0.25	0.20	0.35
7	0.54	0.34	0.20	0.49
8	0.43	0.27	0.38	0.26
9	0.24	0.63		0.39
10	0.31	0.36		0.31
11	0.26	0.22		
12	0.28	0.03		
13	0.30	0.22		
14	0.21			
15	0.44			
Mean	0.32	0.31	0.32	0.38
SD	0.12	0.17	0.12	0.14

Vascular conductance ($ml.100ml^{-1}.min^{-1}.mmHg^{-1}.10$) relative to % force max

	Young men	Older men	Young women	Older women
1	0.0261	0.0334	0.0185	0.0216
2	0.0111	0.0074	0.0152	0.0197
3	0.0183	0.0103	0.0240	0.0401
4	0.0127	0.0238	0.0118	0.0164
5	0.0144	0.0123	0.0291	0.0157
6	0.0129	0.0205	0.0125	0.0293
7	0.0260	0.0191	0.0290	0.0241
8	0.0101	0.0362	0.0220	0.0143
9	0.0286	0.0114		0.0218
10	0.0159	0.0117		0.0099
11	0.0296	0.0194		
12	0.0182	0.0142		
13	0.0190	0.0019		
14	0.0265			
15	0.0240			
Mean	0.020	0.017	0.020	0.021
SD	0.007	0.010	0.007	0.009

Appendix XIII: Heart rate responses at rest, exercise onset (6th s) and end-exercise for subjects who completed the incremental exercise test to maximal exertion

Young men

	HR (beats.min^{-1})		
	Rest	Onset	EE
1	62	65	86
2	55	67	96
3	59	78	88
4	62	80	93
5	80	85	121
6	58	78	93
7	50	59	79
8	50	72	83
9	68	86	107
10	63	60	99
11	67	77	84
12	87	81	86
13	85	94	110
14	76	102	111
15	69	85	128
Mean	66	78	98
SD	12	12	15

Older men

	HR (beats.min^{-1})		
	Rest	Onset	EE
1	63	62	73
2	66	66	68
3	67	79	88
4	63	67	71
5	95	94	103
6	91	100	102
7	60	74	74
8	68	73	86
9	87	83	92
10	85	79	95
11	83	88	93
12	70	69	86
13	47	52	79
Mean	73	76	85
SD	14	13	12

Young women

	HR (beats.min^{-1})		
	Rest	Onset	EE
1	63	64	78
2	76	89	92
3	76	95	112
4	107	110	108
5	70	69	79
6	61	74	86
7	67	89	99
8	58	68	83
Mean	72	82	92
SD	16	16	13

Older women

	HR (beats.min^{-1})		
	Rest	Onset	EE
1	85	86	99
2	113	94	111
3	75	76	85
4	72	85	94
5	73	83	92
6	64	67	83
7	59	71	92
8	59	61	69
9	66	73	96
10	75	75	81
Mean	74	77	90
SD	16	10	11

Appendix XIV: MAP responses at rest, exercise onset (6^{th} s) and end-exercise for subjects who completed the incremental exercise test to maximal exertion

	Young men MAP (mmHg)		
	Rest	Onset	EE
1	78	82	86
2	77	82	84
3	84	94	98
4	81	80	98
5	83	88	121
6	81	83	92
7	85	85	97
8	81	83	91
9	87	92	99
10	82	83	88
11	83	83	91
12	83	65	76
13	88	92	102
14	84	95	88
15	79	97	113
Mean	82	86	95
SD	3	8	11

	Older men MAP (mmHg)		
	Rest	Onset	EE
1	87	93	106
2	102	117	122
3	101	113	117
4	89	100	94
5	115	129	128
6	108	116	92
7	120	124	121
8	90	95	113
9	79	80	117
10	96	102	108
11	110	103	118
12	91	103	118
13	85	88	107
Mean	98	105	112
SD	13	14	11

	Young women MAP (mmHg)		
	Rest	Onset	EE
1	62	63	76
2	74	85	79
3	95	100	112
4	77	75	89
5	68	72	88
6	78	85	84
7	94	110	116
8	85	105	103
Mean	79	87	93
SD	11	17	15

	Older women MAP (mmHg)		
	Rest	Onset	EE
1	70	108	118
2	110	144	143
3	83	96	109
4	66	95	114
5	94	97	99
6	89	120	129
7	77	83	108
8	77	91	97
9	92	109	105
10	95	115	115
Mean	85	106	114
SD	13	18	14

Appendix XV

PARTICIPANT INFORMATION AND CONSENT FORM

1. Title of study: Leg blood flow responses in young and older healthy non-diabetic men and women.

2. Introduction: The main objective is to quantify muscle performance and the rate of increase in blood flow responses at both low and high exercise intensities during a novel calf-exercise in healthy young versus older men and women. Arm and leg vascular functioning will also be assessed.

3. Procedures: You will be one of the 50 volunteers recruited for this study and it is desirable that you have a BMI (BMI = Body weight in Kg / height in meters / height in meters) lower than 32. You will be over 55 years (Group 2, OLDER). Before the commencement of the project, you will undergo a full medical examination by a General Practitioner, where a blood sample will be taken. If found able to participate in this study, you will be required to visit the Cardiovascular Health Unit in the Department of Physiology in Trinity College Dublin on 2 occasions separated at least by 72 hours.

- Visits to the laboratory (TCD):

Visit 1: Familiarisation session of exercise protocol and performance of calf maximum voluntary contractions.

You will be familiarised with the calf ergometer and the exercise protocol to be completed on the second and third days of testing. Then you will try and complete six calf maximal voluntary contractions (MVC) (1 min rest periods between maximal efforts) in the upright position (tilt angle of 67°).

Visit 2: 6 constant-force calf muscle exercise trials (blood flow measurements) (each session will last up to 210 min).

During visit 2, you will try and complete three, 6-min constant-force plantar flexion exercises at two randomly selected intensities of 30%, 45%, 60% or 70% MVC in the upright position (tilt angle of 67°) (total bouts = 6). Prior to each constant force bout, resting blood flow and blood pressure will be simultaneously measured. Following a rest period, forearm and calf muscle reactive hyperaemic blood flow will be determined in the horizontal position. To do so, an arm or thigh cuff will be inflated at a pressure of 200 mmHg for 5 minutes and after arterial occlusion, peak blood flow will be measured in the forearm or calf muscle respectively.

Visit 3: 6 constant-force calf muscle exercise trials (blood flow measurements) (each session will last up to 210 min).

During visit 3, you will try and complete three, 6-min constant-force plantar flexion exercises at the two randomly selected intensities of 30%, 45%, 60% or 70% MVC in the upright position (tilt angle of 67°) (total bouts = 6) following the same procedure employed during visit 2.

4. Benefits:

There are no direct benefits to you in this study. However, you will gain an understanding into the changes that occur in blood flow with age.

5. Risks:

There are very few risks associated with the exercise procedures used during this study:

Calf exercise: Performance of plantar flexion exercise may result in muscle tightness, soreness, fatigue and rarely a pulled muscle.

Blood sampling may make some volunteers feel uneasy, or prove painful to some. Some may experience slight bruising or discomfort around the sampling area.

6. Exclusion from participation:

You will be excluded from the study if you are taking any medications that could affect your peripheral circulation, have an abnormal HbA_{1c}, and have any active medical problems.

7. Confidentiality:

Your identity will remain confidential. Your name will not be published and will not be disclosed to anyone outside the study group. The data or material will be retained after the study is completed. This material will not be used in future unrelated studies without further specific permission being obtained.

8. Compensation:

This study is covered by standard institutional indemnity insurance. Nothing in this document restricts or curtails your rights.

9. Voluntary Participation:

You have volunteered to participate in this study. You may quit at any time. If you decide not to participate, or if you quit, you will not be penalised and will not give up any benefits that you had before entering the study.

10. Stopping the study:

You understand that the investigators may stop your participation in the study at any time without your consent.

11. Permission: This trial has Research Ethics Committee approval from Trinity College Dublin.

12. Further information:

You can get more information or answers to your questions about the study, your participation in the study, and your rights, from Ms Heather Reilly (086-3273872/ heathernreilly@hotmail.com)) or Dr Mikel Egaña, (01-8963728 / megana@tcd.ie). If the study team learns of important new information that might affect your desire to remain in the study, you will be informed at once.

DECLARATION:

I have read, or had read to me, this consent form. 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. 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.

I understand I may withdraw from the study at any time.

(Name of sponsor:)

.....

PARTICIPANT'S NAME:……

CONTACT DETAILS:……..

PARTICIPANT'S SIGNATURE:……

Date:……..

NAME OF CONSENTER, PARENT or GUARDIAN……..

(if under 18 years old)

SIGNATURE:……

RELATION TO PARTICIPANT:……..

Statement of investigator's responsibility: I have explained the nature and purpose of this research study, the procedures to be undertaken and any risks that may be involved. 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.

INVESTIGATOR'S SIGNATURE:…… **Date:**……

Appendix XVI: Anthropometrical and physical activity data for subjects who completed constant force exercise tests at relative exercise intensities of 30%, 45%, 60% and 70% MVC.

Young men

	Age	Height	Weight	BMI	Leg volume	Activity
	yr	cm	kg	$kg.m^{-2}$	mL	$MET.h.week^{-1}$
1	23	171	63.0	21.5	3388	161
2	23	192	79.0	21.4	4207	181
3	21	180	87.0	26.9	3263	185
4	21	188	76.5	21.6	2021	180
5	28	180	97.2	30.0	3029	191
6	24	166	55.1	20.1	1900	221
7	23	183	62.5	18.6	2193	170
8	24	178	65.4	20.7	3234	222
9	23	173	68.6	22.9	3219	240
10	24	176	86.5	28.0	2119	230
11	23	180	66.5	20.5	2628	210
12	21	175	57.9	18.9	2508	190
Mean	23	179	72.1	22.6	2809	198
SD	2	7	13.1	3.7	695	25

Older men

	Age	Height	Weight	BMI	Leg volume	Activity
	yr	cm	kg	$kg.m^{-2}$	mL	$MET.h.week^{-1}$
1	72	173	77.2	25.8	2967	180
2	68	173	71.6	24.0	2877	200
3	73	165	71.1	26.0	2518	179
4	70	160	68.6	26.9	3678	185
5	70	183	90.3	27.0	2827	185
6	89	173	70.1	23.3	2079	270
7	60	165	85.7	31.4	2976	185
8	67	165	71.2	26.3	2506	200
9	60	171	80.5	27.5	2951	210
10	69	165	64.2	23.6	2406	185
Mean	70	169	75.0	26.2	2779	198
SD	8	7	8.2	2.3	434	27

Young women

	Age	Height	Weight	BMI	Leg volume	Activity
	yr	cm	kg	$kg.m^{-2}$	mL	$MET.h.week^{-1}$
1	22	160	56.0	21.9	2321	210
2	22	174	60.8	20.0	2347	245
3	22	165	60.3	22.3	2482	262
4	23	165	53.0	19.6	2087	245
5	23	170	56.0	19.5	2024	155
6	23	166	54.8	19.8	2264	174
7	23	167	62.0	22.3	2712	234
8	23	168	63.6	22.5	2590	225
9	21	159	53.4	21.1	2104	199
10	21	167	64.3	23.2	3406	286
11	23	164	62.3	23.3	3127	231
12	21	166	59.0	21.4	2717	178
Mean	22	166	58.8	21.4	2515	220
SD	1	4	4.0	1.4	424	38

Older women

	Age	Height	Weight	BMI	Leg volume	Activity
	yr	cm	Kg	$Kg.m^{-2}$	mL	$MET.h.week^{-1}$
1	60	164	76.1	28.5	3572	153
2	71	160	59.2	23.3	2339	220
3	70	150	55.0	24.4	1484	131
4	66	153	81.7	34.7	3209	281
5	53	148	65.5	29.8	2450	241
6	64	154	68.6	28.8	2691	274
7	63	168	73.6	26.1	3300	298
8	50	162	57.4	21.8	2699	271
9	67	152	75.6	32.7	2494	183
10	72	166	73.2	26.6	3365	179
Mean	64	158	68.6	27.7	2760	223
SD	7	7	9.0	4.1	623	59

Appendix XVII: Haematological data for subjects who completed constant force exercise tests at relative exercise intensities of 30%, 45%, 60% and 70% MVC.

