The Effects of Exercise Training on Cardiac and Peripheral Function in Men and Women

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Declaration.

I take full responsibility for the material presented within this PhD thesis and confirm that this is all my own work.

Acknowledgements.

I hereby acknowledge my supervisory team; Professor Tim Cable, Professor David Goldspink and Dr Jatin Burniston for their guidance and support throughout my PhD studies. I would in particular, like to thank Professor Goldspink, for the benefit of his experience and his mentorship throughout the 3 years.

I would also like to express my sincere gratitude to every participant who volunteered, but especially to the older men and women who spent nearly a year training and giving their lives to the project.

I am grateful for the support and help from my 'CPO partner', Paul Woods and from our 'on-tap medic', Dr. Ash Patwala, without whom the training study would not have been possible. I would also like to thank Dr. Jos Vanrenteghem, Shellina Skyrme and Mark Black for their collaboration and the supervision of training sessions.

Finally, I would like to extend my thanks to my family. Firstly, to my Mum and Dad, who have always supported me in everything I do and have helped me to get where I am today. They taught me to have ambition and drive to excel myself in whatever venture I undertake. Secondly, I would like to thank my husband Chris for his immense love and support over this time: a shoulder to cry on, always being there to cheer me up, making me realise the value of what I was doing, and always saying the right things. Thank you, my darling. Lastly, to Shadow and Elmo (the cats), for keeping me company throughout my thesis write-up.

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Abstract.

Aerobic power ($\dot{V}O_{2\,max}$) and cardiac output decrease in both sexes with age. Endurance exercise is known to affect cardiac structures and function, and could therefore attenuate the effects of ageing. However, recent studies have suggested that men and women of similar ages adapt differently to exercise training, including adaptations in cardiac function. In younger men and women, training modality is also an important determinant of improvements in cardiac function, but the full effects of exercise training need to be determined both centrally and peripherally.

Cardiac power output (CPO) incorporates measurements of both blood flow ($\dot{Q}$) and mean blood pressure (MAP), and is the most comprehensive method of measuring overall cardiac function. In preliminary studies we elucidated the reliability and reproducibility of the CO_2 rebreathing technique used to determine $\dot{Q}$, and the potential effects of caffeine ingestion on CPO. Then older men and women participated in 30 weeks of training, with step-wise increments in exercise intensity. This programme increased aerobic power and increased the extraction of oxygen in the peripheries, but with no discernable effects on the heart's maximum pumping or reserve capacities. Six week endurance training (interval and continuous) of young men produced similar results. However using proteomics, interval training induced greater expressions of some contractile proteins, creatine kinase-M and heat shock protein 70 kDa, in the vastus lateralis muscle, suggesting possible conversion towards a faster muscle phenotype, but only in men.

We conclude that endurance training with exercise intensities <75 % HRR induces increases in $\dot{V}O_{2\,max}$ and peripheral adaptations in older people, but intensities >75 % HRR are needed to induce changes in cardiac function. We also found no discernable cardiovascular sex-specific differences in either young or older people after either interval or continuous exercise training. However, skeletal muscle exhibited contractile and metabolic adaptations to these training regimes, and these were sex-specific.

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Abbreviations.

2DE	2 Dimensional Electrophoresis
ADP	Adenosine Di-phosphate
ATP	Adenosine Tri-phosphate
a-vO_2 diff	Arterial-venous Difference
Ca^{2+}	Calcium
Ca_{CO2}	Arterial Concentration of CO_2
CI	Cardiac Index
CK	Creatine Kinase
CO	Cardiac Output
CO_2	Carbon Dioxide
COX	Cyclooxygenase
CPO	Cardiac Power Output
CPO_{max}	Maximal Cardiac Power Output
CPO_{rest}	Resting Cardiac Power Output
CR	Cardiac Reserve
CS	Citrate Synthase
CV	Co-efficient of Variance
C_{VCO2}	Venous Concentration of CO_2
DBP	Diastolic Blood Pressure
DEXA	Dual Energy X-ray Absorptiometry
ECG	Electrocardiogram
EDVI	End-diastolic Volume Index
ELC	Essential Light Chain
FADH	flavin adenine dinucleotide
GP	Glucose 3-Phosphate
H^+	Hydrogen ion
HK	Hexose Kinase
HR	Heart Rate
HRT	Hormone Replacement Therapy
HSC	Heat Shock Cognate
HSP	Heat Shock Protein
IEF	Isoelectric focussing

IL	Interleukin
LDH	Lactate Deydrogenase
LV	Left ventricular
MAP	Mean Arterial Pressure
MHC	Myosin Heavy Chain
MLC	Myosin Light Chain
MLCK	Myosin Light Chain Kinase
M_r	Molecular Weight
MS/MS	Tandem Mass Spectrometry
NADH	Nicotinamide adenine dinucleotide
$PaCO_2$	Arterial Carbon Dioxide Partial Pressure
pCO_2	Partial Pressure of Carbon Dioxide
PCr	Phosphocreatine
PDH	Pyruvate dehydrogenase
$P_{ET}CO_2$	End-tidal Partial Pressure of Carbon Dioxide
PFK	Phosphofructokinase
P*I*	Isoelectric Point
PMF	Peptide Mass Fingerprinting
PVR	Peripheral Vascular Resistance
RER	Respiratory Exchange Ratio
RLC	Regulatory Light Chain
RR	Respiratory Rate
SA node	Sinoatrial Node
SBP	Systolic Blood Pressure
SV	Stroke Volume
SVI	Stroke Volume Index
SVR	Systemic Vascular Resistance
TCA	Tricarboxylic acid
TnT	Troponin T
VE	Tidal Ventilation
Vt	Tidal Ventilation
VCO_2	Carbon Dioxide Production
VO_2	Oxygen Uptake
$\dot{V}O_{2\,max}$	Maximal Oxygen Consumption

Chapter 1

Introduction

Aerobic capacity and muscle power generation are the two most important factors for the maintenance of quality of life and functional independence with ageing. Peak oxygen consumption declines around 10 % per decade in sedentary individuals with ageing. This is exacerbated further by an age-associated decline in physical activity levels. The majority of information on the age-related decline in aerobic capacity has been obtained from cross-sectional studies, which due to selection bias, may misrepresent the ageing process. By their nature, longitudinal studies are more accurate in assessing age-related processes and can give a more precise measure of the rate of decline with ageing.

The heart can be perceived as a power generator, by its development of blood flow (Q) and blood pressure (MAP; Bergel *et al.*, 1969), giving rise to the concept of cardiac power output, which provides an accurate measure of myocardial function. Cardiac power output at maximal exercise has been well correlated to aerobic capacity (Cooke et al., 1998) and therefore, the measurement of cardiac power can give an assessment of overall cardiac function in both heart failure and healthy individuals. Changes in central function (i.e. stroke volume and cardiac output) have been found with aerobic exercise training (Spina *et al.*, 1993; Ehsani *et al.*, 1991; Marshall *et al.*, 2001). Given that cardiac power output extends these measurements by accounting for pressure changes, CPO represents the overall measure of cardiac pumping capabilities, compared to measurements of maximal cardiac output or left ventricular stroke volume work index (Tan *et al.*, 1989).

Improvements in aerobic fitness have been seen in both older and younger men and women after endurance-based training. The magnitudes of these improvments in cardiovascular function are dependent on frequency, intensity and duration of the training sessions (Wenger & Bell, 1986) and also the type of training performed. For example, short high-intensity work periods interspersed by longer rest periods (interval training), has recently been popularised, and has been reported to promote similar improvements in aerobic capacity when compared to endurance training (Branch *et al.*, 2000). Improvements in aerobic capacity with interval training have been attributed to either increases in cardiac output (Helgerud *et al.*, 2007), or equal contributions of cardiac output and a-vO_2

difference (Daussin *et al.*, 2007; Makrides *et al.*, 1990). What is also clear is how CPO responds to endurance and interval type training, since there may be differential adaptations in cardiac output and pressure responses with these training modalities. In contrast, short-term endurance training has been shown to increase a-vO_2 difference only.

Given that males have been reported to lose myocyte number with age compared with females (Olivetti et al., 1995), it is unclear whether any differences exist in response to endurance training in males or females. The differences with women adapting to training have been attributed to their sex hormones (i.e. oestrogen), muscle metabolism (i.e. greater fat utilisation), smaller lean body mass and smaller hearts (Mitchell *et al.*, 1992). To date, there have been few studies on both men and women that have directly compared the effects of interval and continuous exercise training on cardiac function. Extending this further, there are no known studies that have measured cardiac function, using the same maintained work-loads between interval and continuous training, in both men and women.

Another research area which has been under investigated is the effect of interval and continuous training on skeletal muscle. Skeletal muscles are heterogeneous with respect to their fibre type compositions, and in consequence possess a range of different contractile and energetic properties, which can be influenced by exercise training. More recently, advances in molecular biology and screening methods (i.e. 2-dimensional electrophoresis) have allowed the analyses of numerous proteins, including spliced variants and post-translational modifications of proteins (Strohman, 1994). These proteomic techniques therefore allow a broad assessment of the expression of muscle proteins and can potentially be used to investigate possible sex-specific differences and the changes induced with exercise training.

Overall, the human body is complex and multifaceted, especially in relation to its responses to exercise training. The majority of current research has a propensity to focus on one particular aspect of the body, e.g. the heart or the vascular system. This thesis investigates both the heart and skeletal muscle, in both

young and older men and women. The main emphasis of this thesis is to provide an insight into 'whole body' responses to exercise training and to allow the comparison of different systems and tissues to different modalities of exercise training.

Therefore, the overall aims of this thesis are to:

i) Investigate potential sex-specific differences in overall cardiac function, by subjecting healthy, but sedentary men and women to a long-term endurance training programme.

ii) Establish whether exercise modalities, comparing interval and continuous exercise training, have the same or different effects on overall cardiac function.

iii) Establish whether interval versus continuous exercise training has differential effects on muscle protein expression.

iv) Establish whether these central and peripheral changes are sex-specific.

It is hoped that these results will enable a more accurate exercise prescription for both younger men and women in order to maintain a healthy lifestyle and attenuate the onset of early cardiovascular diseases.

Due to the diverse nature of this thesis, and in order to allow coherence for the reader, each experimental study will be prefaced by an extended introduction that reviews the literature relevant to the topic of the respective chapter.

Study 1 (chapter 3) reviews the methodological considerations of cardiac power output measurements, both at rest and during maximal exercise.

Study 2 (chapter 4) examines the impact of long-term endurance training on cardiac function in older men and women to determine if sex-specific differences exist and whether exercise might be useful in attenuating the age-related decline in function.

Study 3 (chapter 5) addresses the effects of short-term interval and continuous exercise training on cardiac function in young men and women.

Study 4 (chapter 6) assesses the impact of interval and continuous training on muscle protein expression in men and women.

Chapter 2

Methods.

2.1 Laboratory conditions.

All Cardiac Power Output tests were performed in a specifically designed laboratory within the Research Institute for Sport and Exercise Sciences (John Moores University), with an ambient temperature of 22 °C and relative humidity around 45 %.

2.2 Participant preparation.

Prior to testing, all participants abstained from food (3 hours), caffeine (6 hours), alcohol and exercise (24 hours). This ensured a rested, baseline physiological state for each participant.

On the participant's first visit, a familiarisation session was given with the equipment and protocols, (e.g. facial gas collection, treadmill and CO_2 re-breathing manoeuvre). Any participant who was unfamiliar with using a treadmill was instructed how to use the machine safely without causing injury to themselves. Verbal instructions were given for each procedure and queries and concerns from the participants were answered. Participants were given the right to withdraw from the exercise at any time and any participant that did not complete the whole protocol was excluded from the data set.

2.3 Ethical Approval.

Ethical approval was given by Liverpool John Moores University Human Ethics Committee. Verbal and written explanations of all testing procedures were given to all participants. Participants also signed a written consent form before any testing occurred.

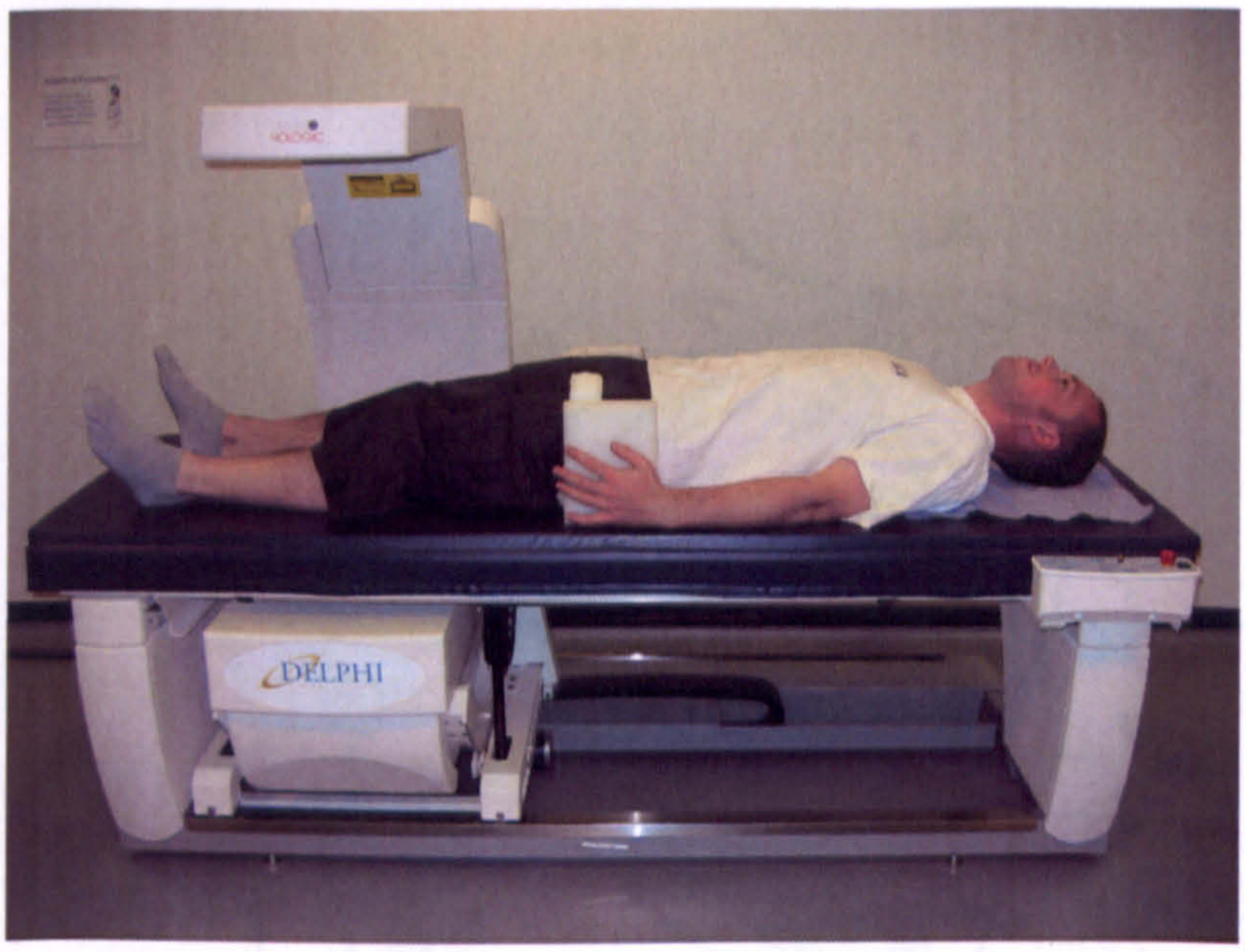
2.4 Measurement of body composition using Dual Energy X-ray Absorptiometry.

Height was measured using a Harpenden stadiometer to the nearest 0.1 cm and body mass was measured using Avery balance beam scales to the nearest 0.1 kg. Height and body mass were used to calculate body surface area according to DuBois and DuBois (1916).

Whole-body composition was measured using Dual Energy X-ray Absorptiometry (Hologic Inc, Horizon Park, Levensesteenweg, Belgium). The DEXA was calibrated on a daily basis using a spine phantom (test precision imaging) and weekly using a step phantom (calibrate soft and lean tissue concentrations). All participants removed any metal objects from their person and wore light clothing for the procedure. The participant laid on the DEXA bed whilst a collimated dual energy x-ray fan beam passed over them three times and the merging X-rays were subsequently detected and measured above the participant. The bed and scanner moved to cover all of the participant's body area. The scan lasted approximately 3 minutes with the participant in a supine position (Figure 2.1). The software (Delphi A S/N 70719) calculated fat free tissue, fat tissue and bone density. The DEXA generated regional default lines (which could be manually adjusted) to isolate specific regions of the body (head, left and right arms and legs, trunk and pelvis).

2.5 ECG.

All participants wore a 12-lead ECG, using the Mason-Likar electrode placement (10 electrodes were used to present 12 views of the heart; Figure 2.2). The participant's chest was prepared using an alcohol wipe and ensuring a clean surface for the electrodes to be placed. Each ECG trace was checked by a cardiologist for any abnormalities. If any abnormalities occurred, participants were referred to their GP and not accepted onto the study. This ECG trace was continued throughout each test to measure resting and maximal heart rate.



DXA Results Summary:

Region	BMC (g)	Lean (g)	Lean+BMC (g)	Total Mass (g)	% Fat
L Arm	237.57	3775.0	4012.6	4923.6	18.5
R Arm	249.90	3989.6	4239.5	5175.7	18.1
Trunk	948.62	34016.1	34964.7	40766.7	14.2
L Leg	606.32	11255.1	11861.5	14493.5	18.2
R Leg	636.25	11864.1	12500.3	15149.9	17.5
Subtotal	2678.67	64899.8	67578.5	80509.3	16.1
Head	627.09	3779.6	4406.7	5497.2	19.8
Total	**3305.76**	**68679.5**	**71985.2**	**86006.5**	**16.3**

TBAR2460

Figure 2.1: DEXA scan and an example of a DEXA summary of results. (bone mineral content, BMC; lean muscle mass, Lean; lean body mass + bone mineral content, Lean + BMC; total percentage body fat, % fat).

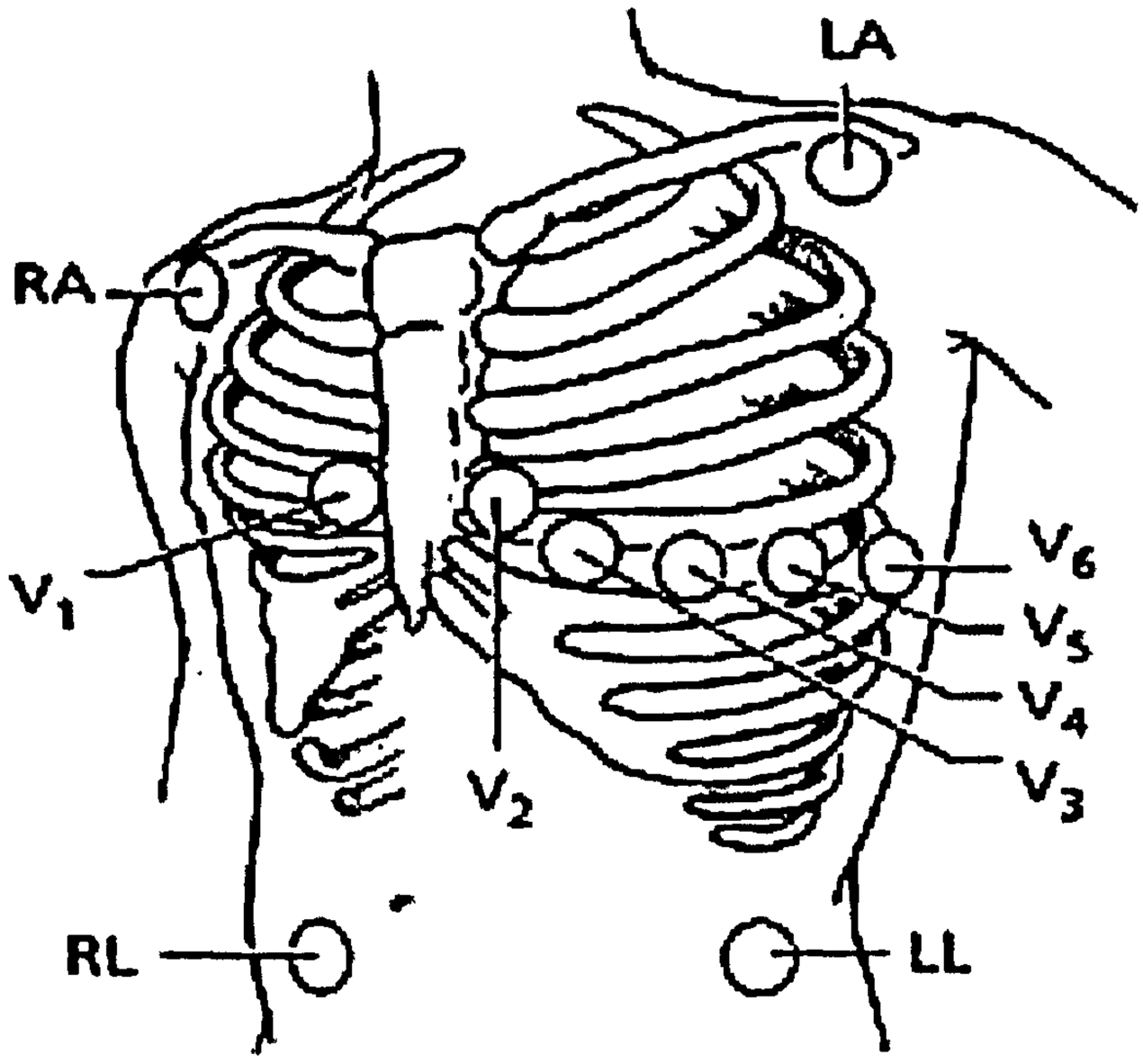


Figure 2.2: Mason-Likar ECG electrode placement.

V1 – V6 standard positioning

LA, RA, LL, RL relocated from the limbs to the torso.

The 10 electrodes give 12 different electrical views of the heart.

2.6 Maximal Oxygen Consumption ($\dot{V}O_{2\,max}$).

This test was performed on a treadmill (Cosmos, Nussdorf-Traustein, Germany) using a modified Bruce (1971) protocol. The treadmill started at a speed of 2.2 kph with a 0 % incline, with both speed and gradient increasing every 1 minute (Table 2.1). The modified Bruce (1971) protocol was used to gain linear increases in VO_2 and heart rate relative to workload to avoid fluctuations in these variables, which had become apparent in the unmodified version. All participants were told not to support themselves on the treadmill bar and were verbally encouraged until volitional exhaustion was reached.

To gain true values for maximal oxygen consumption, the following 3 criteria must be met:

1. Respiratory exchange ratio > 1.1 at maximal exercise.
2. Heart rate > 95 % of age predicted maximum heart rate (220 – age).
3. Plateau in VO_2 occurs (i.e. <150 ml/min increase), despite further increases in workload.

(Johnson, 1998)

Heart rate was determined during exercise from the 12-lead ECG. The CPX/D system (Medgraphics, St.Pauls, Minnesota, USA) measured VO_2 (oxygen uptake), VCO_2 (carbon dioxide production), RER (respiratory exchange ratio), $P_{ET}CO_2$ (end tidal partial pressure of carbon dioxide), VE (tidal ventilation), Vt (tidal volume) and RR (respiratory rate). Across each breathing cycle, VO_2 and VCO_2 were averaged from 5 breaths.

The purpose of this cardio-respiratory exercise stress test ($\dot{V}O_{2\,max}$) was:

a. familiarisation of participants with using a treadmill
b. detection of any underlying heart conditions.
c. to determine aerobic exercise capacity.

Table 2.1: Adapted Bruce (1971) protocol on a treadmill.

Stage	Duration (min)	Speed (kmh^{-1})	Gradient (%)
1	0-1	2.2	0
2	1-2	2.2	0
3	2-3	2.7	5
4	3-4	2.7	10
5	4-5	3.3	11
6	5-6	4	12
7	6-7	4.8	13
8	7-8	5.5	14
9	8-9	6.2	15
10	9-10	6.8	16
11	10-11	7.4	17
12	11-12	8	18
13	12-13	8.4	19
14	13-14	8.8	20
15	14-15	9.2	21
16	15-16	9.6	22

d. acts as the benchmark to re-attain maximal exercise (100 $\%\dot{V}O_{2\,max}$) values when determining CPO_{max}. The $\dot{V}O_{2\,max}$ test was initiated 24 hours prior to CPO_{max} test as previous unpublished data (Chandler et al., 2004; PhD thesis) found a rest period of 24 hours was required to gain reliable $\dot{V}O_{2\,max}$ values.

Previous work from our laboratory has shown coefficient of variances of 5.9 % (Sharp, 2006; PhD thesis) and 4 % (Clements, 2006; PhD thesis) in $\dot{V}O_{2\,max}$.

2.7 Breath-by-breath gas sampling and analysis (CPX/D system).

Breath-by-breath gas sampling and analysis was measured using a CPX/D system (Medgraphics Corporation, St. Paul, Minnesota, USA). This system incorporated a flow module, a gas analyser, 12-lead ECG and a computer host. The 12-lead ECG, recorded by Cardio-Perfect software (Cardio-Perfect, Atlanta) and the respiratory data were recorded and analysed by Breeze Suite (version 6).

Respiratory gases were collected via a line which was attached to the pneumotachograph and then passed through a drying cartridge, via a vacuum system and into the gas analyser. Accuracy of the measurements of these gases is increased by the drying process. Respiratory gas flow was measured through a pneumotach with the flow analyser attached via an umbilical tube.

A zirconia fuel cell measures oxygen and carbon dioxide concentrations. The inner chamber contains a sample gas and the outer chamber contains a reference gas. The movement of oxygen molecules between the chambers causes voltage differences which is subsequently measured and quantified by the cell. Carbon dioxide concentrations are measured via an infrared analyser, with carbon dioxide absorbing more infrared light than any other gas at a wavelength of 4.3 μm. Therefore the amount of light absorbed in the chamber is compared to the reference cell and CO_2 can be quantified.

Before each test, the gas analyser was calibrated using both reference (21 % O_2 and balanced N_2) and calibration (12 % O_2, 5 % CO_2 and balanced N_2) gases (Medgraphics Corporation, St. Paul, Minnesota, USA). The pneumotach was also calibrated prior to each test using a 3 litre syringe, with a baseline established without any flow. This was followed by 5 withdrawals and injections from the syringe at different speeds to represent fluctuations in respiratory rates.

Gas samples, both inspired and expired, were analysed breath-by-breath for VO_2, VCO_2, RER, $P_{ET}CO_2$, VE, Vt and RR.

2.8 Cardiac Power Output.

Cardiac Power Output (CPO) is a measure of overall cardiac function, incorporating the heart's flow (cardiac output; $\dot{Q}$) and pressure (mean arterial pressure; MAP) generating capacities. Cardiac power output was determined both at rest and maximal exercise and involved 3 stages:

Stage 1: Measurement of $\dot{V}O_{2\,max}$, maximal heart rate and carbon dioxide production using an incremental exercise test to exhaustion (as in section 2.6).
Stage 2: Measurement of CPO at rest (CPO_{rest})
Stage 3: Measurement of maximal CPO (CPO_{max}) at the same maximal exercise level as in stage 1 (i.e. 100 % $\dot{V}O_{2\,max}$).

2.8.1 Cardiac Power Output during rest.

The Collier (1956) method is used to determine VO_2, and subsequently $\dot{Q}$. It is a more accurate measurement of $\dot{Q}$ at rest than the method of Defares (1958). Measurements of CPO_{rest} were initiated with the participant in a seated, rested position and lasted approximately 20 minutes. A 5-minute stabilisation period was given to each participant prior to the first measurement of blood pressure. Resting blood pressure measurements were taken using a mercury

sphygmomamater and stethoscope, with the left arm at heart level (the participant used a rail to rest their arm), conforming to the recommendations of the American Heart Association (Frohlich *et al.*, 1988). The participant's upper left arm was fitted with an appropriate sized cuff (width: 40-50 %, length: 80 % upper arm circumference). The stethoscope drum was placed over the brachial artery and the cuff was inflated to 40 mmHg above the disappearance of the Korotkoff sound (point of artery occlusion). The cuff was then gradually released around 2-3 mmHg per second. Systolic blood pressure was determined by the first Korotkoff sound and diastolic blood pressure was determined by the 5^{th} Korotkoff sound.

A CO_2 re-breathing manoeuvre was conducted through a disposable mouthpiece and a 5-litre bag connected to a 3-way valve (2870 series valve, Cranlea and Q, UK). Participants breathed through the mouthpiece, taking in room air for 5 minutes prior to the test (Figure 2.3). A pre-vent pneumotach was attached to the outflow valve so as to measure the flow of breathing. A sample line was placed in the pneumotach to obtain gas concentrations, and was connected to a gas analyser. Once the respiratory variables (VO_2, VCO_2 and RER) had stabilised, the investigator closed the 3-way valve at the end of a normal expiratory tidal breath, so that the participant was taking the gas mixture from the bag. At rest, the Collier (1956) method utilises a medical grade gas mixture of 10 % CO_2, 35 % O_2 and balanced N_2 inside the black bag. The concentration of gases were used to provide a partial pressure of CO_2 to be greater than the participant's mixed venous CO_2 ($PVCO_2$). The 5-litre bag, via closed circuit spirometry, contained a volume of 1.5-2.0 times the participant's resting tidal volume. The participant then took a deep inspiratory breath, to inhale all the gas mixture from the bag. After about 8-10 seconds of breathing, an equilibrium was reached between the gases in the bag, mixing with alveolar air and plasma, causing a fall in pCO_2 levels. Figure 2.4 shows the graph and the plateau maintained for at least 2 breaths.

The capnograph plot is correct if:

a. CO_2 equilibrium reached after 2-3 breaths
b. CO_2 equilibrium maintained for at least 2 breaths

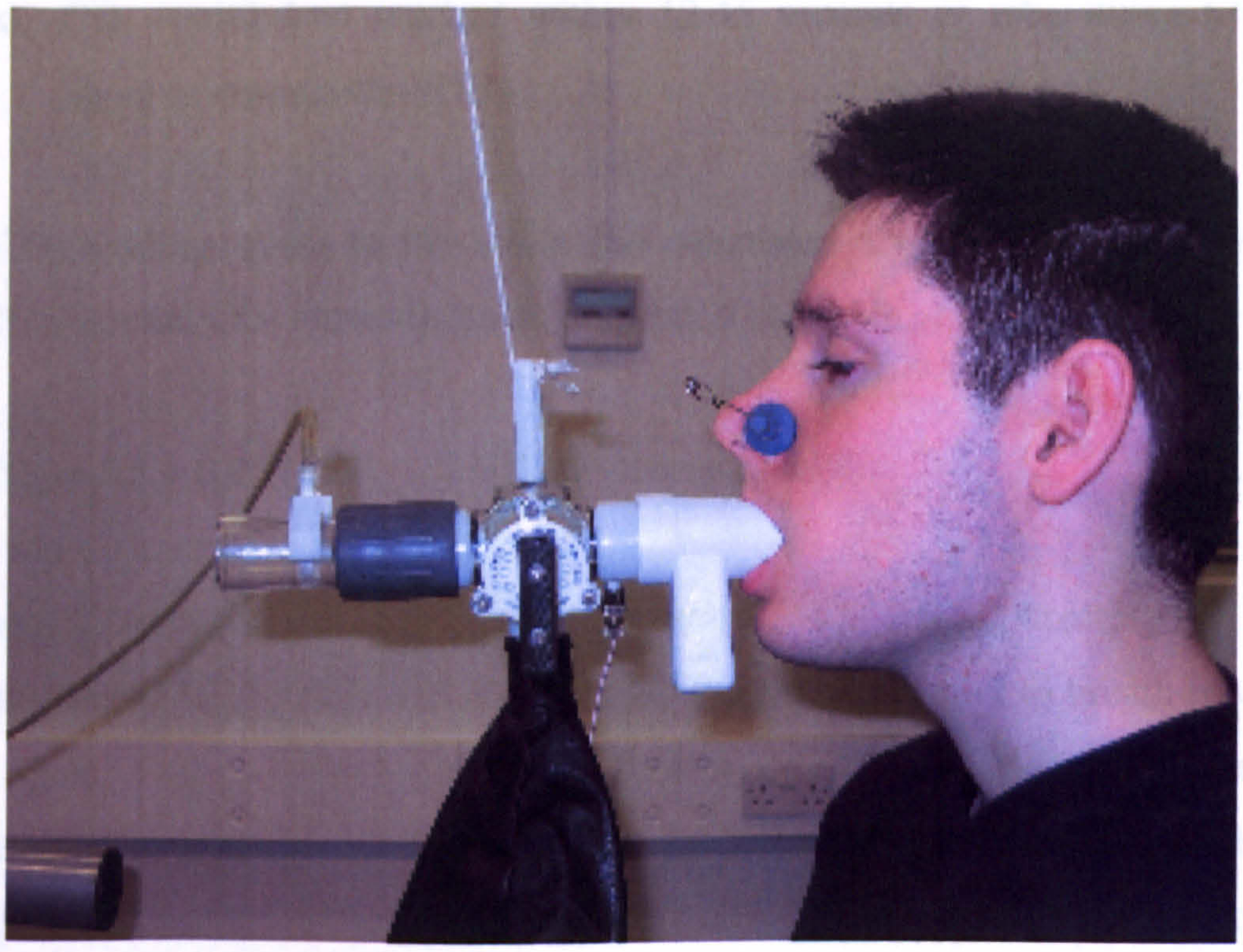


Figure 2.3: 3-way valve mouthpiece used during resting CPO.