Young men

	Hb	HCt	Plasma Glucose	Total Cholesterol	HDL	Triglycerides	LDL	Total Cholesterol/HDL
	$g.dL^{-1}$	%	*	*	*	*	*	*
1	16.0	47.4	4.7	3.5	1.4	1.3	1.8	2.5
2	15.0	44.2	4.4	4.0	0.6	1.3	3.1	6.7
3	15.9	46.2	5.8	3.2	1.4	1.3	1.6	2.3
4	15.6	46.1	5.1	3.2	1.0	1.3	1.9	3.1
5	14.5	43.7	5.1	4.0	1.4	1.3	2.3	2.8
6	16.2	49.6	6.5	4.7	0.7	1.3	3.7	7.0
7	16.3	46.6	4.2	4.4	1.1	1.3	3.0	3.9
8	15.1	45.5	4.2	4.0	1.4	1.3	2.3	2.8
9	15.2	46.3	4.2	2.6	1.1	1.3	1.2	2.4
10	16.3	46.9	4.4	3.1	1.2	1.6	1.5	2.5
11	13.9	40.0	4.7	3.3	1.2	1.3	1.8	3.5
12	15.4	45.0	4.7	4.1	1.1	1.3	2.7	3.7
Mean	15.5	45.6	4.8	3.7	1.1	1.3	2.3	3.6
SD	0.8	2.3	0.7	0.6	0.3	0.1	0.8	1.6

* $mmol.L^{-1}$

Older men

	Hg	Hct	Plasma Glucose	Total Cholesterol	HDL	Triglycerides	LDL	Total Cholesterol/HDL
	$g.dL^{-1}$	%	*	*	*	*	*	*
1	15.0	46.00	3.7	5.2	1.0	2.0	3.8	5.5
2	13.9	43.10	5.0	4.7	1.0	1.3	3.6	5.4
3	14.3	45.70	4.4	3.2	1.0	1.3	1.7	2.2
4	15.3	46.80	6.8	5.1	1.0	2.6	3.9	7.3
5	14.2	44.80	5.7	3.8	1.0	1.3	2.6	3.8
6	15.5	48.00	4.8	4.0	1.0	2.1	2.9	6.7
7	14.7	43.00	3.6	5.2	2.0	1.3	3.2	3.1
8	16.2	47.20	3.7	4.1	1.0	2.9	2.6	4.3
9	14.9	43.50	3.6	5.4	1.0	2.9	3.9	6.1
10	16.0	45.60	4.6	5.0	1.0	2.0	3.0	4.9
Mean	15.0	45.4	4.6	4.6	1.3	2.0	3.1	4.9
SD	0.8	1.8	1.1	0.8	0.3	0.7	0.7	1.6

* $mmol.L^{-1}$

Young women

	Hg	Hct	Plasma Glucose	Total Cholesterol	HDL	Triglycerides	LDL	Total Cholesterol/HDL
	$g.dL^{-1}$	%	*	*	*	*	*	*
1	13.9	39.0	5.4	5.2	1.7	1.3	3.2	3.0
2	13.7	38.8	5.3	3.0	1.0	1.3	1.7	3.0
3	14.2	40.3	4.8	5.2	1.7	1.3	3.2	3.0
4	14.1	40.0	4.5	4.3	1.1	1.3	2.9	3.8
5	13.9	41.5	4.8	4.3	1.1	1.3	2.9	3.8
6	13.7	41.0	5.5	4.9	1.7	1.3	2.9	2.9
7	13.5	38.1	5.2	3.0	1.0	1.3	1.7	3.0
8	14.2	40.7	5.6	5.2	1.7	1.3	3.2	3.0
9	12.1	37.1	4.6	3.0	1.0	1.3	1.7	2.9
10	14.5	41.8	4.3	3.5	1.2	1.3	2.1	3.0
11	13.5	39.7	4.9	4.1	1.2	1.3	2.6	3.5
12	12.6	37.6	4.6	3.0	1.0	1.3	1.7	3.0
Mean	13.7	39.6	5.0	4.1	1.3	1.3	2.5	3.2
SD	0.7	1.5	0.4	0.9	0.3	0.0	0.6	0.4

* $mmol.L^{-1}$

Older women

	Hg	Hct	Plasma Glucose	Total Cholesterol	HDL	Triglycerides	LDL	Total Cholesterol/HDL
	$g.dL^{-1}$	%	*	*	*	*	*	*
1	13.7	40.2	4.8	4.7	1.0	1.3	3.6	4.4
2	14.8	43.8	4.8	4.2	0.9	1.3	3.0	4.4
3	14.4	41.0	5.6	5.2	0.8	1.8	4.0	6.2
4	13.4	39.7	4.0	5.4	1.4	1.3	3.7	4.2
5	13.7	40.2	4.8	4.8	1.1	1.3	3.4	4.4
6	12.3	35.9	4.5	4.8	1.1	1.3	3.4	4.4
7	15.1	44.0	4.5	5.0	1.3	1.3	3.5	4.0
8	12.2	36.0	5.0	3.9	1.6	1.3	2.0	2.4
9	12.8	38.5	4.7	5.5	0.8	2.5	4.1	6.8
10	14.2	42.5	5.0	5.2	1.1	1.3	3.8	4.8
Mean	13.7	40.2	4.8	4.9	1.1	1.5	3.5	4.6
SD	1.0	2.8	0.4	0.5	0.2	0.4	0.6	1.2

* $mmol.L^{-1}$

Appendix XVIII: Maximum voluntary contraction values for subjects who completed constant force exercise tests at relative exercise intensities of 30%, 45%, 60% and 70% MVC.

	MVC (N)			
	Young men	Older men	Young women	Older women
1	800	530	915	550
2	1118	1080	950	1106
3	1200	550	750	700
4	1095	1000	576	900
5	1371	1137	900	620
6	1234	1209	679	600
7	1500	870	860	797
8	1431	1013	920	718
9	1020	500	474	940
10	970	1080	840	620
11	960		1030	
12	1470		960	
Mean	1181	897	821	755
SD	226	271	168	179

Appendix XIX: Resing calf blood flow and vascular conductance responses for subjects who completed constant-force exercise tests at relative exercise intensities of 30%, 45%, 60% and 70% MVC.

Young men

	Resting blood flow	Resting blood flow	Resting vascular conductance	Resting vascular conductance
	($ml.100ml^{-1}.min^{-1}$)	($ml.min^{-1}$)	($ml.100ml^{-1}.min^{-1}.mmHg^{-1}$)	($ml.min^{-1}.mmHg^{-1}$)
1	2.2	47.6	0.030	0.63
2	1.8	59.3	0.021	0.66
3	1.6	29.8	0.023	0.71
4	1.9	57.3	0.023	0.97
5	2.0	84.8	0.026	0.86
6	2.8	95.4	0.021	0.42
7	1.5	30.1	0.010	0.32
8	1.0	32.4	0.029	0.77
9	2.6	68.5	0.022	0.47
10	1.7	37.3	0.019	0.36
11	1.8	57.9	0.022	0.71
12	1.3	32.6	0.016	0.40
Mean	1.9	52.8	0.022	0.61
SD	0.5	22.0	0.005	0.21

Older men

	Resting blood flow	Resting blood flow	Resting vascular conductance	Resting vascular conductance
	($ml.100ml^{-1}.min^{-1}$)	($ml.min^{-1}$)	($ml.100ml^{-1}.min^{-1}.mmHg^{-1}$)	($ml.min^{-1}.mmHg^{-1}$)
1	1.6	48.0	0.017	0.49
2	3.1	89.2	0.034	0.96
3	2.0	49.7	0.020	0.51
4	2.9	108.3	0.026	0.95
5	2.6	73.5	0.024	0.69
6	2.5	52.0	0.034	1.00
7	2.8	83.8	0.025	0.63
8	2.2	54.9	0.020	0.58
9	2.1	62.0	0.030	0.68
10	2.6	59.3	0.025	0.52
11	2.5	89.3	0.036	1.27
12	1.8	43.0	0.017	0.39
Mean	2.4	67.8	0.025	0.72
SD	0.5	20.7	0.007	0.26

Young women

	Resting blood flow ($ml.100ml^{-1}.min^{-1}$)	Resting blood flow ($ml.min^{-1}$)	Resting vascular conductance ($ml.100ml^{-1}min^{-1}.mmHg^{-1}$)	Resting vascular conductance ($ml.min^{-1}.mmHg^{-1}$)
1	1.7	39.3	0.020	0.5
2	1.8	41.6	0.025	0.6
3	1.5	38.3	0.027	0.7
4	2.0	40.9	0.024	0.5
5	2.0	39.7	0.035	0.7
6	1.7	38.3	0.023	0.5
7	2.3	61.4	0.031	0.8
8	1.9	48.0	0.025	0.6
9	1.1	24.0	0.018	0.4
10	1.7	57.6	0.021	0.7
11	1.0	31.1	0.013	0.4
12	2.1	55.9	0.028	0.7
Mean	1.7	43.0	0.024	0.60
SD	0.4	11.0	0.006	0.15

Older women

	Resting blood flow ($ml.min^{1}.100ml^{-1}$)	Resting blood flow ($ml.min^{-1}$)	Resting vascular conductance ($ml.min^{-1}.100ml^{1}.mmHg^{1}$)	Resting vascular conductance ($ml.min^{-1}.mmHg^{-1}$)
1	2.5	89.3	0.036	1.27
2	1.8	43.0	0.017	0.39
3	2.1	67.1	0.025	0.85
4	1.7	40.4	0.017	0.80
5	2.2	59.2	0.022	0.40
6	1.8	60.5	0.026	0.59
7	1.8	48.6	0.026	0.87
8	2.5	61.9	0.036	0.70
9	2.1	70.4	0.023	0.89
10	2.0	68.0	0.022	0.76
Mean	2.1	60.0	0.025	0.75
SD	0.3	15.1	0.007	0.26

Appendix XX: Vascular conductance kinetic responses for subjects who completed constant force exercise tests at relative exercise intensities of 30%, 45%, 60% and 70% MVC.