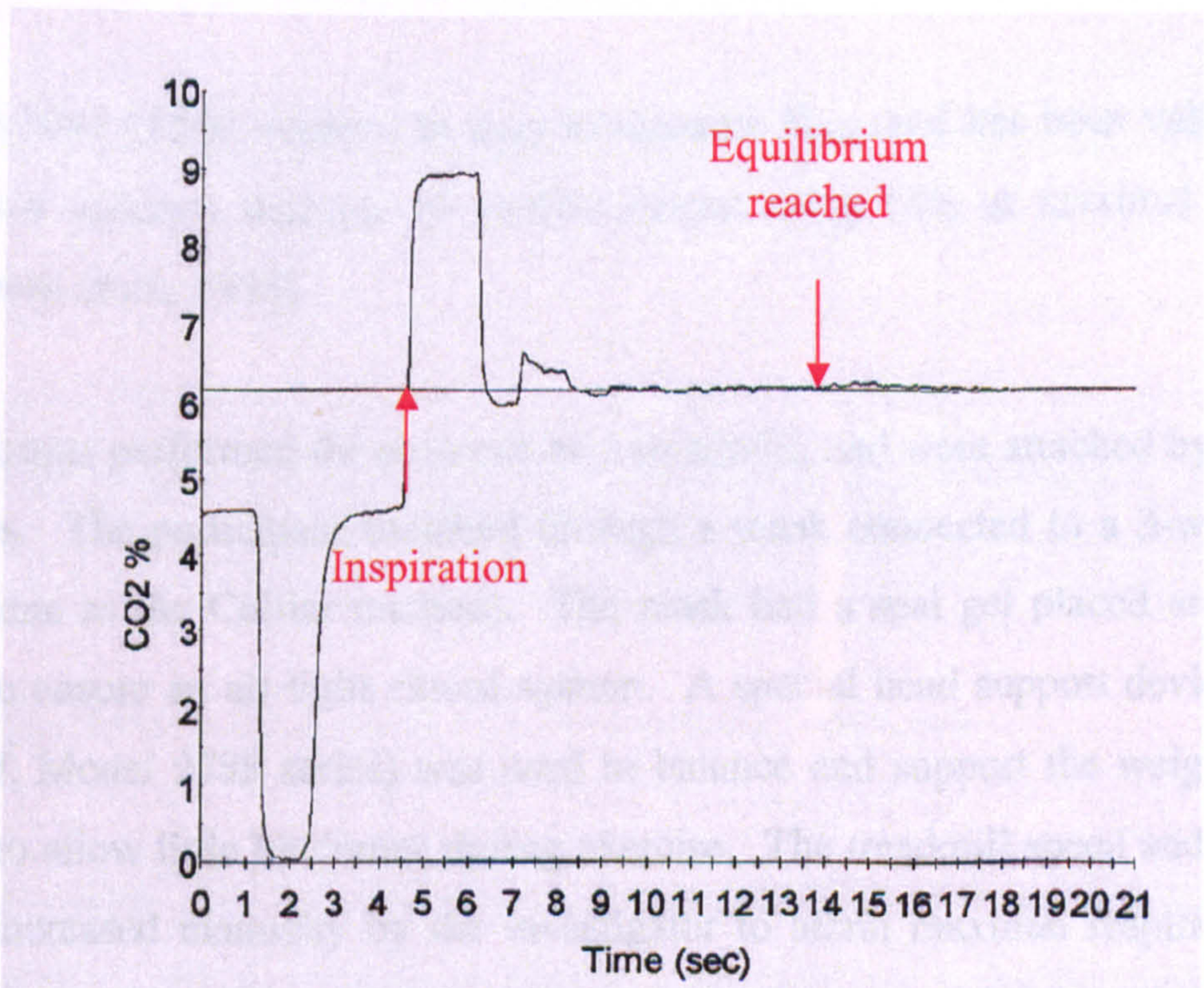


Figure 2.4: Collier (1956) Equilibrium Capnograph.

c. CO_2 equilibrium reached within 12-15 seconds of rebreathing CO_2 to avoid re-circulation of CO_2.

$P_{ET}CO_2$ readings prior to the re-breathe calculated alveolar pCO_2 from which $PaCO_2$ (arterial CO_2 tension) can be calculated using Vt.

$PaCO_2 = 5.5 + 0.9\ P_{ET}CO_2 - 0.0021$ Vt (Jones 1988)

After a CO_2 equilibrium had been maintained for at least 2 breaths, the valve connecting the black bag was closed, allowing the participant to breath room air one again. Cardiac output was measured using the Collier (1956) method and the indirect Fick equation (Fick 1870). At least 3 resting values were collected, separated by at least 4 minutes of quiet breathing, to allow adequate washout of CO_2 from the circulating blood.

2.8.2 Maximum Cardiac Power Output.

The Defares (1958) method is used to measure Q_{max} and has been validated as the most accurate measure of cardiac output using CO_2 at maximal exercise (Ferguson *et al.*, 1968).

Participants performed the protocol on a treadmill, and were attached by a safety harness. The participant breathed through a mask connected to a 3-way valve (the same as the Collier method). The mask had a seal gel placed around the edge to ensure an air tight closed system. A special head support device (Hans Rudolf, Model 2785 series) was used to balance and support the weight of the valve to allow little hindrance during exercise. The treadmill speed and gradient were increased manually by the investigator to attain maximal respiratory and haemodynamic values. This usually occurred between 6-9 minutes, as that is when peak heart function occurs (McCole *et al.*, 2001). Once $\dot{V}O_{2\,max}$ was obtained, blood pressure was taken and $\dot{Q}$ was measured using the Defares (1958) method. Blood pressure was taken with the arm at heart level. Each participant rested his or her arm on the shoulder of the investigator who was on a

stepladder next to the treadmill (Figure 2.5). As the treadmill increased in incline, the investigator moved higher up the stepladder to allow the height of the arm to be the same throughout the test. Once $\dot{V}O_{2\,max}$ was reached, the investigator manually closed the valve so the participant breathed in a gas mixture (4 % CO_2, 35 % O_2, and balanced N_2) from the 5-litre bag. This was done for 8-10 seconds. The valve was then opened to allow the participant to breathe room air. Figure 2.6 shows an exponential CO_2 curve using the Defares (1958) method. The first breath is excluded due to an incomplete mixing of gases in the lungs. Only $P_{ET}CO_2$ values up to 8 seconds were analysed to ensure no potential problems with re-circulation of CO_2.

The whole procedure was untaken again, after a 2 to 3 minute recovery at a low exercise intensity. The protocol was originally designed for assessing cardiac function in heart failure patients, in which it was necessary to complete all the tests within one visit, with only a 40 minute rest between the $\dot{V}O_{2\,max}$ and maximal CPO protocols. Previous unpublished data from our institute (Chandler *et al.*, 2004) found that a 24 hour rest period was required between the $\dot{V}O_{2\,max}$ and maximal CPO protocols to ensure maximal stroke volume, heart rate, systolic blood pressure and diastolic blood pressure values had returned to baseline. With regards to the two maximal CPO tests, we always allowed for all the excess CO_2 to be washed-out before performing the second test and ensured RER was < 0.90 as a set procedure with each test. Maximal cardiac output and MAP values were averaged and CPO_{max} was calculated accordingly.

2.9 Measurement of $\dot{Q}$ using CO_2 re-breathing technique.

Cardiac output ($\dot{Q}$) was determined through the CO_2 re-breathing methods of either Collier (1956) or Defares (1958), and calculated using the indirect Fick equation

$$\dot{Q} = VO_2 / (C_{vCO2} - C_{aCO2}).$$

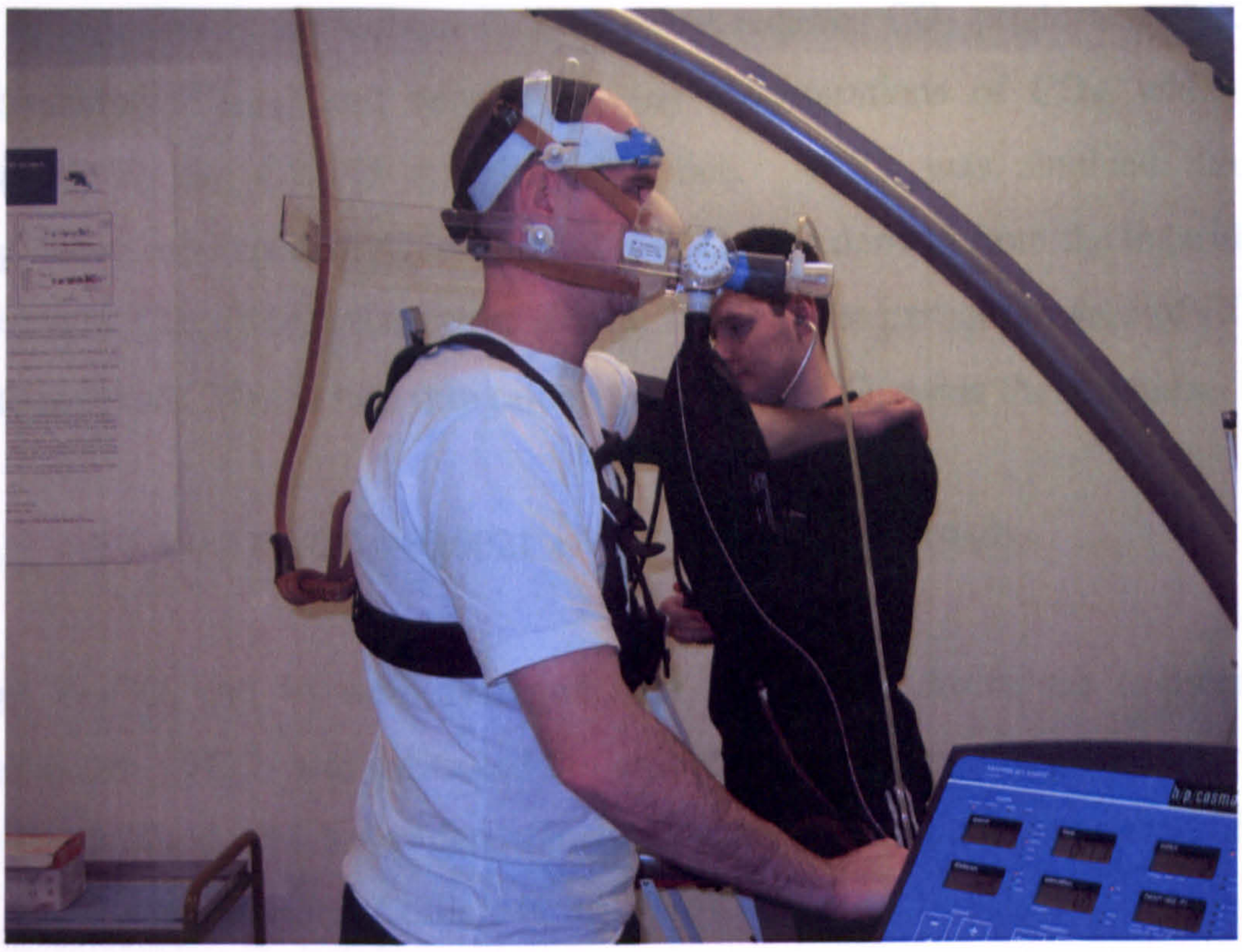


Figure 2.5: CPO during Exercise and blood pressure measurement to enable CPO_{max} to be measured.

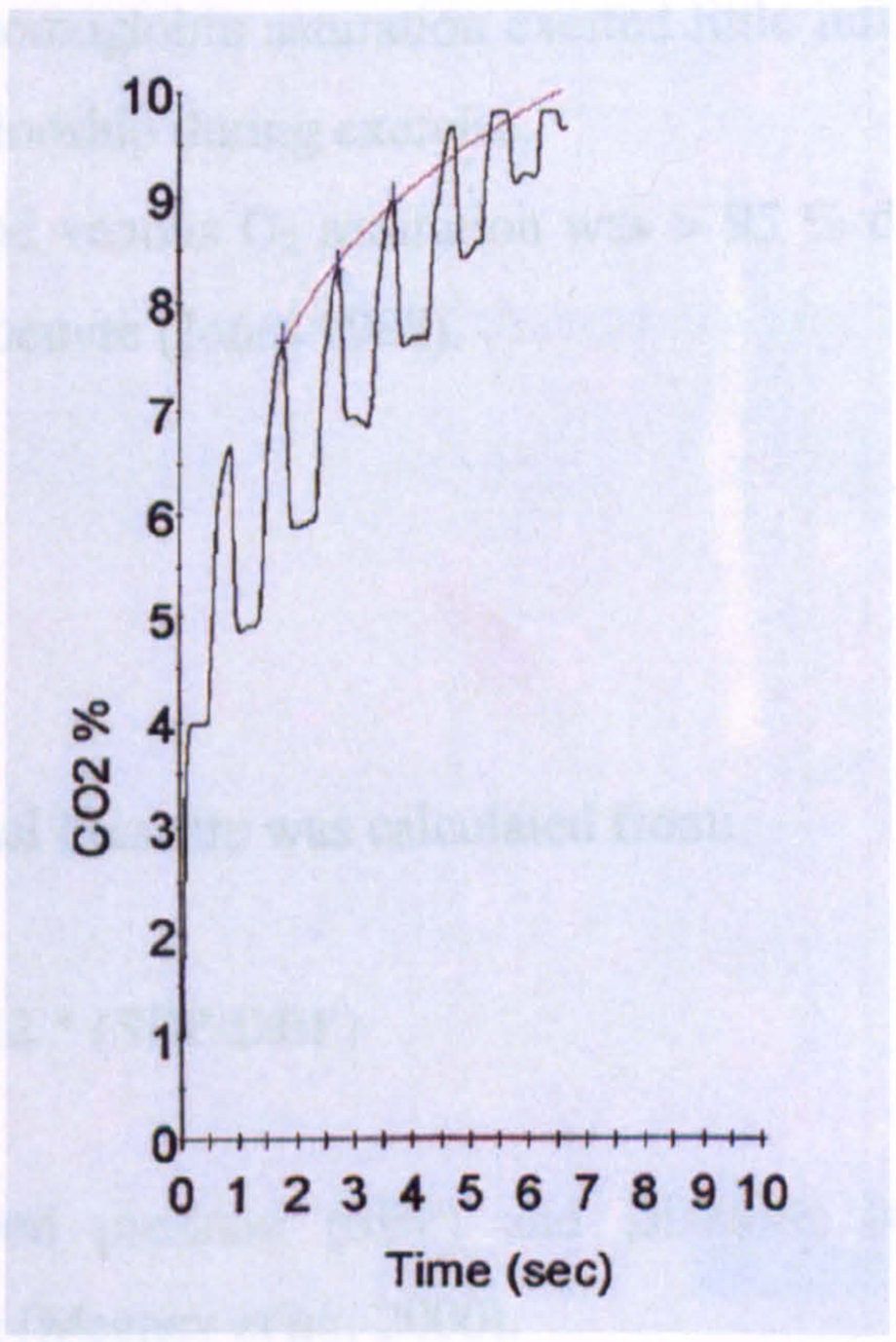


Figure 2.6: Defares Exponential Graph showing an exponential rise in CO_2.

To be calculated, the indirect Fick equation requires CO_2 production (VCO_2), and arterial (Ca_{CO2}) and venous (C_{VCO2}) concentrations of CO_2, which are attained via the CPX/D machine pink line. VCO_2 was obtained through inspiratory and expiratory gas volumes. $CaCO_2$ was derived from the logarithmic version of CO_2 dissociation curve (Jones, 1988) of the partial pressure of CO_2 in arterial blood. This in turn was calculated from $P_{ET}CO_2$ using the equation,

$Pa_{CO2} = 5.5 + 0.9\ P_{ET}CO_2 - 0.0021$ x Vt (BTPS). (Jones, 1988).

Both $P_{ET}CO_2$ and Vt were averaged over 30 seconds during the re-breathing manoeuvre. VCO_2 was measured through obtaining the partial pressure of CO_2 in venous blood, which was determined via either the re-breathing method of,

a. The equilibrium (Collier, 1956)
b. The exponential (Defares, 1958)

Two assumptions were made regarding the formulation of partial pressure:

1. Haemoglobin concentration was at 15 g/ 100ml. Sun *et al.* (2000) stated that changes in haemoglobin levels and blood oxyhemoglobin saturation exerted little influence on PCO_2-CCO_2 relationship during exercise.
2. Mixed venous O_2 saturation was > 95 % during the re-breathing manoeuvre (Jones 1988).

2.10 Calculations.

1) Mean Arterial Pressure was calculated from:

$MAP = DBP + 0.412 * (SBP-DBP)$

With Systolic blood pressure (SBP) and Diastolic blood pressure (DBP) measured in mmHg (Meaney *et al.*, 2000).

2) Cardiac Power Output (Watts) was calculated from:

$$CPO = (\dot{Q} \times MAP) \times K$$

i.e. Cardiac output ($\dot{Q}$; l/min) and K the conversion factor ($2.22*10^{-3}$; Cooke *et al.*,1998) into Watts

3) Cardiac Reserve (Watts) calculated as:

$$CR = CPO_{max} - CPO_{rest}$$

4) Systemic Vascular Resistance (dynes.s.cm^{-5}) was calculated by:

$$SVR = (MAP/\dot{Q}) * 80$$

5) Differences in arterial-venous oxygen (ml O_2/100dl blood) were calculated by:

$$a\text{-}vO_2 \text{ difference} = (VO_2/\dot{Q}) / 10$$

with cardiac output ($\dot{Q}$; l/min) and oxygen consumption (VO_2; ml/min).

Chapter 3

Study 1 – The Methodological considerations for measuring Cardiac Power Output.

This study consists of two parts. Part A will consider the inter-rator and intra-rator reliability of measuring cardiac power output (CPO), as this will be important in establishing reproducibility and in indicating test/re-test reliability. Secondly, part B will examine the potential biological effects of caffeine ingestion on CPO; something that has not been examined previously. Overall this study will assess both the day to day variability of the equipment and investigator, and provide guidance on how to reduce biological variance with regards to measurements of CPO.

3.1 Part A: Reliability.

3.2 Background.

Reliability can be defined as the quality of a reproducible measure (Batterham & George, 2003). Reproducibility is determined by a test or measure, which can repeatedly produce the same scores (Nelson, 1997). Reliability is seen as an important prerequisite for test validity, as a repeatability study should establish reproducibility and indicate test/re-test reliability. There are two main types of reliability – intra-rater and inter-rater reliability. Intra-rater reliability refers to the reliability of one investigator or measurement tool. Inter-rator reliability refers to the reliability between two or more investigators. Cardiac Power Output procedures require both inter-rater and intra-rater reliability. Reliability studies can never be perfect as there is always a percentage of error involved. Random error is regarded as the precision limitations of the measurement device. Systematic error is regarded as reproducible inaccuracies that are consistently in the same direction (Batterham & George, 2003). Random error can be reduced through a large n number, however systematic errors can be hard to recognise.

The aim of this study was therefore to ascertain the intra-rator and inter-rator reliability of measuring cardiac output ($\dot{Q}$), mean arterial pressure (MAP) and cardiac power output (CPO) both at rest and maximal exercise.

3.3 Methods.

Six male participants (20 years ± 1 year with a mean body weight of 72.0 ± 8.6 kg, a mean height of 180 ± 7 cm, a mean body fat of 20 ± 3 % and a mean $\dot{V}O_{2\,max}$ of 53.9 ± 5.8 ml.kg.min^{-1}) were tested for resting and maximal CPO using the techniques described in Chapter 2. Participants were measured on three separate occasions, with a week in between tests. Written consent was gained from each participant prior to testing and all procedures were approved by the University's ethics committee. Results were analysed with a one-way Anova to show 95 % confidence interval of difference, f values and significance levels of the correlation. R values were determined through regression analysis. All of the analyses were analysed using SPSS vs. 14 (Chicago, IL).

3.4 Results.

Maximal values of cardiac output ($\dot{Q}$), mean arterial pressure (MAP) and CPO produced a lower coefficient of variation (CV) than resting values (Table 3.1). However, all resting measurements had high significant Pearson's R correlations, which showed good agreement between tests. Therefore, resting measurements are more prone to error than maximal measurements, but are still reliable.

3.5 Discussion.

Nugent *et al.* (1994) found the CV for cardiac output was around 10 % at rest, but improved with exercise. The present CV for resting cardiac output was 7.8 % and also showed an improvement with exercise (3.6 %; Figures 3.1A and 3.2A). Resting $\dot{Q}$ has a high significant Pearsons R value (r = 0.97), which shows good agreement between tests. These results suggest that resting $\dot{Q}$ measures are more prone to error than maximal measures of $\dot{Q}$, but are still reliable. Resting measured of $\dot{Q}$ have the tendency to be more sensitive to other

Table 3.1: Average values, mean measurement differences, 95 % limits of agreement, CV %, and the correlation between absolute differences for the 3 tests.

n=6	Average mean ± SE	Mean difference between tests	95 % confidence interval (average measures)	CV %	Correlation (R value)
Rest					
$\dot{Q}$	5.1 ± 0.3	0.21	0.831 – 0.993	7.8 %	0.97
CPO	1.05 ± 0.10	0.04	0.956 – 0.998	7.2 %	0.99
MAP	92 ± 5	0.94	0.851 – 0.994	6.5 %	0.95
Max					
$\dot{Q}$	25.18 ± 0.90	0.78	0.404 – 0.977	3.6 %	0.67
CPO	6.3 ± 0.4	0.17	0.896 – 0.996	5.7 %	0.99
MAP	112 ± 3	1.19	0.793 – 0.992	4.4 %	0.89

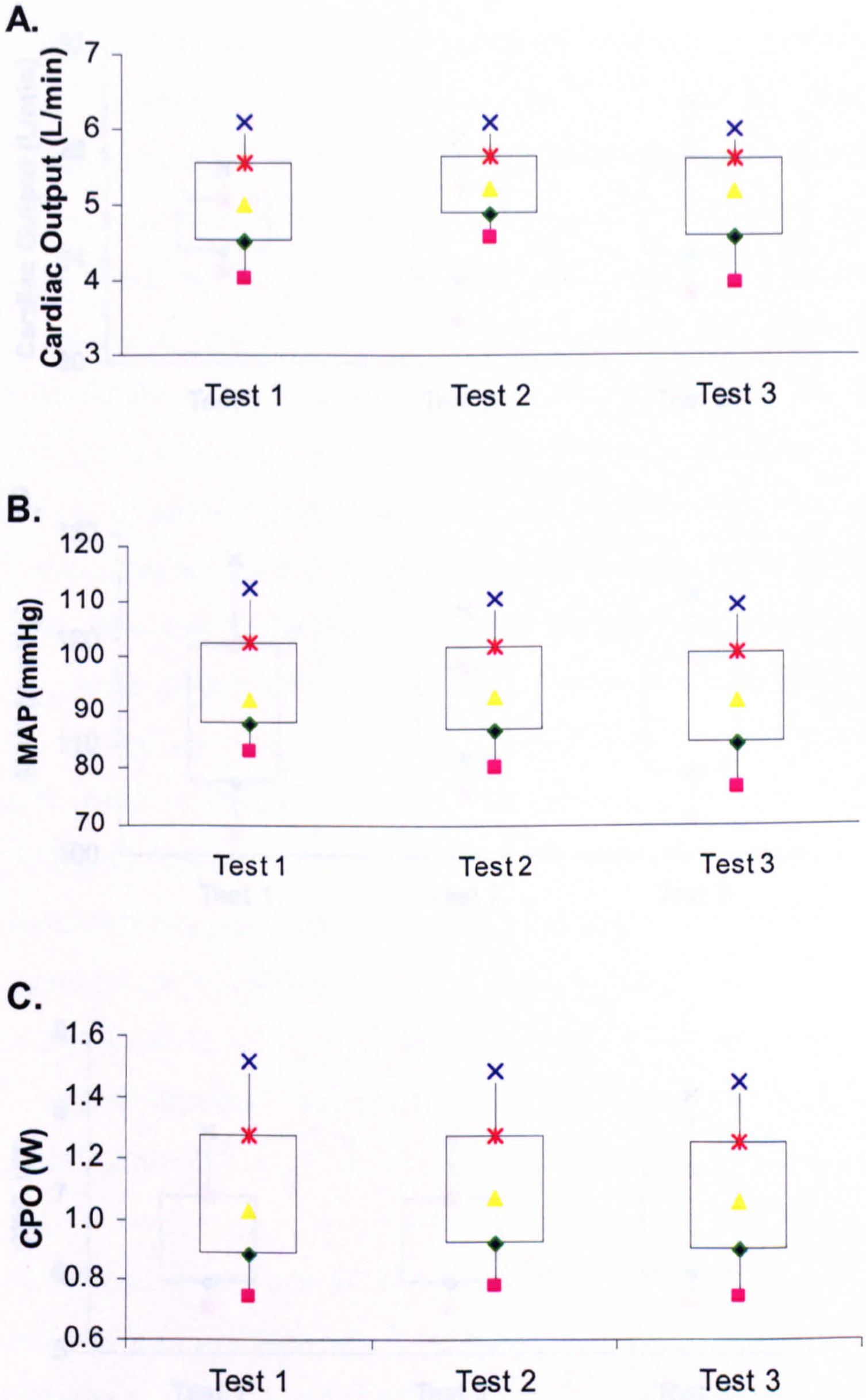


Figure 3.1: Box plots for resting cardiac output (A), maximal mean arterial pressure (B) and maximal cardiac power output (C).

Data are shown as mean (▲), minimum (■) and maximum (x) values, and the inter-quartile ranges (♦ and ∗; n=6).

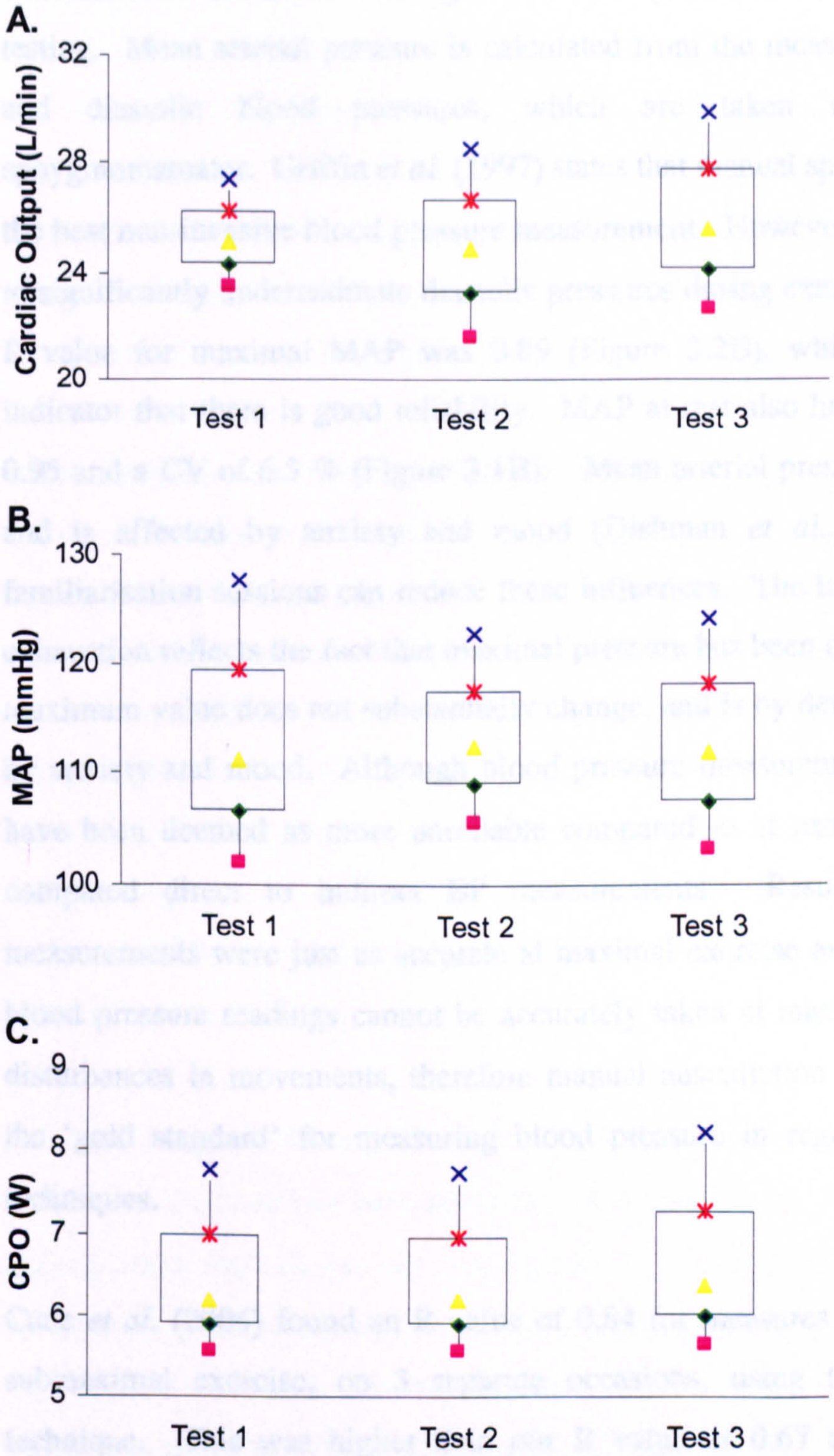


Figure 3.2: Box plots for maximal cardiac output (A), maximal mean arterial pressure (B) and maximal cardiac power output (C).

Data are shown as mean (▲), minimum (■) and maximum (x) values, and the inter-quartile ranges (♦ and ∗; n=6).

physiological or biochemical reactions, e.g. food, caffeine, anxiety. Subjects were asked to refrain from eating food 3 hours prior and caffeine 6 hours prior to testing. Mean arterial pressure is calculated from the measurements of systolic and diastolic blood pressures, which are taken manually using a sphygmomamater. Griffin *et al.* (1997) states that manual sphygmomanometry is the best non-invasive blood pressure measurement. However, it has been known to significantly underestimate diastolic pressures during exercise. The Pearson's R value for maximal MAP was 0.89 (Figure 3.2B), which is also a strong indicator that there is good reliability. MAP at rest also had a high R value of 0.95 and a CV of 6.5 % (Figure 3.1B). Mean arterial pressure can vary at rest and is affected by anxiety and mood (Dishman *et al.*, 2000). However, familiarisation sessions can reduce these influences. The lower CV of 4.4 % at exhaustion reflects the fact that maximal pressure has been obtained and that this maximum value does not substantially change, and is by definition, less affected by anxiety and mood. Although blood pressure measurements during exercise have been deemed as more unreliable compared to at rest, Rasmussen (1985) compared direct to indirect BP measurements. Results showed indirect measurements were just as accurate at maximal exercise as at rest. Automated blood pressure readings cannot be accurately taken at maximal exercise due to disturbances in movements, therefore manual auscultation has been deemed as the 'gold standard' for measuring blood pressure in regards to non-invasive techniques.

Cade *et al.* (2004) found an R value of 0.84 for measures of cardiac output, at submaximal exercise, on 3 separate occasions, using the CO_2 rebreathing technique. This was higher than our R value of 0.67 at maximal exercise. Coefficients of variation of 3 % for resting $\dot{Q}$ and 5 % for maximal $\dot{Q}$ have been reported (Auchincloss *et al.*, 1980) which are the opposite to our results of 3.6 % at maximal exercise and 7.8 % at rest, with these showing an increased reliability with exercise intensity. However, our results do agree with other studies (Claussen *et al.*, 1970; Espersen *et al.*, 1995; Ferguson *et al.*, 1968; Wigle *et al.*, 1979). Beekman *et al.* (1984) found correlations of r = 0.65 for cardiac output at rest and r = 0.81 during exercise, suggesting that the CO_2 rebreathing technique provides reasonably accurate values of cardiac output at maximal

exercise. The assumption made for the greater reliability with exercise is postulated to be the result of a larger a-vO_2 difference in CO_2 during exercise, which minimizes the random errors associated with the estimation of arterial and mixed venous CO_2 (Espersen *et al.*, 1995). With low CV for both maximal cardiac output and maximal MAP, the CV for CPO_{max} was 5.7 %, with an R value of 0.99, demonstrating high reliability (Figure 3.2C). The CV for resting CPO was higher at 7.8 %, but had a high R value of 0.99 showing good reliability within subjects (Figure 3.1C).

When the CO_2 rebreathing technique is compared to other measures of cardiac output, an R value of 0.94 was derived, e.g. when comparing CO_2 rebreathing to the technique of thermodilution (Hargreaves & Jennings, 1983) and an R of 0.94 when compared to impedance cardiography (Edmunds *et al.*, 1982). Also Espersen *et al.* (1995) found the limits of agreement for CO_2 rebreathing were favorable to the direct Fick method, with Reybrouck *et al.* (1978) reporting an R value of 0.96 using the Defares CO_2 rebreathing and direct Fick methods. The alternative gases used in re-breathing techniques are acetylene and nitrous oxide. Farhi *et al.* (1976) found only a 2 % difference in cardiac output measured at rest and maximal exercise with acetylene and the CO_2 rebreathing technique.

Maximal oxygen consumption was not measured in this reliability study, but previous work from our laboratory has shown coefficient of variances of 5.9 % (Sharp, 2006; PhD thesis) and 4 % (Clements, 2006; PhD thesis). With $\dot{V}O_{2\,max}$, the biological (intra-individual) variance consists of 90 % of the total variance, with technical variance contributing only 10 % variance (Armstrong & Costill, 1985; Howley *et al.*, 1995; Katch *et al.*, 1982; Mitchell *et al.*, 1958). A CV of between 2-6 % has been reported in the literature for $\dot{V}O_{2\,max}$ (Froelicher *et al.*, 1974; Katch *et al.*, 1982; McArdle *et al.*, 1973). Conversely, the CPX-D machine does not compare well (95.6 - 106.6 %) when compared to Douglas bags (Porszasz *et al.*, 1994).