	a	A1	TD 1	TAU 1	A2	TD 2	TAU 2	360	MRT	R2
					Young men 30% MVC					
1	0.49	1.48	2.0	1.3	1.05	15.9	20.0	3.02	16.9	0.77
2	0.51	0.57	1.4E-03	0.8	0.62	13.7	16.3	1.71	16.0	0.50
3	0.38	1.49	2.1	1.3	0.33	17.4	8.0	2.20	7.4	0.70
4	0.55	1.71	7.2E-05	1.6	0.58	25.4	35.2	2.84	16.5	0.77
5	0.65	2.85	1.7	2.9	0.44	21.0	3.0	3.95	7.3	0.83
6	060	0.71	1.3	1.4	0.70	17.7	13.9	2.70	13.4	0.67
7	1.09	1.39	0.1	0.7	0.54	18.1	9.9	3.02	8.4	0.72
8	0.60	2.37	1.9	1.3	1.01	17.1	8.0	3.97	9.8	0.73
9	0.68	0.96	4.8	0.5	0.71	17.0	8.1	2.35	13.7	0.66
10	0.74	0.82	1.9E-04	1.3	0.99	17.5	10.5	2.54	15.9	0.59
11	0.50	0.70	2.6E-04	1.3	0.88	18.7	26.7	2.09	25.9	0.67
12	0.36	1.00	2.0	1.8	0.49	12.9	7.0	1.86	9.1	0.45
MEAN	0.60	1.39	1.3	1.4	0.70	17.7	13.9	2.69	13.4	0.67
SD	0.20	0.71	1.5	0.7	0.25	3.4	9.8	0.76	5.7	0.12
					Young women 30% MVC					
1	0.34	1.36	3.2	1.7	1.31	19.8	15.0	3.00	19.6	0.90
2	0.44	0.73	2.6	1.5	0.77	18.0	13.5	1.93	18.2	0.69
3	0.37	1.14	1.4E-06	1.7	0.51	17.2	11.6	2.03	10.1	0.55
4	0.74	0.98	5.2	0.6	0.50	19.8	10.1	2.22	14.0	0.54
5	0.45	0.35	4.9	3.8	0.90	23.6	13.3	1.70	29.0	0.89
6	0.58	0.91	2.2	1.5	0.76	17.4	14.3	2.23	19.3	0.71
7	0.68	0.51	5.1	0.5	0.41	12.6	13.6	1.60	14.8	0.71
8	0.76	0.05	1.5E-03	1.9	0.79	21.4	35.3	1.60	53.2	0.8
9	0.75	2.60	2.0	1.3	1.46	15.0	9.0	4.82	10.7	0.87
10	0.67	1.45	3.7E-04	0.8	0.36	14.6	9.5	2.49	5.4	0.53
11	0.74	0.32	7.0E-04	1.9	0.68	16.7	15.0	1.74	22.2	0.68
12	0.43	0.48	1.8	1.2	0.57	13.6	11.0	1.47	14.8	0.70
MEAN	0.58	0.91	2.2	1.5	0.75	17.5	14.3	2.24	19.3	0.71
SD	0.17	0.72	2.1	0.9	0.36	3.4	7.3	0.97	12.9	0.14
					Older men 30% MVC					
1	0.67	1.80	2.7	1.1	0.42	14	30.9	2.89	11.6	0.68
2	0.93	0.98	2.7	2.4	2.72	17.1	70.0	4.62	65.3	0.84
3	0.66	2.34	2.4	1.4	0.96	13.8	7.0	3.97	8.7	0.79
4	1.04	1.35	4.7	1.4	2.30	30.0	117.9	4.55	95.4	0.83
5	0.97	0.90	2.0	2.0	0.71	12.7	25.0	2.58	18.9	0.78
6	0.75	1.11	2.5	1.9	1.10	17.1	40.6	2.93	33.5	0.72
7	0.78	0.21	9.0E-03	2.2	0.75	14.5	25.2	1.74	31.4	0.55
8	0.64	1.50	2.3	2.0	1.43	19.0	26.0	3.58	24.2	0.89
9	0.59	0.75	2.5	2.3	0.42	18.0	26.7	1.76	19.1	0.58
10	0.44	0.14	3.2	2.6	0.12	15.1	37.0	0.70	27.2	0.50
MEAN	0.75	1.11	2.5	1.9	1.09	17.1	40.6	2.93	33.5	0.72
SD	0.20	0.72	1.2	0.5	0.89	5.3	33.5	1.36	28.5	0.14
					Older women 30% MVC					
1	0.90	0.59	9.6E-04	0.8	0.30	16.5	26.6	1.79	15.1	0.57
2	0.50	1.70	9.4E-04	0.8	0.50	16.6	8.7	2.70	6.3	0.66
3	0.97	0.45	0.4	0.6	1.48	21.1	54. 5	2.90	58.4	0.86
4	0.47	0.98	0.1	0.5	0.41	11.9	26.2	1.85	11.6	0.66
5	0.64	0.39	2.8	2.5	0.37	17.0	25.0	1.40	23.2	0.63
6	0.68	0.71	0.6	1.0	0.53	16.6	31.2	1.92	25.5	0.61
7	0.53	0.12	3.8E-03	1.2	0.52	20.5	55.7	1.17	62.2	0.77
8	0.70	0.67	4.1E-03	0.8	0.75	16.0	34.7	2.11	27.2	0.60
9	0.65	0.33	2.0	1.3	0.08	11.6	15.0	1.06	7.8	0.20
10	0.75	1.19	4.6E-04	0.8	0.39	17.7	34.0	2.34	13.4	0.50
MEAN	0.68	0.71	0.6	1.0	0.53	16.6	31.2	1.92	25.0	0.61
SD	0.17	0.50	1.1	0.6	0.40	3.2	15.9	0.65	21.1	0.19

	a	A1	TD 1	TAU 1	A2	TD 2	TAU 2	360	MRT	R2
					Young men 45% MVC					
1	0.59	2.40	2.2	2.7	1.43	14.0	12	4.42	12.8	0.69
2	0.44	0.87	7.9E-05	2.5	0.60	18.1	18.9	1.91	16.6	0.40
3	0.32	2.26	0.7	1.5	0.32	12.1	7.0	2.90	4.3	0.63
4	0.43	1.51	5.3	0.2	1.68	20.5	14.2	3.62	20.9	0.76
5	0.47	2.71	0.7	2.0	0.60	17.0	7.0	3.77	6.5	0.85
6	0.35	3.01	1.4	2.2	1.26	15.9	7.0	4.62	9.3	0.64
7	0.78	2.63	1.1E-05	1.7	1.27	16.9	8.7	4.69	9.5	0.72
8	0.56	3.53	0.8	2.0	1.39	12.2	10.0	5.48	8.3	0.78
9	0.83	1.71	2.5	1.5	0.81	16.8	9.0	3.34	11.0	0.76
10	0.56	1.38	5.8	0.2	1.42	22.3	20.5	3.35	24.7	0.87
11	0.55	1.34	1.4	1.8	1.49	14.0	37.4	3.38	28.6	0.71
12	0.42	3.41	1.3E-03	0.8	0.44	18.4	14.3	4.27	4.4	0.83
MEAN	0.52	2.23	1.7	1.6	1.06	16.5	13.8	3.81	13.1	0.72
SD	0.16	0.87	2.0	0.8	0.47	3.1	8.7	0.95	8.0	0.13
					Young women 45% MVC					
1	0.61	1.99	0.02	2.8	0.80	25.5	18.4	3.40	14.6	0.69
2	0.48	0.66	1.3	2.2	0.28	15.0	8.0	1.41	9.3	0.62
3	0.40	1.63	0.7	1.9	1.32	16.3	20.0	3.36	17.7	0.71
4	0.51	0.96	5.1	0.7	0.53	16.8	8.8	2.00	12.9	0.86
5	0.45	3.49	0.6	2.0	0.67	12.7	11.0	4.60	6.0	0.73
6	0.71	0.23	1.1	2.8	0.92	15.5	19.0	1.87	28.4	0.78
7	0.71	1.55	2.4	1.4	0.87	14.2	15.0	3.14	12.9	0.68
8	0.73	0.12	5.6	0.58	0.79	15.4	45.3	1.64	53.6	0.82
9	0.94	3.31	3.9E-05	1.7	1.44	14.1	13.1	5.69	9.4	0.77
10	0.69	2.96	1.7	2.3	0.44	16.6	8.0	4.08	6.6	0.71
11	0.77	3.02	0.04	1.9	0.68	16.6	14.0	4.46	7.2	0.81
12	0.43	0.87	1.9	1.3	0.99	17.3	23.0	2.29	22.9	0.88
MEAN	0.62	1.73	1.7	1.8	0.81	16.3	17.0	3.16	16.8	0.76
SD	0.17	1.21	1.9	0.7	0.34	3.2	10.2	1.36	13.4	0.08
					Older men 45% MVC					
1	0.62	2.50	1.9	1.8	0.50	14.0	24.8	3.63	9.5	0.60
2	1.15	2.17	3.2	2.3	1.86	14.4	20.0	5.18	18.9	0.58
3	0.66	2.43	2.5	1.5	1.57	12.7	9.0	4.66	10.9	0.64
4	0.98	1.39	0.6	1.7	2.08	15.6	38.0	4.45	33.1	0.78
5	0.77	0.55	9.0E-05	2.3	1.02	15.1	38.1	2.33	35.3	0.51
6	0.94	1.47	3.4	2.8	1.65	15.9	20.0	4.07	21.9	0.6
7	0.72	1.61	1.9	2.5	2.05	14.1	30.0	4.38	26.6	0.93
8	0.57	2.79	2.2	1.9	2.20	16.0	32.2	5.56	23.5	0.65
9	0.74	1.09	1.1	1.5	0.72	19.0	33.2	2.55	22.2	0.67
10	0.35	0.24	4.7	2.1	0.70	12.0	73.2	1.28	65.2	0.81
Mean	0.75	1.63	2.2	2.0	1.44	14.9	31.8	3.81	26.7	0.68
SD	0.23	0.85	1.4	0.5	0.64	2.0	17.1	1.36	15.8	0.13
					Older women 45% MVC					
1	0.74	0.90	1.8E-04	1.2	0.19	16.2	15.9	1.83	6.6	0.72
2	0.43	2.11	2.9	1.9	1.41	17.0	27.0	3.94	20.5	0.83
3	0.96	0.54	1.4E-03	0.8	0.82	15.8	39.0	2.32	33.4	0.50
4	0.68	0.93	3.0	1.8	1.71	31.0	70.0	3.31	67.1	0.93
5	0.34	0.78	2.3	1.4	1.03	14.6	49.0	2.14	37.9	0.86
6	0.50	0.79	2.5	2.2	0.47	14.4	31.0	1.75	19.9	0.65
7	0.83	0.58	3.0	1.8	0.70	20.0	49.4	2.10	40.1	0.76
8	0.92	0.60	2.3	1.6	0.80	13.4	45.0	2.32	35.0	0.68
9	0.73	2.40	9.9E-03	0.7	0.75	16.2	32.7	3.88	12.3	0.80
Mean	0.68	1.07	1.8	1.5	0.88	17.6	39.9	2.62	30.3	0.75
SD	0.22	0.69	1.4	0.5	0.46	5.4	15.7	0.86	18.1	0.13

				Young men 60% MVC						
	a	A1	TD 1	TAU 1	A2	TD 2	TAU 2	360	MRT	R2
1	0.59	2.76	1.5	2.3	1.67	11.2	15.0	5.03	12.3	0.70
2	0.41	1.44	0.9	2.1	0.86	15.0	20.8	2.71	15.3	0.64
3	0.28	2.21	1.2	1.7	1.05	12.0	13.8	3.54	10.2	0.72
4	0.37	1.90	1.5	1.1	2.00	10.0	49.7	4.27	31.9	0.73
5	0.30	2.46	2.7	4.5	1.11	14.0	13.8	3.87	13.6	0.89
6	0.58	3.11	2.9	2.4	2.00	17.5	15.6	5.70	16.2	0.89
7	0.96	3.14	1.0	1.5	1.47	12.0	14.7	5.57	10.2	0.81
8	0.70	4.06	3.9E-05	1.7	1.40	17.1	33.4	6.17	14.2	0.76
9	0.83	1.72	2.5	1.4	1.61	16.0	18.7	4.15	18.8	0.70
10	0.65	2.39	6.7E-03	1.7	2.00	16.9	15.0	5.04	15.5	0.65
11	0.58	1.67	0.5	1.4	3.33	12.21	19.0	5.58	21.4	0.84
12	0.40	3.76	1.3	1.0	1.69	19.3	23.2	5.85	14.8	0.95
Mean	0.56	2.55	1.3	1.9	1.68	14.4	21.1	4.79	16.2	0.77
SD	0.21	0.84	1.0	0.9	0.64	3.0	10.6	1.07	5.9	0.10
				Young women 60% MVC						
1	0.57	2.51	1.3	3.2	0.81	19.0	18.5	3.88	12.6	0.75
2	0.56	1.21	5.8E-06	1.7	0.68	20.7	16.7	2.45	14.5	0.51
3	0.35	2.38	4.5	0.5	1.56	14.7	28.3	4.29	20.1	0.89
4	0.49	1.68	0.6	1.8	0.46	18.0	26.4	2.63	11.5	0.69
5	0.44	4.36	4.5	0.8	1.83	15.5	15.3	6.63	12.9	0.82
6	0.74	2.00	2.1	1.3	0.80	13.0	26.0	3.54	13.6	0.70
7	0.66	2.11	1.8	1.2	1.10	14.5	18.0	3.86	13.1	0.86
8	0.71	0.21	3.9	3.4	1.33	15.5	26.0	2.25	36.7	0.86
9	0.89	5.56	2.8	1.5	1.72	10.0	13.4	8.17	8.8	0.94
10	0.68	3.35	5.1	0.7	1.25	18.0	9.4	5.28	11.7	0.85
11	0.72	3.04	2.0	1.3	1.69	13.0	17.0	5.45	12.8	0.78
12	0.44	1.88	1.5	1.2	1.80	10.5	30.0	4.12	21.2	0.84
Mean	0.60	2.52	2.5	1.6	1.25	15.2	20.4	4.38	15.8	0.79
SD	0.16	1.42	1.6	0.9	0.48	3.3	6.6	1.76	7.5	0.12
				Older men 60% MVC						
1	0.61	3.39	2.3	2.0	1.29	16.0	43.8	5.30	19.6	0.69
2	1.00	1.93	1.8	1.9	4.22	14.0	52.4	7.15	46.7	0.73
3	0.58	3.04	4.7	0.9	2.08	16.9	15.5	5.70	16.6	0.89
4	0.97	3.00	2.0	2.0	2.81	12.1	42.0	6.78	28.2	0.85
5	0.70	1.10	2.1	2.1	1.31	12.1	43.0	3.11	31.9	0.77
6	0.90	3.20	8.0E-04	1.0	2.01	14.2	22.9	6.11	14.9	0.76
7	0.71	1.91	2.0	2.6	1.75	14.0	36.4	4.36	26.5	0.77
8	0.56	4.29	3.0	1.7	1.80	13.0	42.2	6.65	19.6	0.88
9	0.73	1.97	5.3E-03	1.3	1.20	18.8	39.9	3.88	23.0	0.79
10	0.57	1.39	1.0	1.2	0.80	9.6	36.0	2.76	18.0	0.76
Mean	0.73	2.52	1.9	1.7	1.93	14.1	37.4	5.18	24.5	0.79
SD	0.17	1.01	1.4	0.5	0.98	2.7	10.7	1.57	9.5	0.06
				Older women 60% MVC						
1	0.94	1.45	2.1	2.0	0.43	14.0	32.2	2.82	13.7	0.35
2	0.42	2.80	2.3	1.2	1.90	12.0	32.5	5.11	20.1	0.87
3	0.99	0.54	0.03	0.7	1.47	15.2	81.3	2.97	70.8	0.61
4	0.66	2.40	2.3	1.6	1.10	23.0	69.4	4.15	31.7	0.88
5	0.33	1.80	2.4	1.2	1.40	11.5	50.0	3.53	28.9	0.63
6	0.50	1.59	2.0	2.0	0.40	14.3	19.0	2.49	9.9	0.77
7	0.83	0.72	2.0	2.0	2.02	17.4	68.0	3.56	63.9	0.87
8	0.93	1.52	1.8	1.2	0.72	13.0	45.3	3.17	20.8	0.50
9	0.72	3.26	1.6E-05	0.8	0.54	18.9	42.5	4.52	9.4	0.80
10	0.71	1.80	1.69	1.4	1.09	16.0	49.0	3.60	30.0	0.70
Mean	0.70	1.79	1.7	1.4	1.11	15.5	48.9	3.59	29.9	0.70
SD	0.24	0.90	1.0	0.5	0.62	3.7	20.4	0.86	22.6	0.19

	a	A1	TD 1	TAU 1	A2	TD 2	TAU 2	360	MRT	R2
					Young men 70% MVC					
1	0.52	2.57	2.3E-04	1.5	2.71	15.4	27.9	5.80	23.0	0.77
2	0.51	1.57	2.0	1.3	2.92	12.0	26.7	5.01	26.3	0.65
3	0.23	4.23	2.9	1.6	1.65	12.0	20.3	6.11	12.3	0.80
4	0.29	2.40	4.0	0.1	2.20	10.9	48.1	4.89	30.4	0.76
5	0.29	3.08	2.7	1.6	1.06	14.3	14.0	4.44	10.4	0.88
6	0.50	3.88	1.0	1.6	1.20	10.2	10.0	5.59	6.8	0.70
7	1.02	2.70	0.7	1.9	2.28	14.0	32.9	6.00	22.8	0.61
8	0.82	3.29	0.8	2.0	3.20	16.0	39.2	7.31	28.6	0.79
9	0.79	2.41	1.2	2.2	1.24	13.6	18.0	4.43	13.0	0.67
10	0.65	2.54	2.0	1.3	2.19	12.6	23.0	5.39	18.2	0.65
11	0.70	2.69	4.2	3.0	2.38	13.4	28.5	5.77	23.5	0.75
12	0.33	3.54	1.9	2.0	1.46	14.7	30.0	5.32	15.8	0.81
Mean	0.56	2.91	2.0	1.7	2.04	13.3	26.6	5.51	19.3	0.74
SD	0.25	0.73	1.3	0.7	0.71	1.8	10.6	0.80	7.6	0.08
					Young women 70% MVC					
1	0.39	2.84	0.8	2.4	1.23	17.0	22.4	4.45	14.1	0.71
2	0.61	0.90	2.2	1.4	0.80	15.0	20.4	2.31	18.6	0.72
3	0.38	2.89	2.8	1.6	1.49	14.6	26.0	4.76	16.7	0.82
4	0.50	2.49	0.7	1.9	0.89	12.0	27.7	3.88	12.3	0.71
5	0.56	4.80	2.0	1.3	1.79	12.0	20.7	7.15	11.3	0.84
6	0.62	2.00	2.0	1.3	1.30	11.3	28.0	3.92	17.5	0.69
7	0.47	2.20	2.0	1.3	0.70	12.0	24.0	3.37	11.2	0.71
8	0.62	1.42	2.7E-05	1.7	0.53	15.5	15.3	2.56	9.6	0.70
9	1.04	6.00	2.7	1.3	1.10	11.7	24.0	8.14	8.9	0.95
10	0.69	3.47	0.7	1.9	2.54	13.0	20.3	6.70	15.6	0.85
11	0.75	3.18	0.8	2.0	3.01	14.0	22.6	6.94	19.2	0.89
12	0.34	1.87	5.6	0.2	1.43	20.0	22.2	3.65	21.6	0.90
Mean	0.58	2.84	1.9	1.5	1.40	14.0	22.8	4.82	14.7	0.79
SD	0.19	1.42	1.5	0.6	0.74	2.6	3.5	1.94	4.1	0.09
					Older men 70% MVC					
1	0.65	4.80	2.0E-03	0.8	0.69	9.9	32.6	6.14	6.1	0.68
2	0.90	6.87	1.5E-03	1.1	4.12	12.3	62.4	11.87	28.7	0.68
3	0.62	4.97	2.3	2.2	4.68	12.7	41.0	10.27	28.4	0.90
4	0.98	3.89	2.5	2.1	1.99	14.8	42.0	6.86	22.3	0.76
5	0.70	2.10	2.0	2.0	4.61	12.0	61.1	7.40	51.4	0.86
6	1.01	3.39	2.9	2.5	2.57	14.8	59.0	6.96	34.9	0.90
7	0.82	1.60	2.0	2.0	2.01	12.3	42.0	4.43	32.0	0.90
8	0.62	2.70	2.1	1.6	2.85	12.0	37.6	6.16	27.2	0.81
9	0.74	3.10	2.2	1.9	1.91	14.0	44.2	5.74	24.8	0.71
10	0.54	1.70	2.0E-04	1.1	1.07	9.9	63.2	3.31	28.9	0.70
Mean	0.76	3.51	1.6	1.7	2.65	12.5	48.5	6.91	28.5	0.79
SD	0.16	1.67	1.1	0.6	1.41	1.7	11.6	2.53	11.2	0.09
					Older women 70% MVC					
1	0.94	2.57	1.0	1.6	0.24	15.0	19	3.75	5.3	0.74
2	0.35	1.70	2.3	1.2	2.99	11.2	38.0	5.04	32.7	0.79
3	0.78	1.09	2.2	1.4	2.20	15.0	82.9	4.03	66.7	0.83
4	0.97	2.93	2.1	2.1	1.61	20.1	74.0	5.49	36.0	0.84
5	0.48	1.95	2.3	1.2	2.50	14.0	91.9	4.87	61.0	0.78
6	0.50	1.69	2.0	2.0	0.50	14.1	37.0	2.69	14.7	0.77
7	0.90	1.57	2.6	1.5	1.91	17.0	74.4	4.36	52.1	0.88
8	0.70	2.97	4.1	1.8	1.44	18.0	61.4	5.09	29.8	0.85
9	0.71	1.40	1.9	1.2	1.80	11.0	68.9	3.89	46.3	0.70
10	0.77	3.10	2.2	1.2	1.00	11.6	44.0	4.87	16.2	0.66
Mean	0.71	2.10	2.3	1.5	1.62	14.7	59.2	4.41	36.1	0.78
SD	0.21	0.73	0.8	0.3	0.86	3.0	23.5	0.83	20.5	0.07

Appendix XXI: Mean arterial pressure responses for subjects who completed constant force exercise tests at relative exercise intensities of 30%, 45%, 60% and 70% MVC.

Young men 30% MVC
MAP (mmHg)

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	88	90	97	99	97	100	101
2	85	75	74	76	71	75	75
3	88	84	81	83	86	80	78
4	99	100	96	95	95	94	94
5	84	83	89	88	92	90	85
6	87	110	108	108	109	108	111
7	80	78	72	70	75	80	81
8	79	94	94	92	90	92	92
9	91	86	94	97	98	98	100
10	72	82	83	80	86	90	94
11	73	76	77	76	79	79	75
12	85	78	74	77	84	79	76
Mean	84	86	86	87	89	89	89
SD	7	11	12	12	11	10	12

Older men 30% MVC
MAP (mmHg)

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	98	95	96	97	96	93	97
2	105	105	102	113	109	119	106
3	86	94	96	107	90	94	74
4	104	100	103	105	96	97	94
5	121	119	115	106	107	119	111
6	104	101	98	92	91	95	111
7	95	86	92	96	95	91	102
8	93	92	92	100	102	103	113
9	106	98	104	109	108	116	114
10	89	94	95	92	94	92	92
Mean	100	98	99	102	99	102	101
SD	10	9	7	7	7	12	12

Young women 30% MVC
MAP (mmHg)

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	65	63	65	68	69	67	66
2	86	87	85	88	83	82	80
3	75	67	67	66	69	67	66
4	75	74	72	76	83	81	77
5	81	81	78	77	74	74	80
6	79	70	69	72	78	76	77
7	89	72	74	76	84	75	70
8	87	83	94	92	87	90	87
9	72	72	68	67	65	69	66
10	77	85	87	84	85	84	85
11	88	100	106	107	108	108	108
12	85	87	90	90	90	89	88
Mean	80	78	80	80	81	80	79
SD	7	10	13	12	11	12	12

Older women 30% MVC
MAP (mmHg)

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	76	91	92	92	89	88	87
2	111	92	93	128	126	129	128
3	91	99	103	104	101	102	101
4	90	93	95	94	93	100	98
5	91	89	88	90	91	92	91
6	103	98	90	102	94	98	97
7	67	94	96	94	95	93	93
8	69	80	79	79	78	78	76
9	82	104	106	107	109	107	109
10	83	117	116	119	119	118	112
Mean	86	96	96	101	99	100	99
SD	14	10	11	15	15	15	14

Young men 45% MVC
MAP (mmHg)

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	83	95	95	101	100	96	98
2	87	102	104	104	99	104	103
3	89	72	72	79	77	73	86
4	97	92	98	100	98	96	90
5	84	81	80	83	84	86	86
6	100	105	107	103	102	106	111
7	79	75	77	79	78	77	79
8	87	78	76	91	89	90	91
9	93	87	88	96	98	96	95
10	96	94	95	80	91	81	81
11	79	77	74	73	75	81	83
12	77	68	64	73	75	70	76
Mean	88	86	86	88	89	88	90
SD	8	12	14	12	11	12	10

Older men 45% MVC
MAP (mmHg)

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	92	96	96	96	94	95	96
2	116	104	105	105	113	108	110
3	86	94	96	95	90	94	78
4	95	86	112	103	97	93	93
5	113	127	121	123	86	96	113
6	112	95	93	94	106	94	96
7	97	86	94	91	108	105	103
8	98	100	104	103	102	102	104
9	104	105	108	107	123	131	131
10	86	81	88	85	80	82	83
Mean	100	97	102	100	100	100	101
SD	11	13	10	10	13	13	15

Young women 45% MVC

MAP (mmHg)

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	82	81	82	81	82	84	86
2	79	74	75	75	77	77	76
3	96	69	71	73	71	71	70
4	74	72	71	72	73	74	73
5	67	62	62	67	68	67	64
6	76	77	78	77	80	81	76
7	83	76	72	74	74	74	77
8	91	82	85	93	93	93	82
9	68	65	74	80	79	83	77
10	74	88	89	88	88	89	89
11	81	94	91	91	91	94	91
12	80	101	98	101	104	102	101
Mean	79	79	79	81	82	82	80
SD	9	12	10	10	10	11	10

Older women 45% MVC

MAP (mmHg)

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	68	107	107	110	112	105	109
2	95	95	102	104	105	108	111
3	91	100	93	94	82	76	85
4	80	97	110	102	111	111	110
5	93	104	104	105	108	108	106
6	108	96	102	104	107	108	106
7	72	101	103	105	105	105	102
8	76	96	104	107	102	100	100
9	83	106	109	106	112	110	108
10	83	96	97	100	94	99	97
Mean	85	100	103	104	104	103	103
SD	12	5	5	4	10	10	8