In conclusion, cardiac output, can be readily measured using CO_2 rebreathing, especially during exercise, and has been well validated against other invasive and non-invasive techniques. Manual auscultation at rest and during exercise can

also be reliably used to measure blood pressure, and has been deemed as the best non-invasive technique to use. Overall, these results show that the chosen techniques were highly reproducible at both at rest and during maximal exercise.

3.6 Part B: Caffeine.

3.7 Background.

3.7.1 The Pharmokinetics of Caffeine.

Caffeine (1, 3, 7-trimethylxantine) is a naturally occurring methylxanthine (Vanakowski *et al.*, 1998; Figure 3.3a) and is extensively metabolised throughout the body. Caffeine is an adenosine receptor antagonist, with adenosine receptors found throughout the body, including the cardiovascular and respiratory systems. Caffeine blocks both adenosine receptors A_1 and A_{2a}, thereby inhibiting the actions of adenosine (Figure 3.3b). This antagonistic effect attenuates increases in coronary blood flow created by a decrease in oxygen supply/demand ratio (Hori & Kitakaze, 1991). Caffeine also stimulates the release of dopamine and serotonin, which increase vasoconstriction in the brain (Benowitz, 1990).

Caffeine can affect the central nervous system, relax vascular smooth muscle, decrease peripheral vascular resistance, and increase urine production. These physiological effects depend on the circulating dosage of caffeine for while its half-life is 4 to 6 hours, its clearance is non-linear, and significantly slower after higher doses (Paluska, 2003). The maximal effects of caffeine are usually presented around 1 hour post ingestion (Bell & McLellan, 2002). Extremely high doses of caffeine can cause hypotension due to beta-adrenergic mediated vasodilation, and tachycardia due to high levels of catecholamine release (Benowitz, 1990). D'Urzo *et al.* (1990) found a 20 % increase in respiratory rate when ingesting 650 mg of caffeine. This suggests that caffeine also increases respiratory rate by sensitising the medullary centre to carbon dioxide. Caffeine

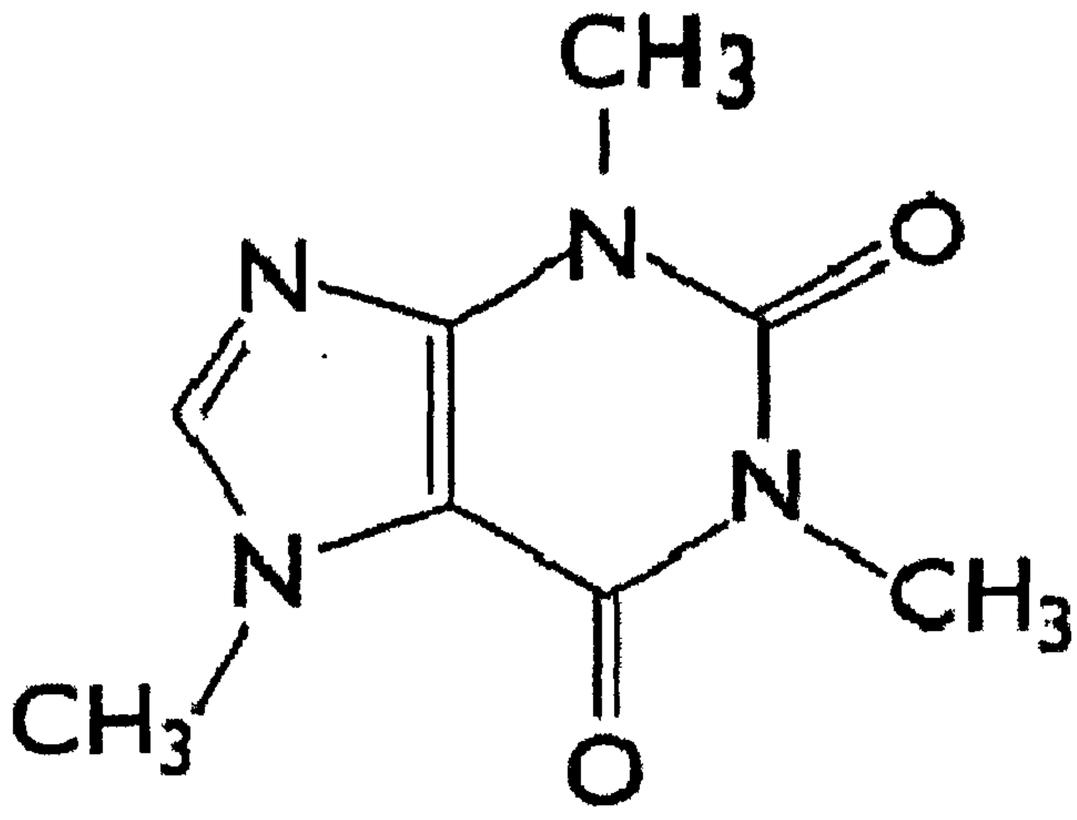


Figure 3.3a: Chemical structure of caffeine.

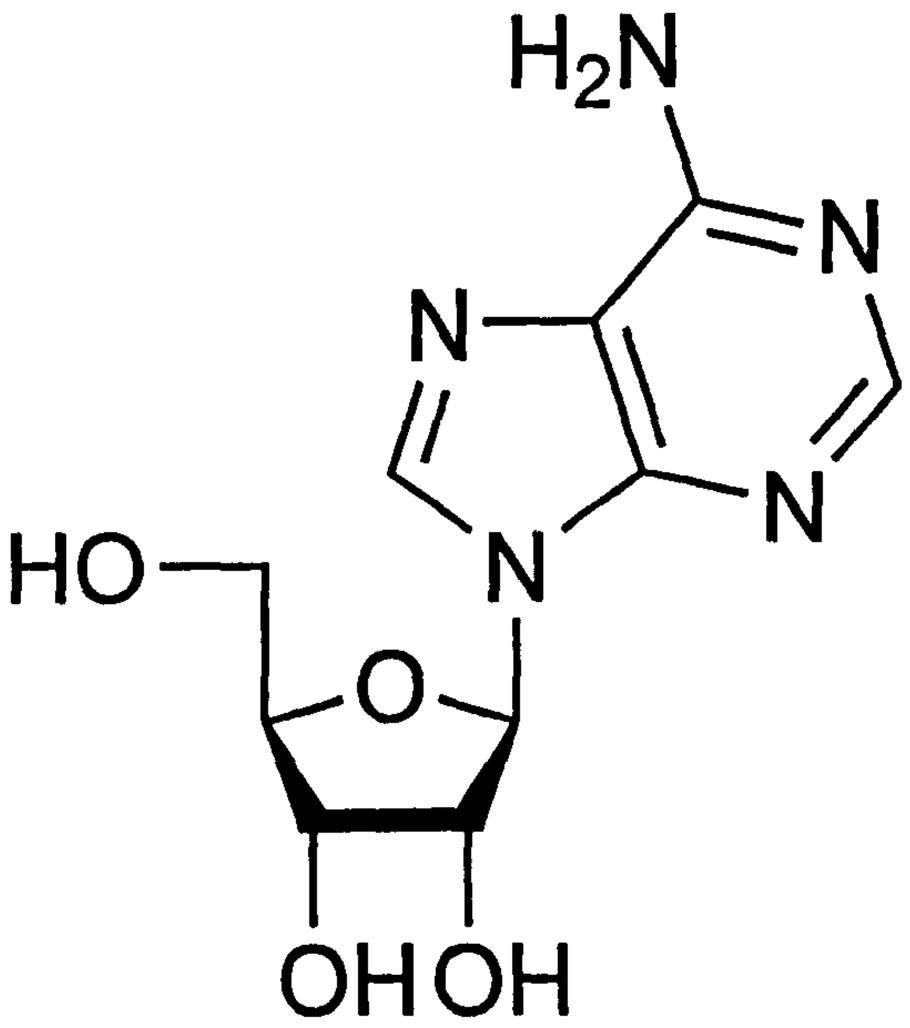


Figure 3.3b: Chemical structure of adenosine.

has also been shown to increase minute ventilation in patients with asthma and chronic obstructive lung disease (Gong *et al.,* 1986).

It has been documented that regular use of caffeine may diminish its own physiological effects by raising the tissues' threshold of responsiveness. Therefore, to gain the same desired effect, higher doses of caffeine may be required for individuals who are regular caffeine drinkers, compared to non-caffeine drinkers (Daniels *et al.,* 1998). However, acute ingestion of caffeine has been found to stimulate both habitual and non-habitual caffeine users (Graham, 2001).

3.7.2 Effects of caffeine on cardiovascular function .

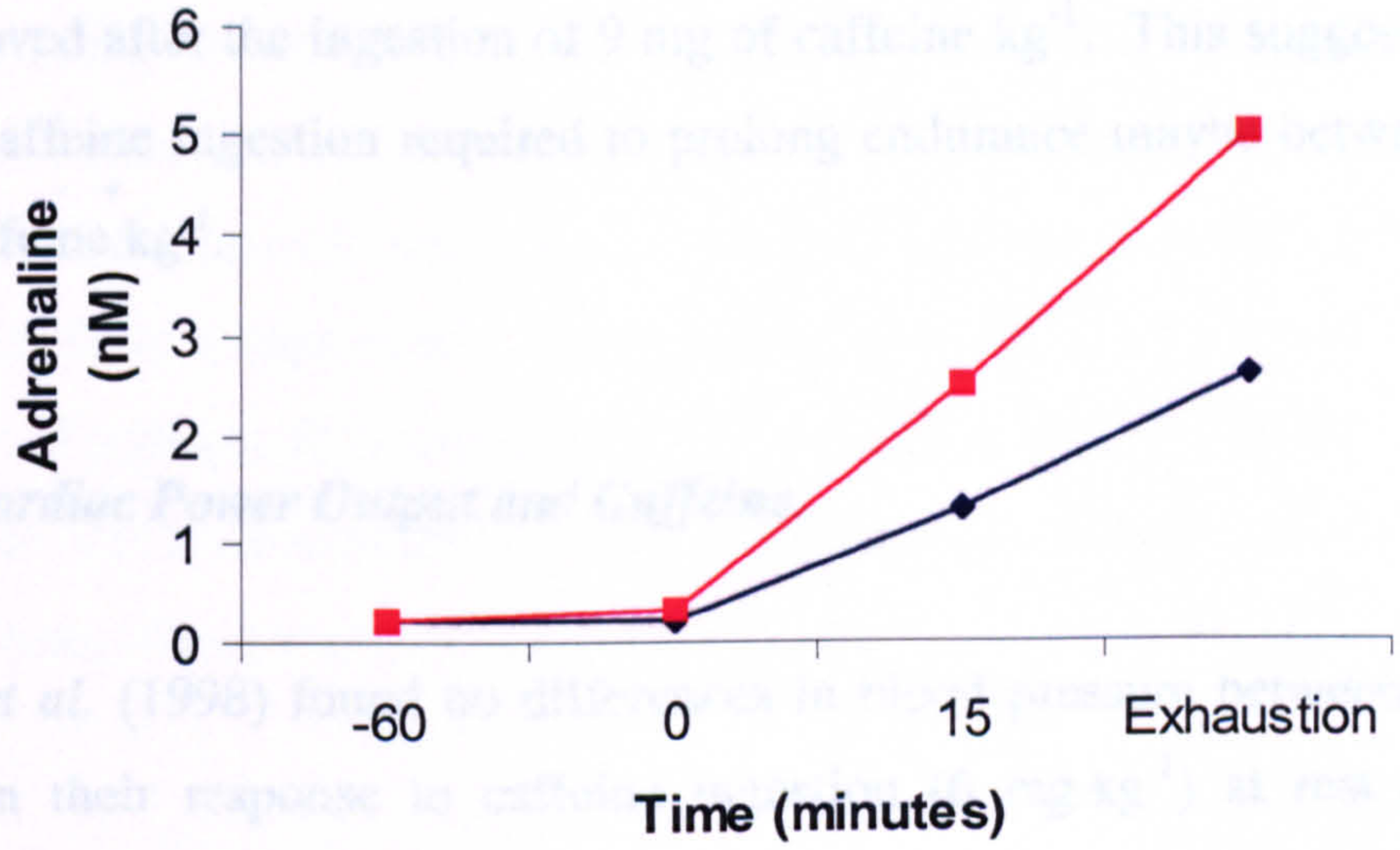
3.7.2.1 Ingestion of caffeine at rest.

Bender *et al.* (1997) found that the acute ingestion of caffeine caused an increase in systolic blood pressure due to an increase in systemic vascular resistance. This increase has been accounted for by an increase in catecholamine induced release of Angiotensin II. Renin is released from the kidneys, which interacts with angiotensinogen (from the liver) in the plasma to form Angiotensin I and then II. This causes an increase in blood pressure via the vasoconstriction of resistance vessels. Corti *et al.* (2002) took 15 healthy young men (6 habitual and 9 non-habitual coffee drinkers) and measured at rest the effect on the sympathetic nervous system of intravenous caffeine. The results showed that caffeine increased systolic and diastolic blood pressure, but heart rate remained the same in all subjects. This suggests that the increase in blood pressure was caused by a baroreceptor-mediated inhibition of cardiac sympathetic activity.

3.7.2.2 Ingestion of caffeine followed by exercise.

Waring *et al.* (2003) studied 20 healthy subjects (male and female, 18 - 25 years old and regular caffeine drinkers) who performed an isometric handgrip exercise for 5 minutes, 1 hour post ingestion of 300 mg of caffeine. Post exercise results showed that there was a significant decrease in heart rate, which was proposed to be related to an enhanced parasympathetic chronotropic action. Conversely, Sondermaijer *et al.* (2002) found acute caffeine ingestion caused a reduction in parasympathetic nervous activity, which was related to the shift in sympathovagal balance, contributing to an increase in blood pressure as heart rate was not altered by the caffeine supplement during exercise. Caffeine has also been shown to elicit no effect on heart rate in elite athletes who ingested 7 mg of caffeine kg^{-1} after exercising for 45 minutes on a cycle ergometer (Vanakowski *et al.*, 1998).

Costill *et al.* (1978) first reported increased performance amongst male cyclists when ingesting caffeine 1 hour prior to exhaustive exercise. Numerous papers have shown that caffeine supplementation can increase skeletal muscle contractility, work output, performance in endurance exercise and a decreased time to exhaustion (Applegate, 1999; Graham, 2001; Graham & Spriet, 1995; Tarnopolsky & Cupido, 2000). Only a small number of studies have shown no enhancement of performance with caffeine supplementation (Bell *et al.*, 2001; Ferrauti *et al.*, 1997). Numerous studies have also been undertaken with caffeine ingestion during high intensity exercise. Bell *et al.* (2001) studied untrained men who performed an oxygen deficit cycling test. Results showed they significantly improved their exercise times to exhaustion following a supplement of 5 mg of caffeine kg^{-1}. This was also found by Graham *et al.* (1998) who studied 9 healthy young fit adult men that ran at 85 % $\dot{V}O_{2\,max}$ until exhausted. The results showed that adrenaline levels were significantly higher with a caffeine supplement of 4.45 mg of caffeine kg^{-1} compared to a placebo supplement, and thus the increase in adrenaline levels were associated with a 31 % increase in exercise duration (Figure 3.4).