Young men 60% MVC

MAP (mmHg)

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	93	95	105	103	100	100	108
2	87	102	106	113	105	107	107
3	92	90	84	88	81	83	93
4	90	88	80	85	82	91	95
5	79	86	87	86	82	86	88
6	94	111	104	109	110	107	107
7	86	76	66	64	70	72	69
8	91	94	89	85	99	97	74
9	94	99	96	93	88	92	103
10	83	95	94	93	82	83	92
11	86	76	68	83	83	86	84
12	78	79	79	78	82	84	79
Mean	88	91	88	90	89	91	91
SD	6	11	14	14	12	10	13

Older men 60% MVC

MAP (mmHg)

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	102	91	89	85	94	102	104
2	93	90	89	90	91	96	93
3	101	120	111	117	125	107	126
4	87	75	105	100	97	93	93
5	88	150	146	128	130	134	139
6	98	100	109	110	114	105	104
7	94	80	91	97	108	107	115
8	99	102	103	106	112	114	113
9	94	97	105	114	120	129	128
10	88	90	84	97	95	89	91
Mean	94	99	103	104	109	108	111
SD	6	22	18	13	14	14	17

Young women 60% MVC

MAP (mmHg)

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	84	87	89	90	98	100	101
2	81	73	72	75	75	77	77
3	96	76	75	74	67	70	68
4	79	77	78	78	79	72	73
5	73	60	65	69	69	67	64
6	83	80	77	79	79	73	72
7	77	74	77	75	78	77	75
8	93	82	82	85	82	82	84
9	70	80	77	76	77	77	76
10	77	87	89	89	87	90	87
11	87	109	114	111	111	111	115
12	85	102	102	103	101	101	100
Mean	82	82	83	84	84	83	83
SD	8	13	14	13	13	14	15

Older women 60% MVC

MAP (mmHg)

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	68	98	101	105	106	106	106
2	95	98	105	111	106	104	121
3	91	92	99	97	90	85	93
4	80	95	103	103	101	100	107
5	93	113	114	119	118	115	112
6	90	100	112	102	110	112	112
7	72	103	99	102	107	106	106
8	108	104	107	110	107	110	105
9	94	94	96	101	102	100	98
10	83	93	96	100	100	98	98
Mean	87	99	103	105	105	104	106
SD	12	6	6	7	7	8	8

Young men 70% MVC

MAP (mmHg)

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	83	93	93	87	84	89	105
2	87	111	112	117	113	111	112
3	87	91	90	84	85	84	86
4	122	113	116	117	119	119	116
5	80	81	92	96	98	94	85
6	92	94	99	101	91	91	89
7	84	73	79	85	91	96	95
8	92	87	83	79	77	78	79
9	87	82	86	89	93	105	107
10	70	72	75	76	78	72	79
11	86	76	84	82	83	89	85
12	72	72	80	88	87	91	92
Mean	87	87	91	92	92	93	94
SD	13	14	13	13	13	13	13

Older men 70% MVC

MAP (mmHg)

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	99	92	97	101	108	97	95
2	89	94	98	104	107	109	109
3	110	107	103	90	89	104	100
4	95	80	88	94	104	101	101
5	108	111	107	104	104	95	123
6	104	98	100	102	107	106	103
7	93	89	97	106	102	102	97
8	93	94	94	100	104	121	124
9	87	94	98	115	111	105	109
10	95	94	95	102	104	106	109
Mean	97	95	98	102	104	105	107
SD	8	9	5	7	6	7	10

Young women 70% MVC

MAP (mmHg)

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	74	62	64	69	67	69	70
2	87	94	82	88	91	87	112
3	72	74	74	78	84	87	85
4	80	78	85	79	80	79	83
5	78	79	80	79	77	79	77
6	76	69	74	71	72	75	72
7	82	80	81	81	82	80	76
8	85	88	91	94	93	90	95
9	73	65	65	69	71	70	63
10	77	75	77	77	80	81	80
11	81	92	91	91	90	93	92
12	80	109	112	113	113	114	115
Mean	79	80	81	83	83	84	85
SD	5	13	13	13	12	12	16

Older women 70% MVC

MAP (mmHg)

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	68	88	96	95	112	103	102
2	111	102	100	97	102	99	102
3	81	99	105	94	90	99	100
4	90	95	97	94	103	101	108
5	91	105	106	109	109	108	111
6	96	102	103	103	103	105	103
7	67	99	98	102	102	104	105
8	69	86	84	104	107	107	102
9	88	103	102	108	98	109	95
10	83	107	118	126	121	123	127
Mean	85	99	101	103	105	106	105
SD	14	7	9	10	8	7	9

Appendix XXII: Heart rate responses for subjects who completed constant force exercise tests at relative exercise intensities of 30%, 45%, 60% and 70% MVC.

Young men 30% MVC

HR (beats.min^{-1})

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	65	73	73	72	73	74	73
2	60	65	68	75	73	74	74
3	59	77	77	81	77	80	80
4	65	75	73	76	74	75	76
5	61	66	67	67	67	67	68
6	72	67	68	67	65	64	64
7	60	61	58	62	62	61	62
8	85	83	84	89	90	89	95
9	91	94	92	94	94	95	96
10	92	92	92	94	94	97	95
11	64	69	71	75	75	76	73
12	67	71	69	71	72	71	74
Mean	70	74	74	77	76	77	78
SD	12	11	10	11	11	12	12

Older men 30% MVC

HR (beats.min^{-1})

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	73	71	73	73	73	73	72
2	56	57	55	58	60	62	58
3	78	82	87	87	85	83	77
4	88	86	87	86	85	85	86
5	82	76	76	75	74	76	76
6	71	71	73	75	76	75	76
7	61	67	74	77	76	79	77
8	70	73	73	75	73	77	74
9	67	73	75	75	74	74	74
10	57	61	61	61	60	62	61
Mean	70	72	74	74	74	75	73
SD	11	9	10	9	8	8	8

Young women 30% MVC

HR (beats.min^{-1})

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	70	64	65	66	66	68	66
2	67	80	77	81	80	80	77
3	51	69	76	80	82	84	86
4	66	82	83	87	89	86	87
5	67	82	84	85	82	84	86
6	93	91	96	93	90	92	91
7	97	97	96	100	100	104	104
8	89	88	87	91	90	86	87
9	63	78	75	77	83	80	75
10	76	76	77	76	80	80	79
11	78	86	89	92	91	90	92
12	67	66	68	68	72	67	70
Mean	74	80	81	83	84	83	83
SD	14	10	10	10	9	10	11

Older women 30% MVC

HR (beats.min^{-1})

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	72	71	79	80	77	76	79
2	96	116	121	105	114	116	124
3	73	74	76	75	74	75	74
4	77	77	77	77	79	83	79
5	80	76	80	83	79	82	81
6	66	72	76	73	73	71	71
7	69	69	72	70	72	72	74
8	58	61	63	66	64	64	64
9	87	85	87	76	85	83	77
10	71	78	81	82	79	83	68
Mean	75	78	81	79	80	80	79
SD	11	15	15	11	13	14	17

Young men 45% MVC

HR (beats.min^{-1})

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	68	75	78	78	77	79	80
2	64	59	60	63	58	61	64
3	59	77	77	81	77	80	80
4	73	78	79	78	78	75	74
5	66	71	69	66	68	70	68
6	73	74	72	73	74	74	78
7	61	61	59	60	63	63	64
8	80	86	90	90	92	92	93
9	85	97	97	97	99	99	97
10	81	84	82	84	84	86	86
11	63	71	72	72	79	81	84
12	113	103	114	105	112	115	100
Mean	74	78	79	79	80	81	81
SD	15	13	16	14	15	15	12

Older men 45% MVC

HR (beats.min^{-1})

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	82	80	78	78	78	77	79
2	70	61	60	61	63	61	63
3	82	79	80	82	81	83	74
4	92	90	91	92	93	95	93
5	84	77	79	80	78	80	79
6	74	72	71	73	73	73	72
7	73	77	84	86	85	84	82
8	71	75	76	75	74	75	76
9	74	83	84	85	84	81	84
10	62	66	70	69	71	71	71
Mean	76	76	77	78	78	78	77
SD	8	8	9	9	9	9	8

Young women 45% MVC

HR (beats.min^{-1})

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	76	69	66	70	67	68	67
2	68	83	80	82	79	82	79
3	59	72	77	78	80	81	83
4	69	86	87	82	83	83	83
5	66	81	76	83	83	84	84
6	70	81	89	89	89	90	80
7	102	109	108	111	110	114	112
8	85	83	82	82	84	84	84
9	82	72	81	79	77	81	73
10	65	77	77	78	77	77	81
11	82	87	87	93	90	89	91
12	73	80	72	71	75	76	77
Mean	75	82	82	83	83	84	83
SD	12	10	11	11	11	11	11

Older women 45% MVC

HR (beats.min^{-1})

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	71	71	71	71	71	71	139
2	70	72	75	74	75	75	76
3	71	72	72	72	70	73	70
4	82	82	88	84	83	82	89
5	97	92	96	97	94	97	76
6	75	79	79	84	77	78	65
7	72	74	74	75	73	74	76
8	60	62	67	59	58	57	88
9	79	84	81	89	77	82	71
10	74	76	76	72	75	67	70
Mean	75	76	78	78	75	76	82
SD	10	8	9	11	9	11	21

Young men 60% MVC

HR (beats.min^{-1})

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	70	76	78	82	81	81	80
2	65	68	70	70	74	77	78
3	63	82	80	80	82	78	83
4	57	76	77	76	78	79	78
5	64	71	73	73	72	73	73
6	75	75	73	73	77	74	76
7	62	65	66	67	67	70	68
8	76	81	86	93	95	94	93
9	100	103	103	106	109	109	110
10	81	85	86	88	83	86	88
11	81	85	87	88	91	93	93
12	101	115	107	120	117	109	114
Mean	74	82	82	85	86	85	86
SD	14	14	13	16	15	13	14

Older men 60% MVC

HR (beats.min^{-1})

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	81	74	74	74	75	74	74
2	67	62	61	62	58	56	58
3	71	83	85	86	86	85	77
4	98	95	97	98	96	99	98
5	85	86	89	88	89	87	88
6	79	81	84	84	81	84	84
7	62	77	89	95	94	93	94
8	68	69	67	68	69	68	69
9	75	83	87	88	87	85	86
10	53	65	69	70	74	75	77
Mean	74	77	80	81	81	81	81
SD	13	10	12	12	12	12	12

Young women 60% MVC

HR (beats.min^{-1})

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	76	72	75	71	71	65	67
2	68	89	89	86	86	92	83
3	57	74	79	83	80	86	86
4	70	86	85	86	87	91	88
5	67	83	77	74	72	72	75
6	82	79	75	84	80	83	76
7	98	105	111	114	115	118	122
8	110	92	95	94	91	95	95
9	69	73	75	76	74	72	80
10	89	88	81	81	80	81	85
11	87	81	82	79	77	76	77
12	67	77	83	79	85	81	89
Mean	78	83	84	84	83	84	85
SD	15	9	11	11	12	14	14

Older women 60% MVC

HR (beats.min^{-1})

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	72	75	72	75	72	73	75
2	69	70	74	73	75	72	82
3	84	85	85	85	85	87	89
4	88	85	84	86	88	86	97
5	100	98	100	97	98	72	78
6	96	86	81	83	85	61	68
7	70	74	68	71	71	51	59
8	56	62	61	61	63	88	79
9	84	86	86	77	83	76	75
10	78	78	80	80	76	81	84
Mean	80	80	79	79	80	75	79
SD	13	10	11	10	10	12	11

Young men 70% MVC

HR (beats.min^{-1})

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	63	88	90	95	94	94	96
2	65	85	85	85	88	88	88
3	59	81	84	84	90	85	86
4	72	77	78	78	77	80	79
5	59	71	68	69	70	69	69
6	75	74	73	73	77	74	76
7	72	73	78	78	78	75	75
8	99	107	107	117	126	129	134
9	99	106	109	111	113	112	118
10	102	120	120	123	120	117	120
11	78	86	84	87	87	92	85
12	69	76	78	76	78	76	73
Mean	76	87	88	90	92	91	92
SD	16	16	16	18	18	19	21

Older men 70% MVC

HR (beats.min^{-1})

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	77	77	78	79	78	78	77
2	63	58	62	62	60	61	62
3	71	83	85	86	86	85	77
4	84	87	89	89	87	90	88
5	84	80	83	83	83	85	85
6	89	92	95	95	95	96	96
7	70	67	83	86	87	90	88
8	79	84	84	83	82	84	83
9	82	90	89	89	91	89	88
10	45	57	67	68	72	77	79
Mean	74	77	81	82	82	83	82
SD	13	13	10	10	10	10	9

Young women 70% MVC

HR (beats.min^{-1})

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	71	65	61	64	64	62	64
2	74	82	88	90	96	72	50
3	53	72	78	83	85	88	88
4	67	80	85	83	84	84	86
5	67	83	89	87	84	85	88
6	74	85	84	88	90	91	100
7	86	97	97	102	103	101	101
8	95	96	92	95	104	102	109
9	67	81	75	73	77	70	75
10	76	83	84	84	88	90	86
11	83	86	86	88	86	94	84
12	66	83	88	80	84	84	80
Mean	73	83	84	85	87	85	84
SD	11	9	9	10	11	12	16

Older women 70% MVC

HR (beats.min^{-1})

	Rest	Min 1	Min 2	Min 3	Min 4	Min 5	Min 6
1	83	89	87	91	109	122	130
2	70	71	70	72	74	72	78
3	81	82	81	80	74	82	82
4	83	83	82	84	93	94	95
5	95	98	98	101	97	89	86
6	85	83	86	84	84	84	86
7	76	74	75	76	75	76	75
8	62	61	64	61	70	87	92
9	93	89	94	92	71	78	81
10	82	79	83	85	84	86	85
Mean	81	81	82	83	83	86	87
SD	10	10	10	11	13	11	10

Appendix XXIII: Arm vascular conductance responses (reactive hyperaemia) for subjects who completed constant force exercise tests at relative exercise intensities of 30%, 45%, 60% and 70% MVC.

Young men

	a	A	Decay constant	Peak VC	A + a
	*	*		*	*
1	2.9	22.3	0.0397	25.9	25.3
2	2.1	20.6	0.0671	20.6	22.7
3	2.6	25.0	0.0741	20.3	27.6
4	4.3	24.2	0.0594	29.8	28.5
5	3.4	15.8	0.0302	19.8	19.2
6	2.9	14.9	0.0277	17.8	17.8
7	1.4	12.9	0.0387	12.8	14.2
8	2.5	32.0	0.0414	33.8	34.5
9	4.3	40.9	0.0308	42.9	45.2
10	1.4	28.0	0.0313	26.0	29.4
11	1.8	18.0	0.0784	16.0	19.8
12	2.1	18.3	0.0376	15.2	20.4
Mean	2.6	22.7	0.046	23.4	25.4
SD	1.0	8.0	0.018	8.7	8.5

* =ml.100ml^{-1}.mmHg^{-1}.min^{-1}.10

Older men

	a	A	Decay constant	Peak VC	A + a
	*	*		*	*
1	2.0	18.0	0.020	20.1	20.0
2	2.7	32.6	0.030	37.5	35.3
3	3.2	13.5	0.108	14.9	16.7
4	4.9	17.0	0.014	19.8	21.9
5	1.6	10.0	0.010	11.0	11.6
6	2.2	12.3	0.027	13.6	14.5
7	4.3	22.0	0.021	32.1	26.3
8	2.9	23.0	0.026	25.3	25.9
9	2.1	24.2	0.034	25.0	26.3
10	2.0	10.0	0.102	11.3	12.0
11	1.2	15.2	0.125	17.0	16.4
Mean	2.7	18.0	0.047	20.7	20.6
SD	1.1	6.9	0.043	8.6	7.3

* =ml.100ml^{-1}.mmHg^{-1}.min^{-1}.10

Young women

	a	A	Decay constant	Peak VC	A + a
	*	*		*	*
1	1.4	20.0	0.064	19.9	21.4
2	3.2	22.0	0.052	16.5	25.2
3	1.6	21.0	0.053	16.7	22.6
4	2.2	19.0	0.031	16.4	21.2
5	1.9	16.0	0.053	16.8	17.9
6	2.1	17.0	0.049	20.0	19.1
7	1.3	22.0	0.061	21.1	23.3
8	1.3	15.0	0.054	14.5	16.3
9	0.8	17.0	0.053	18.0	17.8
10	2.1	17.0	0.036	20.1	19.1
11	5.1	14.4	0.033	18.0	19.5
Mean	2.1	18.2	0.049	18.0	20.3
SD	1.2	2.7	0.011	2.0	2.7

* =ml.100ml^{-1}.mmHg^{-1}.min^{-1}.10

Older women

	a	A	Decay constant	Peak VC	A + a
	*	*		*	*
1	1.8	19.0	0.021	22.5	20.8
2	4.1	23.3	0.084	19.8	27.3
3	1.5	21.0	0.040	23.1	22.5
4	3.0	10.0	0.022	10.6	13.0
5	6.2	14.0	0.022	20.7	20.2
6	4.5	24.0	0.030	24.4	28.5
7	4.7	26.0	0.025	37.6	30.7
8	2.4	13.0	0.076	12.2	15.4
9	1.8	12.1	0.062	12.8	14.0
Mean	3.0	18.0	0.042	20.4	21.4
SD	1.6	5.9	0.025	8.3	6.5

* =ml.100ml^{-1}.mmHg^{-1}.min^{-1}.10

Appendix XXIV: Leg vascular conductance responses (reactive hyperaemia) for subjects who completed constant force exercise tests at relative exercise intensities of 30%, 45%, 60% and 70% MVC.

Young men

	a	A	Decay constant	Peak VC	A + a
	*	*		*	*
1	1.2	10.5	0.093	6.4	11.7
2	0.8	7.0	0.064	10.9	12.1
3	1.5	14.4	0.019	14.4	15.9
4	4.0	24.0	0.018	27.0	28.0
5	2.9	39.7	0.056	39.9	42.5
6	2.4	10.4	0.033	9.6	12.8
7	2.5	12.5	0.023	14.8	15.0
8	1.9	26.0	0.040	26.3	27.9
Mean	2.1	18.1	0.043	18.7	20.8
SD	1.0	11.0	0.026	11.4	10.9

* =ml.100ml^{-1}.mmHg^{-1}.min^{-1}.10

Older men

	a	A	Decay constant	Peak VC	A + a
	*	*		*	*
1	2.2	35.0	0.035	34.7	37.2
2	1.5	17.5	0.027	18.7	19.0
3	0.8	7.0	0.098	7.5	7.8
4	2.4	20.0	0.034	13.9	22.3
5	2.0	24.4	0.028	24.3	26.4
6	1.2	24.0	0.051	24.7	25.2
7	1.6	21.6	0.044	20.8	23.1
8	2.2	39.0	0.060	38.5	41.2
Mean	1.7	23.6	0.047	22.9	25.3
SD	0.6	10.0	0.023	10.2	10.4

* =ml.100ml^{-1}.mmHg^{-1}.min^{-1}.10

Young women

	a	A	Decay constant	Peak VC	A + a
	*	*	s	*	*
1	1.9	27.0	0.066	26.0	28.9
2	0.8	15.8	0.019	16.5	16.6
3	1.1	24.0	0.032	24.9	25.1
4	1.7	13.0	0.027	15.3	14.8
5	2.2	36.0	0.020	35.5	38.2
6	0.9	12.0	0.017	10.5	12.9
7	2.8	11.5	0.051	12.2	14.3
8	1.7	14.0	0.023	11.0	15.7
Mean	1.6	19.2	0.032	19.0	20.8
SD	0.7	8.9	0.018	8.90	9.0

* =ml.100ml^{-1}.mmHg^{-1}.min^{-1}.10

Older women

	a	A	Decay constant	Peak VC	A + a
	*	*	s	*	*
1	1.4	19.0	0.054	18.2	20.4
2	1.5	19.0	0.035	20.6	20.5
3	4.1	32.0	0.020	31.9	36.1
4	3.5	21.3	0.044	18.8	24.8
Mean	2.6	22.8	0.038	22.4	25.5
SD	1.4	6.2	0.014	6.4	7.4

* =ml.100ml^{-1}.mmHg^{-1}.min^{-1}.10

Appendix XXV

PARTICIPANT INFORMATION AND CONSENT FORM

1. Title of study: Muscle fatigue profiles during constant-force calf exercise in young and older healthy non-diabetic men and women.

2. Introduction: The main objective is to quantify muscle fatigue and muscle performance during a novel calf-exercise in healthy young versus old men and women.

3. Procedures: You will be one of the 50 volunteers recruited for this study. You will be over 55 years (Group 2, OLDER). Before the commencement of the project, you will undergo a full medical examination by a General Practitioner, where a blood sample will be taken. If found able to participate in this study, you will be required to visit the Cardiovascular Health Unit in the Department of Physiology in Trinity College Dublin on 2 occasions separated at least by 72 hours.

- Visits to the laboratory (TCD):

Visit 1: Familiarisation session and calf maximum voluntary contractions.

You will be familiarised with the calf ergometer and the fatigue exercise protocol and then you will try and complete six calf maximal voluntary contractions (MVC) (1 min rest periods between maximal efforts) in the upright position (tilt angle of 67°).

Visit 2: 2 fatiguing constant-force calf muscle exercises at two randomly selected exercise intensities (session will last up to 210 min).

On visit 2, you will try and complete a fatiguing constant-force plantar flexion exercises to failure at a randomly selected intensity of 30%, 45%, 60% or 70% MVC seperated by 45 minutes of passive rest. You will then try and complete another fatiguing constant-force plantar flexion exercise test to failure at a different randomly selected intensity of 30%, 45%, 60% or 70% MVC. Visit 2:

Visit 3: 2 fatiguing constant-force calf muscle exercises at two randomly selected exercise intensities (session will last up to 210 min).

On visit 3, you will again try and complete two fatiguing more constant-force plantar flexion exercise tests to failure at the remaining two randomly selected intensities of 30%, 45%, 60% or 70% MVC.

4. Benefits:

There are no direct benefits to you in this study. However, you will gain an understanding into the changes that occur in muscle fatigue with age.

5. Risks:

There are very few risks associated with the exercise procedures used during this study:

Calf exercise: Performance of plantar flexion exercise may result in muscle tightness, soreness, fatigue and rarely a pulled muscle.

Blood sampling may make some volunteers feel uneasy, or prove painful to some. Some may experience slight bruising or discomfort around the sampling area.

6. Exclusion from participation:

You will be excluded from the study if you are taking any medications that could affect your peripheral circulation, have an abnormal HbA_{1c}, and have any active medical problems.

7. Confidentiality:

Your identity will remain confidential. Your name will not be published and will not be disclosed to anyone outside the study group. The data or material will be retained after the study is completed. This material will not be used in future unrelated studies without further specific permission being obtained.

8. Compensation:

This study is covered by standard institutional indemnity insurance. Nothing in this document restricts or curtails your rights.

9. Voluntary Participation:

You have volunteered to participate in this study. You may quit at any time. If you decide not to participate, or if you quit, you will not be penalised and will not give up any benefits that you had before entering the study.

10. Stopping the study:

You understand that the investigators may stop your participation in the study at any time without your consent.

11. Permission: This trial has Research Ethics Committee approval from Trinity College Dublin.

12. Further information:

You can get more information or answers to your questions about the study, your participation in the study, and your rights, from Ms Heather Reilly (086-3273872/ heathernreilly@hotmail.com)) or Dr Mikel Egaña, (01-8963728 / megana@tcd.ie). If the study team learns of important new information that might affect your desire to remain in the study, you will be informed at once.

DECLARATION:

I have read, or had read to me, this consent form. 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. 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.

I understand I may withdraw from the study at any time.

(Name of sponsor:)

……

PARTICIPANT'S NAME:……

CONTACT DETAILS:……

PARTICIPANT'S SIGNATURE:……

Date:……..

NAME OF CONSENTER, PARENT or GUARDIAN……..

(if under 18 years old)

SIGNATURE:……

RELATION TO PARTICIPANT:……..

Statement of investigator's responsibility: I have explained the nature and purpose of this research study, the procedures to be undertaken and any risks that may be involved. 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.

INVESTIGATOR'S SIGNATURE:..... **Date:**.....