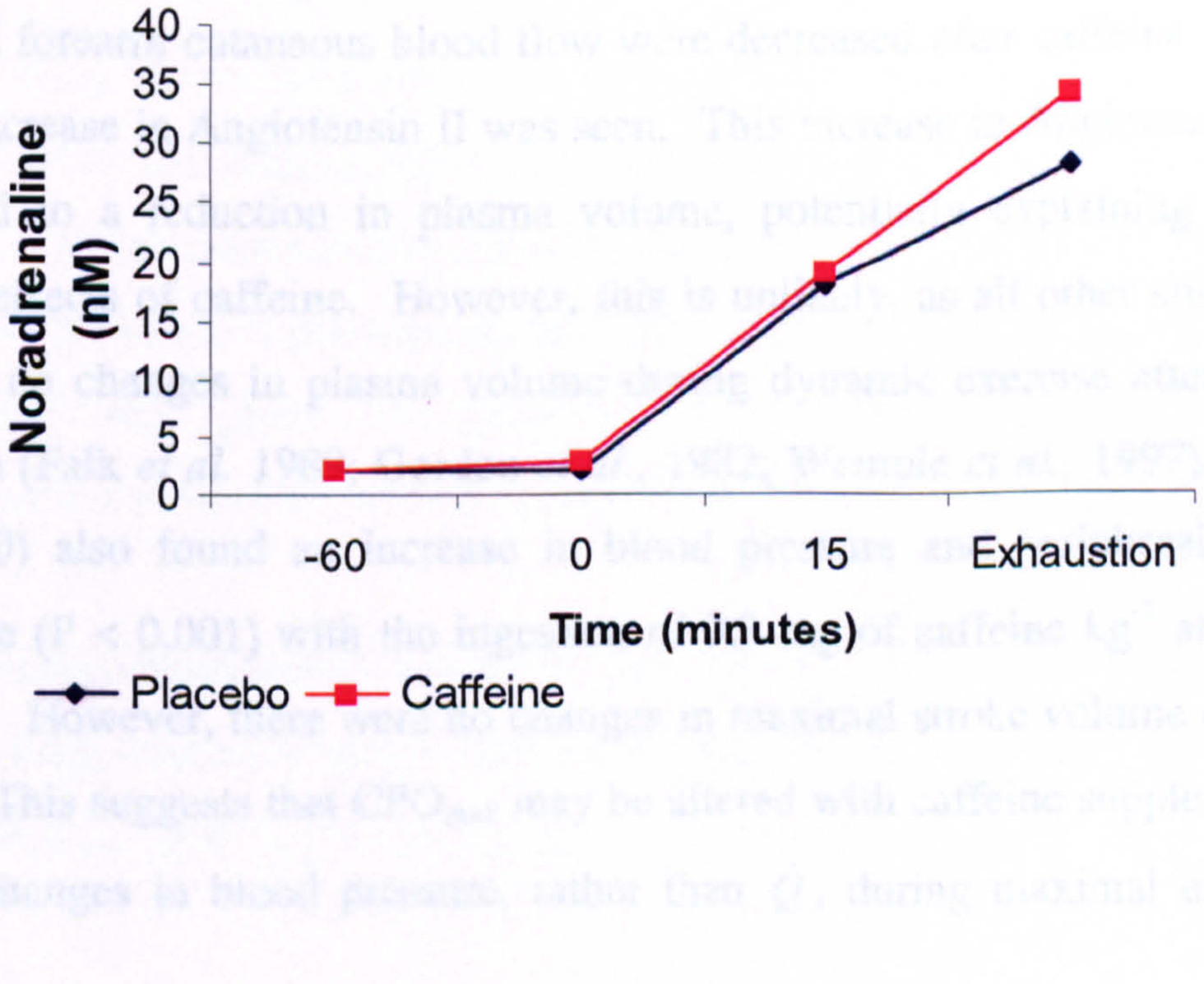


Figure 3.4: Catecholamine levels with caffeine supplementation before, at the start of exercise (0 minutes), and during exercise.
n=9; Taken from Graham *et al.,* 1998.

However, larger doses of caffeine in studies have not always enhanced performance further during endurance exercise. Graham & Spriet (1995) showed that the consumption of 3 or 6 mg of caffeine kg^{-1} enhanced the time to exhaustion of trained distance runners, but this enhanced time to exhaustion was not improved after the ingestion of 9 mg of caffeine kg^{-1}. This suggests that the optimal caffeine ingestion required to prolong endurance maybe between 3 to 6 mg of caffeine kg^{-1}.

3.7.2.3 Cardiac Power Output and Caffeine.

Daniels *et al.* (1998) found no differences in blood pressure between men and women in their response to caffeine ingestion (6 $mg.kg^{-1}$) at rest or during dynamic leg exercise. Prior to exercise, caffeine increased SBP and MAP (40 minutes post ingestion), but had no effect on DBP or heart rate. During exercise, the ingestion of caffeine also increased SBP and MAP compared to the placebo group. These increases in blood pressure at rest and during exercise could potentially cause significant increases in CPO. During exercise, forearm blood flow and forearm cutaneous blood flow were decreased after caffeine ingestion, but an increase in Angiotensin II was seen. This increase in Angiotensin II was attributed to a reduction in plasma volume, potentially explaining the mild diuretic effects of caffeine. However, this is unlikely, as all other studies have reported no changes in plasma volume during dynamic exercise after caffeine ingestion (Falk *et al.* 1989; Gordon *et al.*, 1982; Wemple *et al.*, 1997). Sung *et al.* (1990) also found an increase in blood pressure and peripheral vascular resistance ($P < 0.001$) with the ingestion of 3.3 mg of caffeine kg^{-1} at maximal exercise. However, there were no changes in maximal stroke volume or cardiac output. This suggests that CPO_{max} may be altered with caffeine supplementation due to changes in blood pressure, rather than $\dot{Q}$, during maximal exercise in men.

3.8 Rationale.

Caffeine is a regularly ingested drug within the population. Caffeine can increase blood pressure, and therefore caffeine could potentially have an effect on cardiac power output. To ensure reliability between all our measurements of CPO, our protocol needed to be carefully standardised with respect to caffeine ingestion to eliminate any effect it might have on measuring CPO. Hence, it is important to ascertain if the dose of caffeine, and the duration of any effect, could potentially compromise our measurements of CPO, both at rest and during exercise. Therefore the aim of this study was to assess the impact of caffeine dosage on CPO measurements and determine the caffeine abstinence time required before these measurements were made.

3.9 Protocol.

3.9.1 Recruitment.

Recreationally active men (n = 11), between the ages of 18-23 years, were recruited from the student population. This recruitment was initiated through academic lecture presentations and individual contact. A meeting was held to familiarise prospective participants with the equipment and laboratories. Ethical compliance was given by Liverpool John Moores Ethics Committee. Participant's also signed a written consent form before any testing occurred.

Eleven recreationally active males (20 $\pm$ 1 years old, with a mean body weight of 72.0 $\pm$ 8.6 kg, a mean height of 180.6 $\pm$ 6.9 cm, a mean body fat of 20 $\pm$ 3 % and a mean $\dot{V}O_{2\,max}$ of 53.9 $\pm$ 5.8 ml.kg.min^{-1}) completed the study.

3.9.2 Health screening.

To ascertain subject suitability for the project a medical health questionnaire was completed by each subject (appendix 9.1). Exclusion criteria included any past history of cardiovascular disease, hypertension, neuromuscular problems limiting exercise, and taking any dietary supplements. If subjects met the inclusion criteria, they then attended a further health screening session at the University. This consisted of a treadmill exercise stress test. Prior to this exercise, resting blood pressure was measured in a seated position. Throughout the exercise test, a 12-lead ECG was continually monitored to detect any underlying heart abnormalities. To ensure the exclusion of obese individuals, BMI and a Dual Energy X-ray Absorptiometry (DEXA) scan was used to ascertain percentage body fat. Any male subjects with a percentage body fat of over 26 % were excluded as being obese, according to the ACSM guidelines for the age group. At the end of the screening, all of the participants met the strict criteria to be included in the study.

3.9.3 Measurements.

The CPX/D system (Medgraphics, St.Pauls, Minnesota, USA) measured breath-by-breath VO_2, VCO_2, $P_{ET}CO_2$, VE and RR. VO_2 and VCO_2 were averaged from 5 breaths. Maximal oxygen consumption was measured using an adapted Bruce (1971) protocol. Cardiac outputs were measured at rest and during exercise using the CO_2 rebreathing method (see Chapter 2 for more details). Blood pressure was taken manually, at rest and during exercise, using manual auscultation. Exercise was undertaken with a mean room temperature of 22 °C ± 0.1 °C and room humidity of 50 ± 10 %. Cardiac power output and cardiac reserve were subsequently calculated as previously described. All these measurements were taken on 3 separate occasions. All subjects had at least 72 hours between CPO tests to allow a physiological baseline state to be re-attained.

3.9.4 Caffeine dosages.

Each caffeine dose was administered orally via a capsule and was randomised.

Condition 1: Placebo (Control) - Identical placebo capsules were filled with cornflour so as not to bias results based on participants' prior knowledge of the experimental conditions.

Condition 2: Caffeine dose of 5 $mg.kg^{-1}$ body weight, 3 hours prior to testing (3 hour low dose)

Condition 3: Caffeine dose 10 $mg.kg^{-1}$ body weight, 3 hours prior to testing (3 hour high dose)

Condition 4: Caffeine dose 10 $mg.kg^{-1}$ body weight, 6 hours prior to testing (6 hour high dose)

3.9.5 Statistical Analyses.

The Kolmogorov-Smirnov test was used to ascertain normality of distribution of data. Once established, repeated measures analysis of variance was used (ANOVA) between each condition. Bonferroni was used after the ANOVA to determine the differences between each condition. Statistics were analysed using SPSS (vs. 14, Chicago, Illinois) and all data are presented as mean ± SE.

3.10 Results.

3.10.1 VO_2 and VCO_2.

VO_2 and $VO_2.kg^{-1}$ at rest were significantly ($P < 0.05$) increased by 8 % between the control and the 10 mg of caffeine kg^{-1} dose which was taken 3 hours prior to testing (Figure 3.5). Despite this, RER_{rest} was not significantly changed, particularly because VCO_2 was not affected by the caffeine. Neither VO_2, VCO_2 nor RER were affected by any of the other doses (placebo, 5 $mg.kg^{-1}$ or 10

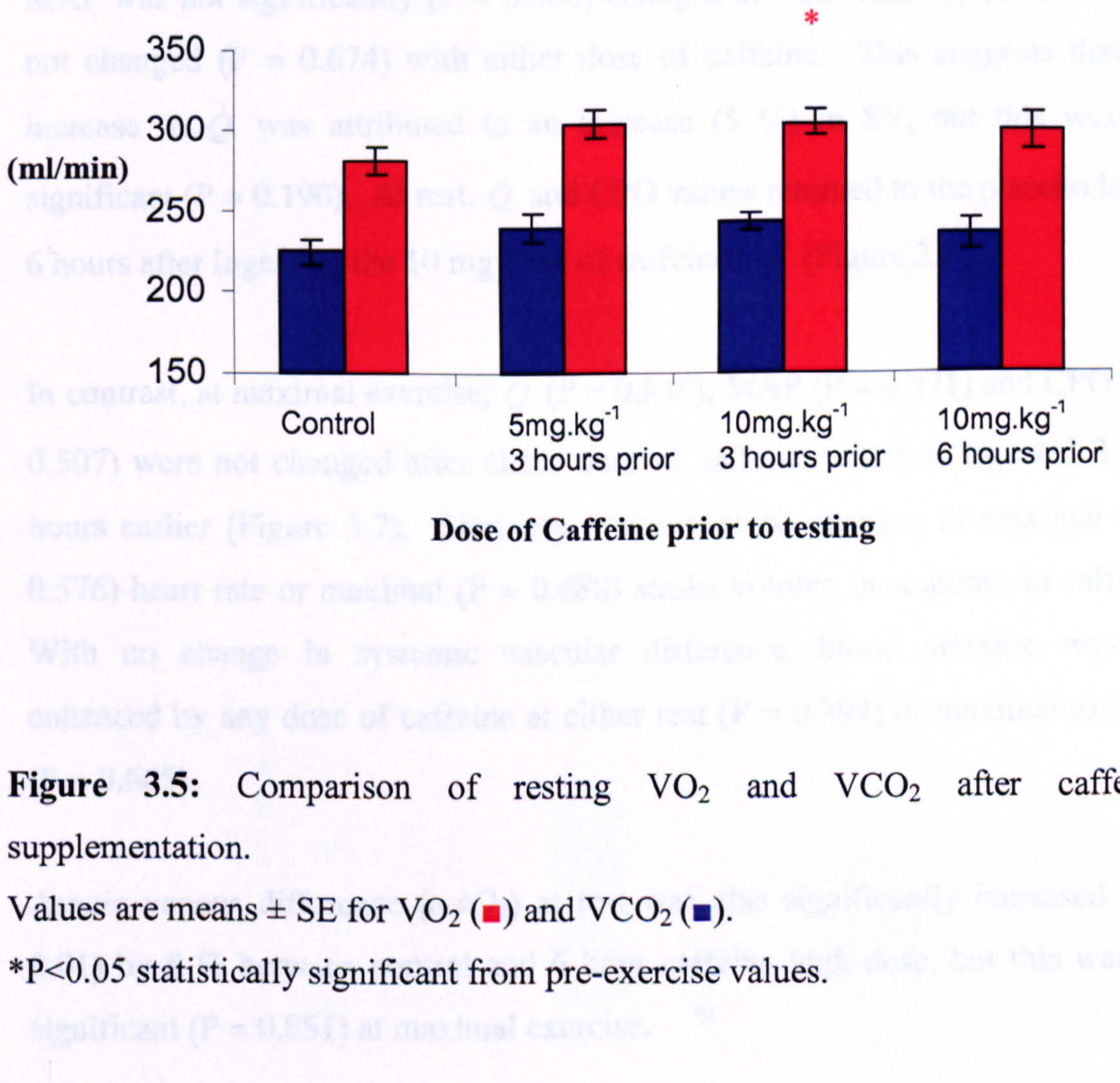


Figure 3.5: Comparison of resting VO_2 and VCO_2 after caffeine supplementation.

Values are means ± SE for VO_2 (■) and VCO_2 (■).

*P<0.05 statistically significant from pre-exercise values.

mg.kg^{-1} taken 6 hours prior) of caffeine at maximal exercise.

3.10.2 Cardiac Power Output.

There was a significant (P = 0.009) increase of 6 % in resting cardiac output between placebo and the 10 mg of caffeine kg^{-1} taken 3 hours prior to testing (Figure 3.6). This increase in $\dot{Q}$ probably explains the significant (P < 0.05) increase of 9 % in CPO_{rest} between placebo and this dose of caffeine, because MAP was not significantly (P = 0.308) changed at rest. Resting heart rate was not changed (P = 0.674) with either dose of caffeine. This suggests that the increase in $\dot{Q}$ was attributed to an increase (5 %) in SV, but this was not significant (P = 0.190). At rest, $\dot{Q}$ and CPO values returned to the placebo levels 6 hours after ingesting the 10 mg dose of caffeine kg^{-1} (Figure 3.6).

In contrast, at maximal exercise, $\dot{Q}$ (P = 0.640), MAP (P = 0.471) and CPO (P = 0.507) were not changed after either dose of caffeine, whether ingested 3 or 6 hours earlier (Figure 3.7). Similarly, there were no changes in maximal (P = 0.576) heart rate or maximal (P = 0.688) stroke volume in response to caffeine. With no change in systemic vascular difference, blood pressure was not enhanced by any dose of caffeine at either rest (P = 0.394) or maximal exercise (P = 0.665).

Arterio-venous difference (a-vO_2) at rest was also significantly increased (P < 0.01) by 8 % between control and 6 hour caffeine high dose, but this was not significant (P = 0.851) at maximal exercise.

3.10.3 Summary.

The results suggest that a high dose (around 10 mg.kg^{-1}) of caffeine taken 3 hours prior to testing, affects measurements of resting cardiac power output, due to increases in cardiac output. These affects however disappear 6 hours after

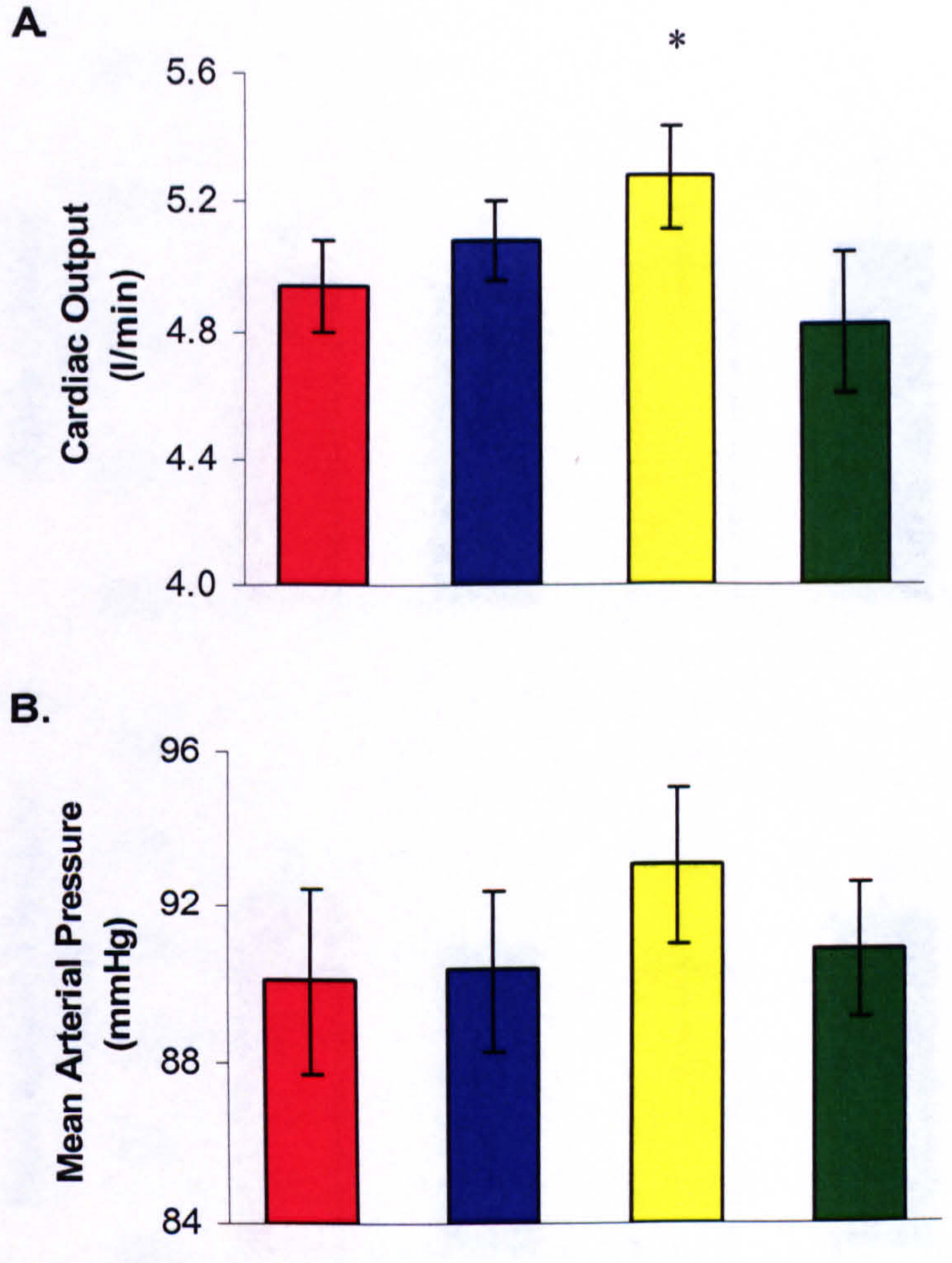


Figure 3.6: Effects of caffeine ingestion on resting cardiac output (A), mean arterial pressure (B) and CPO (C).