Appendix XXVI: Anthropometrical and physical activity data for subjects who completed fatigue tests performed at relative exercise intensities of 30%, 45%, 60% and 70% MVC.

Young men

	Age	Height	Weight	BMI	Leg volume	Activity
	yr	cm	Kg	$Kg.m^{-2}$	mL	$MET.h.week^{-1}$
1	23	192	79	21.4	3388	161.8
2	21	188	77	21.6	2021	192.9
3	28	183	62	18.6	3029	227.3
4	24	178	65	20.7	1900	211.6
5	23	173	69	22.9	2192	186.0
6	24	176	87	28.0	3234	169.0
7	23	180	66	20.5	3219	193.1
8	21	175	58	18.9	2508	225.3
Mean	23	181	70.3	21.6	2686	196
SD	2	7	9.5	2.9	601	24

Older men

	Age	Height	Weight	BMI	Leg volume	Activity
	yr	cm	Kg	$Kg.m^{-2}$	mL	$MET.h.week^{-1}$
1	72	173	77.2	25.8	2967	250
2	68	173	71.6	24.0	2877	195.5
3	73	160	68.6	26.9	2518	162.5
4	70	183	90.3	27.0	3678	173.5
5	60	173	70.1	23.3	2976	269.725
6	67	165	85.7	31.4	2506	160.75
7	60	171	80.5	27.5	29501	189.25
8	69	165	64.2	23.6	2406	173.42
Mean	67	170	76.0	26.2	2860	197
SD	5	7	9	2.7	405	41

Young women

	Age	Height	Weight	BMI	Leg volume	Activity
	yr	cm	Kg	$Kg.m^{-2}$	mL	$MET.h.week^{-1}$
1	23	170	56.0	19.5	2024	155
2	23	166	54.8	19.8	2264	174
3	23	167	62.0	22.3	2712	234
4	23	168	63.6	22.5	2590	225
5	21	159	53.4	21.1	2104	199
6	21	167	64.3	23.2	3406	286
7	23	164	62.3	23.3	3127	231
8	21	166	59.0	21.4	2717	178
Mean	22	166	59.4	21.6	2618	210
SD	1	3	4.2	1.5	484	42

Older women

	Age	Height	Weight	BMI	Leg volume	Activity
	yr	cm	Kg	$Kg.m^{-2}$	mL	$MET.h.week^{-1}$
1	60	164	76.1	28.5	3572	153
2	71	160	59.2	23.3	2339	220
3	70	150	55.0	24.4	1484	131
4	66	153	81.7	34.7	3209	234
5	53	148	65.5	29.8	2450	241
6	64	154	68.6	28.8	2691	212
7	63	168	73.6	26.1	3300	240
8	50	162	57.4	21.8	2699	271
9	67	152	75.6	32.7	2494	183
10	72	166	73.2	26.6	3365	179
Mean	64	158	68.6	27.7	2760	206
SD	7	7	9.0	4.1	623	44

Appendix XXVII: Haematological data for subjects who completed fatigue tests performed at relative exercise intensities of 30%, 45%, 60% and 70% MVC.

Young men

	Hb	Hct	Plasma Glucose	Total Cholesterol	HDL	LDL	Triglycerides	Total Cholesterol/HDL
	$g.dL^{-1}$	%	*	*	*	*	*	*
1	15.0	44.2	4.4	4.0	0.6	3.1	1.3	6.7
2	15.6	46.1	5.1	3.2	1.0	1.9	1.3	3.1
3	16.3	46.6	4.2	4.4	1.1	3.0	1.3	3.9
4	15.1	45.5	4.2	4.0	1.4	2.3	1.3	2.8
5	15.2	46.3	4.2	2.6	1.1	1.2	1.3	2.4
6	16.3	46.9	4.4	3.1	1.2	1.5	1.6	2.5
7	13.9	40.0	4.7	3.3	1.2	1.8	1.3	3.5
8	15.4	45.0	4.7	4.1	1.1	2.7	1.3	3.7
Mean	15.4	45.1	4.5	3.6	1.1	2.2	1.3	3.6
SD	0.8	2.2	0.3	0.6	0.2	0.7	0.1	1.4

*$mmol.L^{-1}$

Older men

	Hg	Hct	Plasma Glucose	Total Cholesterol	HDL	Triglycerides	LDL	Total Cholesterol/HDL
	$g.dL^{-1}$	%	*	*	*	*	*	*
1	15	46.0	3.7	5.2	1	2.0	3.8	5.5
2	13.9	43.1	5.0	4.7	1	1.3	3.6	5.4
3	14.2	44.8	5.7	3.8	1	1.3	2.6	3.8
4	15.5	48.0	4.8	4.0	1	2.1	2.9	6.7
5	14.7	43.0	3.6	5.2	2	1.3	3.2	3.1
6	16.2	47.2	3.7	4.1	1	2.9	2.6	4.3
7	14.9	43.5	3.6	5.4	1	2.9	3.9	6.1
8	16	45.6	3.9	4.6	1	2.9	3.9	6.1
Mean	15.1	45.2	4.3	4.6	1.0	2.1	3.3	5.1
SD	0.8	1.9	0.8	0.6	0.3	0.7	0.6	1.3

*$mmol.L^{-1}$

Young women

	Hg	Hct	Plasma Glucose	Total Cholesterol	HDL	Triglycerides	LDL	Total Cholesterol/HDL
	g.dL^{-1}	%	*	*	*	*	*	*
1	13.9	41.5	4.8	4.3	1.1	1.3	2.9	3.8
2	13.7	41.0	5.5	4.9	1.7	1.3	2.9	2.9
3	13.5	38.1	5.2	3.0	1.0	1.3	1.7	3.0
4	14.2	40.7	5.6	5.2	1.7	1.3	3.2	3.0
5	12.1	37.1	4.6	3.0	1.0	1.3	1.7	2.9
6	14.5	41.8	4.3	3.5	1.2	1.3	2.1	3.0
7	13.5	39.7	4.9	4.1	1.2	1.3	2.6	3.5
8	12.6	37.6	4.6	3.0	1.0	1.3	1.7	3.0
Mean	13.5	39.7	4.9	3.9	1.2	1.3	2.4	3.1
SD	0.8	1.9	0.5	0.9	0.3	0.0	0.6	0.3

*mmol.L^{-1}

Older women

	Hg	Hct	Plasma Glucose	Total Cholesterol	HDL	Triglycerides	LDL	Total Cholesterol/HDL
	g.dL^{-1}	%	*	*	*	*	*	*
1	13.7	40.2	4.8	4.7	1.0	1.3	3.6	4.4
2	14.8	43.8	4.8	4.2	0.9	1.3	3.0	4.4
3	14.4	41.0	5.6	5.2	0.8	1.8	4.0	6.2
4	13.4	39.7	4.0	5.4	1.4	1.3	3.7	4.2
5	13.7	40.2	4.8	4.8	1.1	1.3	3.4	4.4
6	12.3	35.9	4.5	4.8	1.1	1.3	3.4	4.4
7	15.1	44.0	4.5	5.0	1.3	1.3	3.5	4.0
8	12.2	36.0	5.0	3.9	1.6	1.3	2.0	2.4
9	12.8	38.5	4.7	5.5	0.8	2.5	4.1	6.8
10	14.2	42.5	5.0	5.2	1.1	1.3	3.8	4.8
Mean	13.7	40.2	4.8	4.9	1.1	1.5	3.5	4.6
SD	1.0	2.8	0.4	0.5	0.2	0.4	0.6	1.2

*mmol.L^{-1}

Appendix XXVIII: Time to failure data for subjects who completed fatigue exercise tests performed at relative exercise intensities of 30%, 45%, 60% and 70% MVC.

	Young men	Older men	Young women	Older women
	TTF	TTF	TTF	TTF
	min	min	min	min
1	6.2	6.4	11.5	7.1
2	12.2	20.0	14.5	13.3
3	13.0	20.0	22.0	10.3
4	8.5	19.0	7.2	5.1
5	10.0	9.0	11.5	10.1
6	10.0	18.3	20.0	20.0
7	12.3	11.1	11.5	12.5
8	12.5	20.2	12.0	13.6
9				7.0
10				20.0
Mean	10.6	15.5	13.8	11.9
SD	2.4	5.7	4.9	5.1

Appendix XXIX: Maximum voluntary contraction (MVC) values immediately prior to the fatigue tests for subjects who completed fatigue exercise tests at relative exercise intensities of 30%, 45%, 60% and 70% MVC

	Young men	Older men	Young women	Older women
	Pre 30% MVC fatigue test (N)			
1	740	515	555	588
2	1162	1080	908	1129
3	1085	451	860	710
4	1055	1034	929	869
5	1291	1103	428	743
6	1344	1250	840	650
7	1360	750	1006	710
8	1050	988	1030	773
9				940
10				618
Mean	1136	896	820	773
SD	204	291	215	165

	Young men	Older men	Young women	Older women
	Pre 45% MVC fatigue test (N)			
1	740	535	565	495
2	1200	1100	952	1152
3	1280	451	870	639
4	1011	1100	929	927
5	1417	1200	288	748
6	1203	1250	800	630
7	1386	710	1050	819
8	1050	888	1073	679
9				857
10				676
Mean	1161	904	816	762
SD	222	308	266	186

	Young men	Older men	Young women	Older women
	Pre 60% MVC fatigue test (N)			
1	740	534	555	491
2	1213	1100	964	1152
3	1360	557	858	639
4	1054	1034	850	927
5	1386	1290	486	748
6	1205	1250	840	650
7	1386	709	1050	819
8	1050	900	1030	773
9				857
10				675
Mean	1174	922	829	773
SD	222	297	208	182

	Young men	Older men	Young women	Older women
	Pre 70% MVC fatigue test (N)			
1	673	550	809	589
2	1213	923	887	1129
3	1405	558	878	732
4	1055	1100	906	927
5	1384	1200	662	743
6	1146	1295	800	565
7	1390	827	1006	709
8	1050	951	1030	679
9				940
10				618
Mean	1165	926	872	763
SD	247	275	118	181

Appendix XXX: Rate of fatigue values for subjects who completed fatigue exercise tests at relative exercise intensities of 30%, 45%, 60% and 70% MVC.

30% MVC

	Young men	Older men	Young women	Older women
	Ns^{-1}	Ns^{-1}	Ns^{-1}	Ns^{-1}
1	-0.100	-0.166	-0.131	-0.031
2	-0.032	-0.063	-0.011	-0.073
3	-0.443	-0.021	-0.095	-0.125
4	-0.135	-0.068	-0.120	-0.035
5	-0.084	-0.052	-0.010	-0.026
6	-0.142	7.40E-03	-0.079	-1.50E-03
7	-0.059	-0.080	-0.055	-0.022
8	-0.020	-2.50E-03	-0.017	-1.08E-01
9				-0.051
10				-0.030
Mean	-0.127	-0.056	-0.065	-0.050
SD	0.135	0.055	0.049	0.040

45% MVC

	Young men	Older men	Young women	Older women
	Ns^{-1}	Ns^{-1}	Ns^{-1}	Ns^{-1}
1	-0.115	-0.123	-0.054	-0.058
2	0.0343	-0.045	-0.128	-0.074
3	-0.275	-0.021	-0.079	-0.152
4	-0.416	-0.074	-0.118	-0.588
5	-0.068	-0.064	-0.017	-0.072
6	-0.219	-0.045	-0.065	-0.051
7	-0.130	-0.134	-0.115	-0.019
8	-0.075	-5.30E-03	-0.084	-4.59E-02
9				-0.085
10				-0.055
Mean	-0.158	-0.064	-0.083	-0.120
SD	0.141	0.046	0.037	0.168

60% MVC

	Young men	Older men	Young women	Older women
	Ns^{-1}	Ns^{-1}	Ns^{-1}	Ns^{-1}
1	-0.126	-0.296	-0.173	-0.065
2	-0.417	-0.068	-0.016	-0.193
3	-0.363	-0.051	-0.090	-0.118
4	-0.234	-0.081	-0.268	-0.104
5	-0.144	-0.090	-0.017	-0.067
6	-0.240	-0.053	-0.117	-0.054
7	-0.124	-0.128	-0.114	-0.080
8	-0.106	-6.40E-03	-0.075	-0.049
9				-0.093
10				-0.057
Mean	-0.219	-0.097	-0.109	-0.088
SD	0.118	0.088	0.083	0.043