Data are means ± SE for placebo (■), 5mg.kg^{-1} 3 hour dose (■), 10 mg.kg^{-1} 3 hour dose (■) and 10 mg.kg^{-1} 6 hour dose (■).

*P<0.05 statistically significant from pre-exercise values.

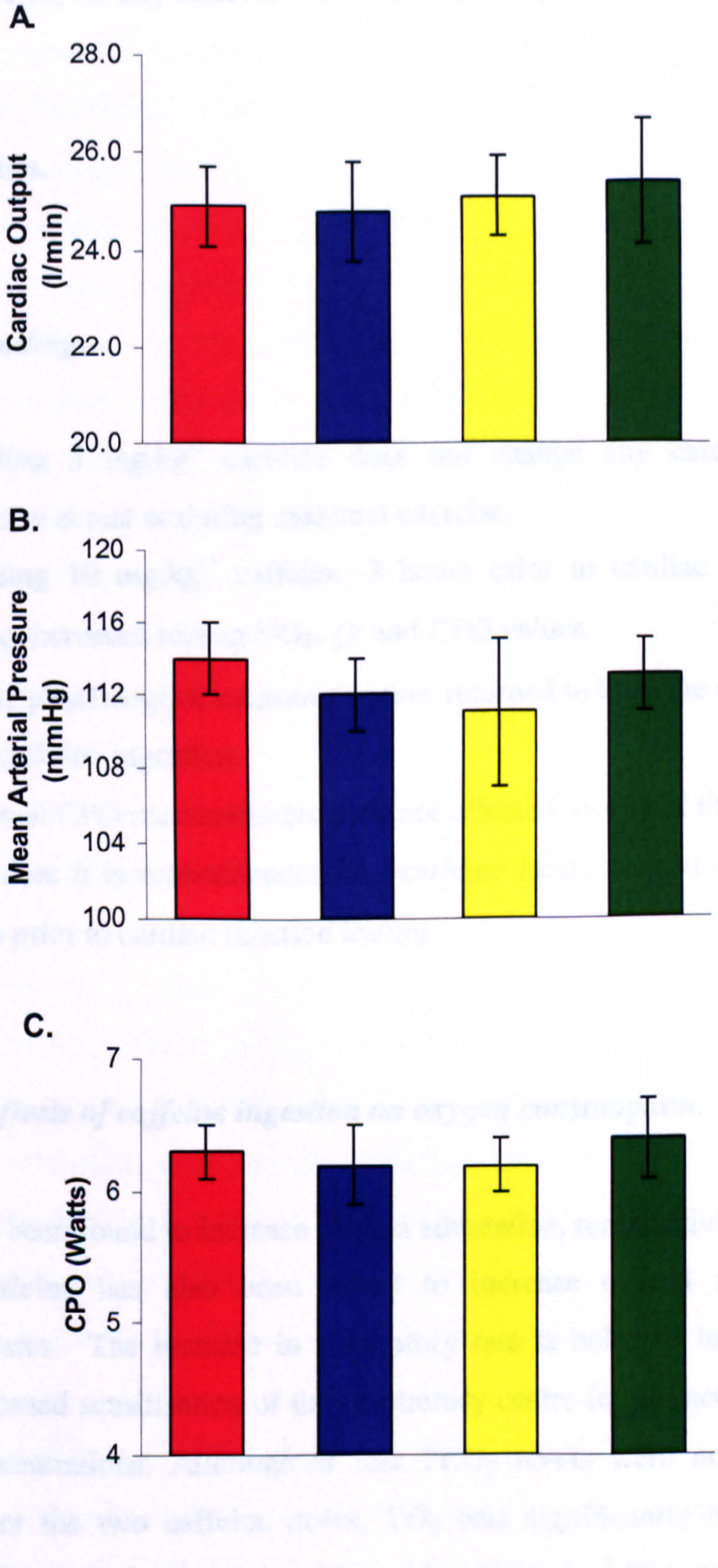


Figure 3.7: Effects of caffeine ingestion on maximal cardiac output (A), mean arterial pressure (B) and CPO (C).

Data are means ± SE for placebo (■), 5mg.kg^{-1} 3 hour dose (■), 10 mg.kg^{-1} 3 hour dose (■) and 10 mg.kg^{-1} 6 hour dose (■).

caffeine abstinence. Conversely, caffeine does not affect measures made at maximal exercise, for any caffeine dose or abstinence period.

3.11 Discussion.

3.11.1 Key findings.

1. Ingesting 5 $mg.kg^{-1}$ caffeine does not change any cardio-respiratory variables at rest or during maximal exercise.
2. Ingesting 10 $mg.kg^{-1}$ caffeine, 3 hours prior to cardiac power output testing increased resting VO_2, $\dot{Q}$ and CPO values.
3. All the physiological measured values returned to baseline values 6 hours after caffeine ingestion.
4. Maximal CPO measurements were not affected by any of the conditions.
5. Therefore it is recommended that caffeine intake should be abstained 6 hours prior to cardiac function testing.

3.11.2 The effects of caffeine ingestion on oxygen consumption.

Caffeine has been found to increase plasma adrenaline, renin activity and cortisol levels. Caffeine has also been found to increase overall metabolic and respiratory rates. The increase in respiratory rate is believed to be associated with an increased sensitisation of the respiratory centre in the medulla to carbon dioxide concentrations. Although at rest VCO_2 levels were not significantly different after the two caffeine doses, VO_2 was significantly higher between control and 3 hours after ingesting 10 mg of caffeine kg^{-1} (Figure 3.5). Chad & Quigley (1989) found at rest an increase in VO_2 from 0.23 $l.min^{-1}$ to 0.33 $l.min^{-1}$ 1 hour post ingestion of 5 mg of caffeine kg^{-1}. This increase was consistent with our findings and suggests that caffeine increases resting metabolic rate. Chad & Quigley (1989) also found that 5 mg of caffeine kg^{-1} caused a slight increase in fat oxidation, but this was not statistically significant from the placebo control.

There was no effect on RER from either of the doses ingested in the present study. Donelly *et al.* (1992) found that 5 mg of caffeine kg^{-1} and 10 mg of caffeine kg^{-1} increased VO_2 compared to baseline. This effect at rest was noted after one hour, but did not change beyond an hour. This suggests that either a higher level of caffeine beyond one hour was required to sustain an increase in VO_2 or that the effect was optimal at one hour. VO_2 did increase in the present study, but after 3 hours it was not ascertained if plasma caffeine was at its peak concentration. However, 10 mg of caffeine kg^{-1} is a large dose, since two to three cups of instant coffee contains 6 mg of caffeine kg^{-1} (Lamarine, 1994). Coffee is not the only drink which contains caffeine, as many sports drinks (i.e. lucozade containing 12 mg/ 100 ml), and energy drinks (i.e. redbull containing 33 mg/ 100 ml) contain higher caffeine levels. Therefore, the increase in VO_2 after the ingestion of 10 mg of caffeine kg^{-1} could represent normal levels of caffeine ingestion.

3.11.3 The effect of caffeine on cardiac output and CPO.

Acute administration of caffeine (300 mg) has been shown to significantly affect the cardiovascular system (Nishijima *et al.*, 2002; Pincomb *et al.*, 1985; Sondermajer *et al.*, 2002). Waring *et al.* (2003) found a maximal haemodynamic response after 45 minutes of ingesting caffeine; a time which also corresponded to caffeine's peak plasma concentration. When compared with our results, the study of Waring *et al.* (2003) suggests that much of our caffeine-induced haemodynamic effects may have disappeared by 3 hours post caffeine ingestion.

In contrast, Bender *et al.* (1997) showed that a consumption of 5 mg of caffeine kg^{-1} increased cardiac contractility, which returned to normal within 4.5 hours. In contrast, Pincomb *et al.* (1985) found no enhanced cardiac contractility and no change in cardiac output or stroke volume after 3.3 mg of caffeine kg^{-1} ingestion. Instead their results showed an increased afterload, which can be attributed to an increase in vascular resistance. Although Smits *et al.* (1983) found no change in either femoral blood flow or forearm vascular resistance after caffeine ingestion, they did find an increase in diastolic blood pressure, suggesting an increase in

total SVR. This is consistent with other studies, which found increases in peripheral vascular resistance after caffeine ingestion, both at rest and during exercise (Pincomb *et al.*, 1991; Sung *et al.*, 1990). We found no increase in maximal $\dot{Q}$ (Figure 3.7A), but our measurements were taken 1.5 hours later than Pincomb *et al.* (1991) and therefore caffeine levels could have peaked earlier. It is also important to note that the previous studies used echocardiographic techniques to measure cardiac output rather than the CO_2 rebreathing manoeuvre used here.

Heart rates were not affected by either low or high doses of caffeine, at both rest and during exercise. This conflicts with Whitsett *et al.* (1984) as they observed a slight decrease in heart rate (10 bpm) from 30 minutes to 3 hours after receiving 8.8 mg of caffeine kg^{-1}. This decrease in resting heart rate was attributed to a rise in blood pressure, caused by an increase in baroreceptor response (Corti *et al.*, 2002; Sondermaijer *et al.*, 2002) and direct vagal stimulation (Whitsett *et al.*, 1984). Their rise in blood pressure occurred 1 hour post caffeine consumption, but was not apparent in our study after 3 hours. Conversely, Namdar *et al.* (2006) found that 200 mg of caffeine did not affect heart rate or blood pressure 50 minutes post consumption. This suggests that the dose and temporal peak of caffeine is important when considering physiological responses.

Hartley *et al.* (2004) found that men consuming 3.3 mg of caffeine kg^{-1} had a higher total peripheral resistance at rest compared to an age-matched control group. Hartley *et al.* (2004) also investigated women in the same study, showing that women increased their resting stroke volume to a greater extent compared to men, and therefore produced a larger increase in resting cardiac output. These results suggest that there may be interesting sex-specific differences in response to caffeine ingestion.

3.11.4 Limitations.

3.11.4.1 Caffeine ingestion.

Due to early testing times, some subjects had to administer the 6 hour dose during the night. Therefore, they were given the capsules the previous day and thus we had to rely on the subject to administer the caffeine dose at the correct time. This we believe they did. Studying more time points and doses would have helped us to establish a better knowledge of the optimal dose and time course of caffeine action. Despite this, the emphasis here was to determine if caffeine had an effect on CPO and how best to eliminate this from our measurements.

3.11.5 Summary.

Caffeine can affect resting cardiac indices of function. Therefore it is essential that guidelines are enforced to prevent the ingestion of caffeine prior to cardiac function testing. These guidelines should recommend the abstinence of caffeine for 6 hours prior to testing to ensure no affect on physiological components.

Chapter 4

Study 2 - The effects of a 30-week exercise training intervention on cardiac function in men and women aged 55-65 years.

4.1 Review of Literature.

4.1.1 Cardiovascular function at rest in older populations.

The heart is the muscular pump within the circulatory system, providing tissues and their cells with vital oxygen and nutrients. The rate of blood flow from the heart ($\dot{Q}$) is determined by the heart's dimension, frequency of pumping (heart rate) and contractility (stroke volume). An index of contractility has been determined to be an accurate measure of cardiac function (Bergel *et al.*, 1969). However, measuring contractility does not exclude peripheral influences on cardiac function. In order to generate blood flow effectively, the heart must generate adequate pressure to overcome vascular impedance. Therefore, to gain a true representation of overall cardiac function, central and peripheral factors should both be incorporated within the measure. Previous studies have investigated whole body flow ($\dot{Q}$) or pressure (MAP), but not both together.

The ageing process integrates numerous changes in physiological factors, including within the vasculature, heart and skeletal muscle. Blood pressure is significantly lower in young women compared to young men, but as ageing progresses, similar increases in blood pressure levels occur in both sexes. Arterial blood pressure is influenced by the stiffness or compliance of large elastic arteries. Ageing is associated with a decreased compliance of the large elastic and peripheral arteries system. These changes can be attributed to the localised accumulations of lipid, collagen and mineral deposits in the arteries. Also, elastin fibres fragment and decrease in overall number, while smooth muscle components thicken in the media of the vessel (Robert *et al.*, 1999). In addition, endothelial cells become more heterogeneous in size, shape and orientation, and fatty deposits become more common (Marin, 1995). As a consequence, there is an increase in systolic blood pressure due to an augmentation in the pulse wave and its velocity (Lakatta & Sollot, 2002). Although the aortic artery becomes stiffer with age, the left ventricle continues to pump the same amount of blood into the aorta. As a result, the velocity of the blood in the aorta increases with age, thus accelerating pulse wave velocity down

the entire arterial tree. Vaitkevicius *et al.* (1993) showed that between 21 years and 96 years, there is a 5-fold increase in pulse wave velocity. In younger people, this pulse wave is reflected slowly and systole is completed before the wave returns to the heart. However, in older people, the wave returns more rapidly before the aortic valve closes, thus elevating both systolic left ventricular pressure and arterial blood pressure, and possibly increasing the work-load on the left ventricle (Weisfeldt, 1998).

The increase in left ventricular loading with ageing causes an increase in left ventricular wall tension, resulting in a prolonged myocardial contraction. Similar, increased myocardial relaxation time is understood to be due to changes in calcium ATPase (SERCA) in the sarcoplasmic reticulum. There are different isoforms of SERCA, with SERCA 2a being predominant in cardiomyocytes. Senescence instigates a reduction in SERCA 2a messenger RNA and protein levels, thus causing a slower removal of calcium back into the sarcoplasmic reticulum, and consequently a slower cardiac relaxation time. The increased myocardial relaxation time also causes a decrease in early diastolic filling. Conversely, the reduction in resting end diastolic volume is maintained through an increase in late left ventricular filling (Miller *et al.*, 1986) or enhanced active atrial contribution to left ventricular filling (Arora *et al.*,1987). This results in an uncompromised resting functional capacity.

Increased left ventricular (LV) wall tension also instigates LV hypertrophy. Echocardiographic techniques have determined that women between 20 and 90 years old show a 9 % shortening along the long axis of the left ventricle. This change is counteracted by an increase in wall thickness, causing a change in shape but no increase in left ventricular mass (Hees *et al.*, 2002). Men's left ventricular long axis decrease by 11 % over the same time period. However, wall thickness does not increase, therefore resulting in a decrease of overall mass by 10 % throughout the ageing process (Hees *et al.*, 2002; Olivetti *et al.*, 1995). The decrease in LV mass in normotensive men is attributed to a loss of LV myocytes, despite some cellular hypertrophy and multinucleation. With ageing, myocyte number is reduced by 30 % in men, and has been attributed to an increase in oxygen free radical production (Sabbah, 2000). Therefore, to try and

compensate for this loss in cell number, cardiac myocyte size increases, to allow cardiac output and stroke volume to remain reasonably stable in men (Ferrari *et al.*, 2003). Interestingly in women over the same age range there is little, or no, loss of myocytes (Olivetti *et al.*, 1995). It is still unclear why myocyte loss is greater in men than women as age progresses, but a link to oestrogen protection seems highly likely.

Left ventricular hypertrophy causes a decreased myocardial contraction velocity, resulting in the maintenance of ejection time and consequently a preserved systolic volume and ejection fraction (Lakatta & Sollot, 2002). Myocardial contraction velocity can be diminished by a reduction in the number of sino-atrial (SA) node cells. In both sexes, sinoatrial pacemaker cells decrease to 50 % during youth to less than 30 % in older populations. The SA node can also suffer from fibrosis, fatty infiltrations and calcification of the conduction system, reducing electrical propagation with ageing (Pugh & Wei, 2001).