70% MVC

	Young men	Older men	Young women	Older women
	Ns^{-1}	Ns^{-1}	Ns^{-1}	Ns^{-1}
1	-0.289	-0.304	-0.357	-0.215
2	-0.251	-0.166	-0.173	-0.236
3	-0.534	-0.057	-0.192	-0.338
4	-0.483	-0.085	-0.350	-0.865
5	-0.432	-0.140	-0.260	-0.094
6	-0.391	-0.147	-0.102	-0.056
7	-0.249	-0.206	-0.190	-0.089
8	-0.235	-0.008	-0.099	-0.127
9				-0.264
10				-0.027
Mean	-0.358	-0.139	-0.215	-0.231
SD	0.117	0.092	0.100	0.244

Appendix XXXI: End exercise % force (relative to initial MVC):

End exercise % force 30%

	Young men	Older men	Young women	Older women
1	88	90	90	95
2	96	75	84	83
3	80	91	98	90
4	95	95	100	96
5	82	89	89	98
6	60	101	89	78
7	72	100	81	95
8	78	94	94	82
9				97
10				92
Mean	80	92	91	91
SD	16	8	7	7

End exercise % force 45%

	Young men	Older men	Young women	Older women
1	97	93	86	87
2	92	67	86	88
3	81	91	98	90
4	95	93	89	92
5	70	76	85	95
6	66	97	89	75
7	77	98	99	86
8	60	93	78	94
9				86
10				87
Mean	80	89	89	88
SD	14	11	7	6

End exercise % force 60%

	Young men	Older men	Young women	Older women
1	87	85	76	80
2	85	78	86	87
3	84	90	95	85
4	66	87	88	90
5	86	74	82	91
6	76	96	87	80
7	81	93	72	92
8	72	92	94	90
9				92
10				89
Mean	80	87	85	88
SD	8	8	8	5

End exercise % force 70%

	Young men	Older men	Young women	Older women
1	69	72	73	80
2	73	59	66	72
3	73	75	62	82
4	64	77	84	87
5	86	80	71	81
6	70	89	71	66
7	75	85	49	84
8	73	91	78	83
9				85
10				81
Mean	73	79	69	80
SD	6	10	11	6

Appendix XXXII: Predicted maximum values for subjects who completed fatigue exercise tests at relative exercise intensities of 30%, 45%, 60% and 70% MVC.

PREDICTED MAXIMUM 30% MVC (N)

	Young men	Older men	Young women	Older women
1	679.0	362	607	583
2	1161	958	890	1316
3	1230	459	892	696
4	1039	1004	979	830
5	1276	1037	399	794
6	1039	1270	826	641
7	1174	709	1025	726
8	1058	1060	954	854
9				908
10				632
Mean	1082	857	822	798
SD	185	316	213	211

PREDICTED MAXIMUM 45% MVC (N)

	Young men	Older men	Young women	Older women
1	772	479	531	541
2	1043	959	988	1178
3	1217	501	887	617
4	1120	1097	910	930
5	1375	1262	430	787
6	1152	1314	750	750
7	1063	713	1105	710
8	1029	886	966	652
9				912
10				641
Mean	1096	901	821	772
SD	173	320	234	189

PREDICTED MAXIMUM 60% MVC (N)

	Young men	Older men	Young women	Older women
1	739	541	663	536
2	1163	955	949	1161
3	1328	574	899	602
4	1153	963	876	854
5	1284	1343	474	768
6	1158	1291	838	697
7	1281	688	1051	773
8	1005	846	1002	755
9				936
10				703
Mean	1139	900	844	779
SD	191	302	190	176

PREDICTED MAXIMUM 70% MVC (N)

	Young men	Older men	Young women	Older women
1	641	369	604	604
2	1099	953	862	1227
3	1395	579	922	858
4	1034	1074	857	944
5	1329	1343	654	814
6	1094	1246	748	619
7	1491	754	1034	756
8	1045	823	994	833
9				933
10				631
Mean	1141	893	834	822
SD	266	330	155	189

Appendix XXXIII: Heart rate and MAP responses at rest, onset of exercise (6 s) and end-exercise for subjects who completed fatigue exercise tests at relative exercise intensities of 30%, 45%, 60% and 70% MVC.

Young men 30% MVC

	HR (beats.min^{-1})			MAP (mmHg)		
	Rest	Onset	EE	Rest	Onset	EE
1	74	76	82	97	82	99
2	55	58	68	100	110	115
3	66	75	70	94	89	79
4	81	83	90	125	118	120
5	90	100	100	85	74	92
6	84	90	97	87	85	83
7	66	70	96	66	78	74
8	79	82	92	72	89	76
Mean	74	79	87	91	91	92
SD	11	13	12	18	15	18

Older men 30% MVC

	HR (beats.min^{-1})			MAP (mmHg)		
	Rest	Onset	EE	Rest	Onset	EE
1	70	72	77	72	95	86
2	67	61	66	107	105	124
3	82	86	87	120	118	91
4	74	87	81	96	107	122
5	86	92	81	103	81	98
6	88	101	102	98	99	118
7	80	83	75	131	127	126
8	79	85	83	78	107	78
Mean	78	83	82	101	105	105
SD	7	12	10	20	14	19

Young women 30% MVC

	HR (beats.min^{-1})			MAP (mmHg)		
	Rest	Onset	EE	Rest	Onset	EE
1	98	97	99	54	60	57
2	86	92	102	87	76	77
3	86	87	91	91	86	71
4	66	72	68	88	76	87
5	100	100	101	78	71	68
6	82	94	94	67	75	71
7	89	91	100	94	108	103
8	67	87	86	93	95	88
Mean	84	90	93	82	81	78
SD	13	6	11	14	15	14

Older women 30% MVC

	HR (beats.min^{-1})			MAP (mmHg)		
	Rest	Onset	EE	Rest	Onset	EE
1	71	80	83	80	104	119
2	92	95	86	96	114	107
3	72	78	76	77	91	76
4	73	82	84	101	100	108
5	71	92	82	89	97	98
6	79	76	68	106	104	123
7	66	83	84	71	65	89
8	71	78	69	94	84	87
9	73	89	85	88	87	99
10	78	76	73	85	89	97
Mean	75	83	79	89	94	100
SD	7	7	7	11	14	14

Young men 45% MVC

	HR (beats.min^{-1})			MAP (mmHg)		
	Rest	Onset	EE	Rest	Onset	EE
1	75	80	82	95	97	110
2	53	65	72	71	105	93
3	63	68	78	84	93	81
4	74	82	94	110	113	94
5	94	98	108	71	79	75
6	83	83	82	80	78	78
7	67	101	108	75	74	91
8	81	89	91	72	68	78
Mean	74	83	89	82	88	88
SD	13	13	13	14	16	12

Older men 45% MVC

	HR (beats.min^{-1})			MAP (mmHg)		
	Rest	Onset	EE	Rest	Onset	EE
1	63	65	68	93	83	93
2	63	67	64	71	84	96
3	105	109	110	96	98	100
4	80	80	95	98	101	128
5	79	93	84	81	104	118
6	85	96	101	67	85	92
7	85	86	86	91	101	135
8	71	79	71	76	73	89
Mean	79	84	85	84	91	106
SD	14	15	17	12	11	18

Young women 45% MVC

	HR (beats.min^{-1})			MAP (mmHg)		
	Rest	Onset	EE	Rest	Onset	EE
1	97	106	107	64	69	66
2	89	108	102	87	79	70
3	82	95	93	81	71	82
4	72	91	77	59	78	75
5	90	104	94	71	66	79
6	79	97	89	60	76	73
7	76	93	79	71	71	74
8	60	90	74	104	97	115
Mean	81	98	89	75	76	79
SD	12	7	12	15	10	15

Older women 45% MVC

	HR (beats.min^{-1})			MAP (mmHg)		
	Rest	Onset	EE	Rest	Onset	EE
1	69	77	79	74	79	80
2	94	96	100	110	107	134
3	77	81	83	95	111	101
4	79	79	84	94	92	96
5	84	94	109	96	84	108
6	74	83	79	93	105	121
7	70	81	75	73	65	89
8	65	68	66	68	66	100
9	71	80	78	86	96	97
10	78	85	81	96	98	99
Mean	76	82	83	89	90	103
SD	8	8	12	13	17	15

Young men 60% MVC

	HR (beats.min^{-1})			MAP (mmHg)		
	Rest	Onset	EE	Rest	Onset	EE
1	74	78	80	93	93	106
2	53	60	79	71	100	115
3	63	78	81	84	89	73
4	74	90	94	112	117	126
5	84	102	100	94	88	92
6	86	90	85	80	97	84
7	67	101	108	71	93	100
8	80	87	94	86	97	99
Mean	73	86	90	86	97	99
SD	11	14	10	13	9	17

Older men 60% MVC

	HR (beats.min^{-1})			MAP (mmHg)		
	Rest	Onset	EE	Rest	Onset	EE
1	69	76	74	93	84	90
2	63	69	67	90	87	93
3	104	101	94	104	110	110
4	82	82	83	93	98	128
5	80	90	87	104	97	107
6	86	97	112	67	91	125
7	74	82	76	97	92	102
8	73	80	79	76	92	96
Mean	79	85	84	91	94	106
SD	13	11	14	13	8	14

Young women 60% MVC

	HR (beats.min^{-1})			MAP (mmHg)		
	Rest	Onset	EE	Rest	Onset	EE
1	98	100	99	75	67	64
2	88	104	101	87	77	66
3	77	85	97	99	71	72
4	66	79	76	74	82	83
5	85	95	94	74	69	72
6	72	91	90	61	83	75
7	66	93	84	71	82	86
8	70	75	80	91	111	122
Mean	78	90	90	79	80	80
SD	12	10	9	12	14	19

Older women 60% MVC

	HR (beats.min^{-1})			MAP (mmHg)		
	Rest	Onset	EE	Rest	Onset	EE
1	69	87	80	70	86	90
2	94	96	108	126	123	140
3	77	94	95	85	83	93
4	79	90	90	94	93	109
5	83	85	95	102	101	117
6	74	84	80	93	112	124
7	70	79	74	68	91	99
8	64	71	71	61	63	65
9	71	87	82	93	85	85
10	70	77	75	87	98	104
Mean	75	85	85	88	93	103
SD	9	8	12	19	16	21

Young men 70% MVC

	HR (beats.min^{-1})			MAP (mmHg)		
	Rest	Onset	EE	Rest	Onset	EE
1	78	94	115	100	80	110
2	60	74	87	100	125	131
3	75	73	73	86	95	82
4	81	81	117	125	134	129
5	84	98	113	98	83	88
6	84	86	93	66	78	97
7	65	99	103	92	81	88
8	79	83	80	72	81	86
Mean	76	86	98	92	94	101
SD	9	10	17	18	22	20

Older men 70% MVC

	HR (beats.min^{-1})			MAP (mmHg)		
	Rest	Onset	EE	Rest	Onset	EE
1	76	79	75	72	101	112
2	61	60	67	118	117	123
3	105	96	95	101	124	109
4	75	80	95	106	99	121
5	81	86	87	104	112	103
6	92	100	99	105	95	125
7	81	90	87	110	122	140
8	66	81	107	90	87	93
Mean	80	84	89	101	107	116
SD	14	12	13	14	13	15

Young women 70% MVC

	HR (beats.min^{-1})			MAP (mmHg)		
	Rest	Onset	EE	Rest	Onset	EE
1	72	69	86	75	70	73
2	59	94	113	84	85	82
3	73	83	95	82	80	72
4	69	95	102	82	83	77
5	106	126	121	57	82	83
6	82	100	96	77	68	70
7	80	97	109	82	82	97
8	70	97	87	75	65	70
9	104	104	99	67	76	78
10	82	98	95	94	88	111
11	70	99	100	112	111	118
Mean	79	97	100	81	81	85
SD	14	14	11	14	12	17

Older women 70% MVC

	HR (beats.min^{-1})			MAP (mmHg)		
	Rest	Onset	EE	Rest	Onset	EE
1	71	83	85	80	106	120
2	91	86	124	138	134	144
3	79	78	75	87	88	92
4	78	85	80	101	93	111
5	71	83	85	89	107	108
6	79	68	75	106	105	122
7	68	85	93	62	81	95
8	71	64	74	94	98	90
9	67	91	91	68	93	104
10	73	71	69	99	97	101
Mean	75	80	85	92	100	109
SD	7	9	16	21	15	17

X Publications

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