Due to the reduction in LV early diastolic filling, more filling occurs in late diastole due to a greater atrial contraction. In fact, end-diastolic volume index (end-diastolic volume normalised to body surface area [EDVI]) increases in men with age, which causes an increase in SVI (stroke volume index; Figure 4.1E), in order to compensate for the reduction in the heart rate at rest (Figure 4.1C). This therefore allows cardiac index (CI) to remain stable at rest (Figure 4.1D). However, women's SVI at rest does not increase, therefore EDVI decreases (Figure 4.1E) causing a reduction in CI (Figure 4.1D), with an associated increase in peripheral vascular resistance (PVR) as referred to in figure 4.1B.

At rest, cardiac output is preserved in both sexes with ageing due to small decreases (~17 % in women, ~13 % in men) in heart rate and an increase in stroke volume. Heart rate is decreased due to a lower ß-adrenergic stimulation, as ß-adrenergic receptors become more desensitised with increasing age (Lakatta, 1994). Parasympathetic control has also been found to decrease with age, resulting in a decline in heart rate variability and baroreflex sensitivity (Craft *et*

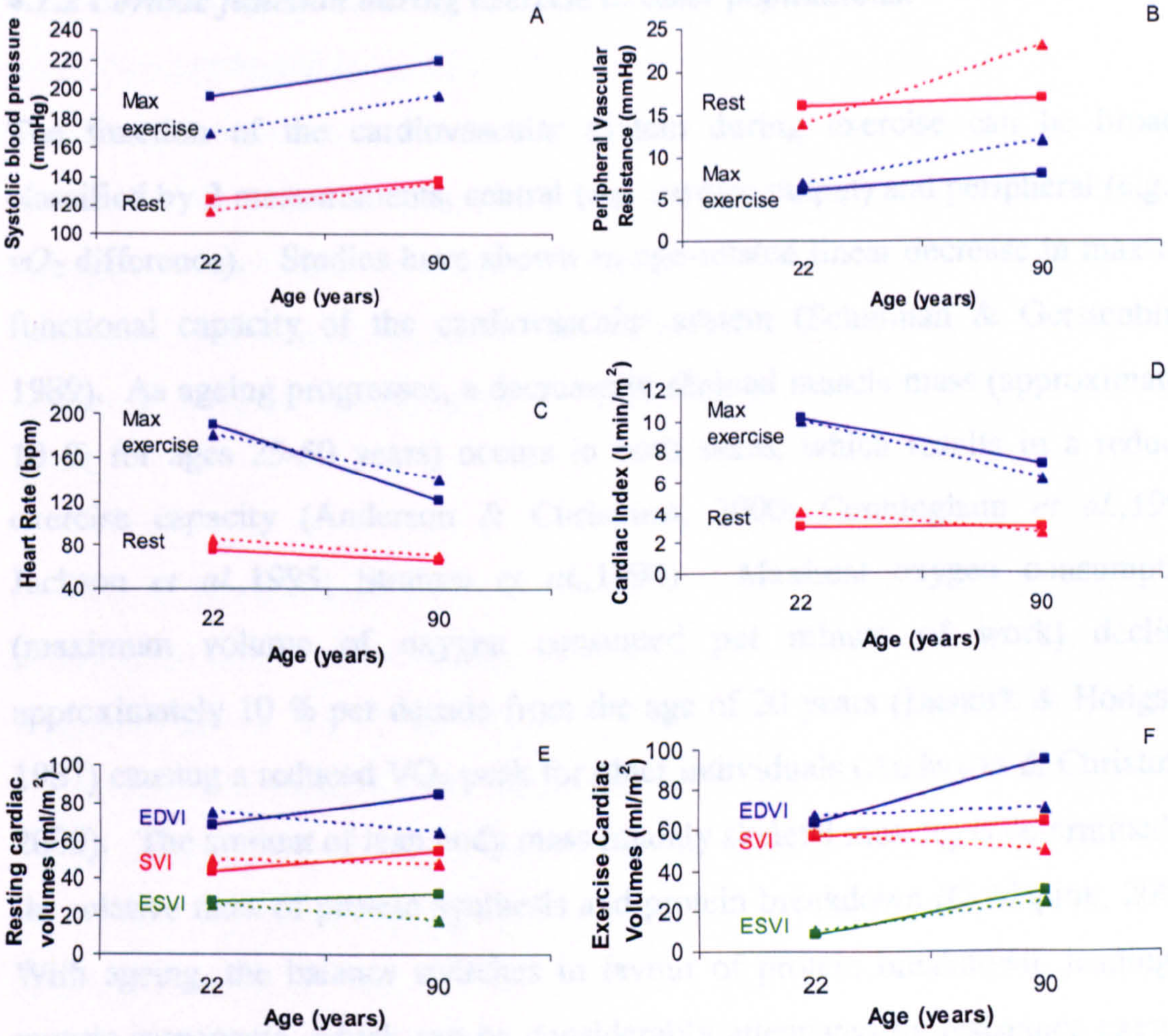


Figure 4.1: Linear regression of systolic blood pressure (A), peripheral vascular resistance (B), heart rate (C), cardiac index (D), resting cardiac volume (E), and exercise cardiac volume (F). EDVI (end-diastolic volume index), SVI (stroke volume index), ESVI (end-systolic volume index) with ageing at rest and during exercise.

▲ Female (n = 95) ■ Male (n = 50)

Taken from Lakatta (1994).

al., 1995). However, older females have been found to preserve diastolic filling in response to parasympathetic withdrawal (Stratton *et al.*, 2003).

4.1.2 Cardiac function during exercise in older populations.

The function of the cardiovascular system during exercise can be broadly classified by 2 measurements, central (e.g. cardiac output) and peripheral (e.g. a-vO_2 difference). Studies have shown an age-related linear decrease in maximal functional capacity of the cardiovascular system (Schulman & Gerstenblith, 1989). As ageing progresses, a decrease in skeletal muscle mass (approximately 10 % for ages 25-50 years) occurs in both sexes, which results in a reduced exercise capacity (Anderson & Christmas, 2000; Cunningham *et al.*,1997; Jackson *et al.*,1995; Stratton *et al.*,1994). Maximal oxygen consumption (maximum volume of oxygen consumed per minute of work) declines approximately 10 % per decade from the age of 20 years (Buskirk & Hodgson, 1987) causing a reduced VO_2 peak for older individuals (Anderson & Christmas, 2000). The amount of lean body mass (mainly skeletal muscle) is determined by the relative rates of protein synthesis and protein breakdown (Goldspink, 2005). With ageing, the balance switches in favour of protein breakdown, leading to muscle sarcopenia, which can be considerably attenuated by resistance exercise (Balagopal *et al.*, 2001; Hasten *et al.*, 2000; Tipton & Wolfe, 1998; Yarasheski *et al.*, 1995). The reduction in muscle mass and oxidative capacity results in a decline in a-vO_2 difference at rest and with exercise, due to a reduced oxygen extraction. Within muscles, ageing is associated with a decline in mitochondria size and number and a shift in fibre types from type IIa to more type IIb fibres. All of these changes cause a decreased oxidative capacity and lower fatigue resistance during exercise (Anderson & Christmas, 2000). Consequently, a greater reliance is placed on anaerobic pathways to generate ATP, and subsequently cause a greater accumulation of lactate.

A decline in $\dot{V}O_{2\,max}$ has also be attributed to a decrease in maximal cardiac output with ageing (Ogawa *et al.*, 1992). This decrease in $\dot{Q}_{max}$ has been ascribed to a decrease in maximal heart rate. The parasympathetic nervous

system is affected with age due to an age-related decrease in vagal baroreflex sensitivity and vagal modulation of resting heart rate (R-R interval; Seals *et al.*, 2001). At the start of exercise, an older individual produces less vagal tone causing an increase in resting heart rate, with a further increase in heart rate facilitated through an increase in sympathetic activity (Seals *et al.*, 1994). With age, resting sympathetic nervous activity is enhanced, supported by elevations in plasma adrenaline and noradrenaline concentrations. These changes result in the over-activation of vascular β-adrenergic receptors and consequently a background desensitisation and de-regulation of β-adrenergic receptors. The progressive decreased response of the whole circulatory system to β-adrenergic stimulation causes a progressive reduction in maximal heart rate (Weisfeldt, 1998).

Several studies have shown that maximal cardiac output declines with age due to decreases in both maximal stroke volume and heart rate (Dehn & Bruce, 1972; Higginbotham *et al.*, 1986; Hossack & Bruce, 1982; Julius *et al.*, 1967). However, more recent studies on ageing suggest that a rise in stroke volume during exercise, which is achieved through an increase in end-diastolic volume (Frank-Starling mechanism), preserves cardiac output during exercise (Fitzgerald *et al.* 1997; Pugh & Wei, 2001). It has been proposed that the discrepancies between studies regarding maximal stroke volume during exercise are due to sex-specific differences. Lakatta (2002) stated that stroke volume index was increased in ageing men during exercise due to an increased end-diastolic volume index (EDVI). Conversely in women, maximal stroke volume index declines with age, due to a decline in EDVI, which results in a reduction in cardiac index, along with an associated increase in peripheral vascular resistance (Figure 4.1).

4.1.3 Aerobic power and cardiac function with training in older populations.

As previously described, aerobic capacity declines with age. However, endurance training has been found to improve aerobic capacity and diminish the effects of ageing. Blumenthal *et al.* (1991) established that after 4 months of

aerobic exercise at 70 % HRR (heart rate reserve), both older men and women increased their cardiorespiratory fitness by ~12 %. An additional 4 months produced more gradual improvements in aerobic power (~4 %), eventually reaching an 18 % improvement after 14 months. Other studies have shown similar increases of 11 % (Cunningham *et al.*, 1987) and 10 % (Niinimaa and Shephard, 1978) in $\dot{V}O_{2\,max}$ after 12 months of endurance exercise in men aged 55-65 years old. However, some studies have found larger increases. Sidney and Shephard (1978) found a 24 % increase in $\dot{V}O_{2\,max}$ with training, but their rigorous inclusion criteria may not be typical of the general population as participants were completely free from disease. A higher intensity exercise programme has also shown large improvements of 25-30 % in $\dot{V}O_{2\,max}$ (Seals *et al.*, 1984). Numerous exercise training studies have found no sex-specific differences in relation to improvements in $\dot{V}O_{2\,max}$, with both sexes improving in like manner (Blumenthal *et al.* 1989; Hagberg *et al.*, 1989a; Kohrt *et al.*, 1991; Seals *et al.*, 1984). However, the mechanisms through which $\dot{V}O_{2\,max}$ is improved have been found to be sex-specific. Spina *et al.* (1993) found that with a 9-12 months endurance exercise programme, two-thirds of the increase in $\dot{V}O_{2\,max}$ for older men was due to an increase in maximal cardiac output and one-third due to an increase in maximal a-vO_2 difference. In women, results showed that the similar increases in $\dot{V}O_{2\,max}$ were solely due to an increase in maximal a-vO_2 difference. Studies performed with men tend to agree that central changes are apparent with aerobic exercise training.

Endurance training has been attributed to preventing the age-related decline in stroke volume (Spina *et al.*, 1992), with reports suggesting that individuals may increase their stroke volume as exercise intensity increases (Gledhill *et al.*, 1994). Short-term training also increases LV functional response to exercise, with increases in stroke volume being secondary to the Frank-Starling mechanism, hence bradycardia and increased left ventricular filling (Goodman *et al.*, 2005). Schulman *et al.* (1996) also found that a few months of aerobic training in older men resulted in improvements in peak ejection fraction, cardiac index and left ventricular contractility index. This improved cardiac function has also been noted in older endurance trained men. Here the larger peak blood volumes, contribute to a greater peak left ventricular end-diastolic volume during exercise,

through the utilisation of the Frank-Starling effect, increasing stroke volume and cardiac output during exercise (Hagberg *et al.*, 1998; Stratton *et al.*, 1994).

This Frank-Starling phenomenon appears to be used less in age-matched females due to both LV end-diastolic volume and end-systolic volume being decreased with age. This again suggests that there is no appearance of hypertrophy in the female heart (Spina *et al.*, 1993). Two studies have shown that increases in diastolic filling, stroke volume and cardiac output in response to training do not occur in women (Spina *et al.*, 1993, 1996a). This has been attributed to an absence of β-adrenergic-mediated enhancement of LV systolic function (Spina *et al.*, 2000). A noted decrease in diastolic blood pressure with training, has suggested an improvement in peripheral vascular resistance to exercise in women. Collectively the facts suggest that enhancement of cardiac function is required less in women to improve aerobic power in response to endurance exercise training.

4.1.4 Peripheral vascular function with training in older populations.

As previously described, older populations have been found to have an increased arterial stiffness, typically demonstrated through an increased augmentation index, increased pulse-wave velocity, and reduced arterial elastic compliance (Gates *et al.*, 2004; Rajkumar *et al.*, 1997; Sutton-Tyrrell *et al*, 2001; Vaitkevicus *et al.*, 1993). Around 85 % of cardiac output to the active musculature during exercise is provided by resistance vessels. Therefore, resistance vascular function and peripheral vasodilatory capacity will influence total peripheral resistance and blood pressure during exercise (Mellander & Johansson, 1968). Increased vasodilatory response to exercise training has been attributed to an up-regulation of nitric oxide (NO) synthesis, due to increased sheer stresses on the vascular endothelium during exercise.

Cross-sectional studies have shown that endurance trained men have a 50 % greater endothelium-dependent vasoreactivity (DeSouza *et al.*, 2000; Rywik *et al.*, 1999) and more pronounced increases in peripheral blood flow (Ho *et al.*,

1997; Thomas *et al.*, 1999) compared to their sedentary counterparts. The preservation in brachial flow-mediated dilation with endurance training is believed to be due to the bioavailability of tetrahydrobioprotein (involved in the synthesis of NO from L-arginine) or the absence of oxidative stress (Eskurza *et al.*, 2004; Taddei *et al.*, 2000). This suggests that regular exercise prevents the age-related decline in endothelial function. Endurance trained men also exhibit a lower augmentation index, aortic pulse wave velocity and systolic blood pressure compared to normotensive men (Vaitkevicius *et al.*, 1993). Perini *et al.* (2002) found that after 6 months endurance training in men, there was a decrease in resting diastolic blood pressure, suggesting a decrease in total peripheral resistance at rest. A recent study found that carotid arterial compliance can be increased through a 12-week moderate intensity aerobic exercise programme, and thus lower blood pressure. The study also showed that men who perform regular vigorous exercise have a reduction in the age-related decline of arterial compliance, compared to their sedentary counterparts (Tanaka *et al.*, 2000). This suggests that habitual exercise can prevent or reduce the age-related changes in arterial compliance. Exercising rats showed a higher elastin content and reduced calcium content of elastin in the aorta (Matsunda *et al.*, 1993). Also, old trained rats exhibited 50 % less collagen cross-linking in the left ventricle compared with non-trained (Thomas *et al.*, 2000). This suggests that in rats at least, exercise reduces both arterial and cardiac wall compliance. Also in older men, carotid arterial compliance has been found to be lower, compared to younger men regardless of fitness (Tanaka *et al.*, 2000), while women displayed no change in arterial compliance with ageing (Moreau *et al.*, 2003b).

Cross-sectional studies select only aged participants who have successfully aged, while longitudinal endurance training programmes can give a clearer perspective on ageing and the benefits of exercise. Three months of endurance exercise has been shown to increase peak O_2 consumption through a greater re-distribution of the cardiac output to exercising limbs, therefore increasing systemic a-vO_2 differences (Beere *et al.*, 1999). Central arterial compliance has also been shown to improve by 25 % and beta stiffness index reduced by 20 %, after 14 weeks of exercise training in older men. Due to biochemical changes in collagen and elastin taking years to manifest, the decreases in arterial compliance with

endurance training have been attributed to enhancing the sympathoinhibitory effect of nitric oxide (Tanaka *et al.*, 2000). In older men, the increase in NO availability with training has been seen, with increases in maximal cutaneous blood flow after just 4 weeks of rigorous endurance training (Ho *et al.*, 1997) and increases in maximal cutaneous vascular conductance after 16 weeks of endurance exercise (Thomas al., 1999). Moreau *et al.* (2003a) found that post-menopausal women on hormone replacement therapy (HRT) exhibited greater carotid compliance by 40 % and a decreased beta stiffness index of 25 % after endurance training. Hence these effects were similar to the adaptations in men. However, no known studies to date have examined non-HRT post-menopausal women with endurance training in regards to blood flow.

4.1.4 Cardiac function in older females.

Recent studies have suggested that men and women of a similar age adapt differently with regards to cardiac function and ageing. Females have been found to have an increased cardio-protective mechanism, with lower levels of necrosis and apoptosis of myocytes, which have been attributed to levels of female reproductive hormones (Du, 2002). Oestrogen has been suggested to be cardio-protective, as cardiac myocytes and cardiac fibroblasts contain isoforms of oestrogen receptors (ERα and ERβ; Grohe *et al.*, 1998). Oestrogen phosphorylates insulin-like growth factor-1 receptors, which results in the phosphorylation of BAD (Bcle-2 antagonist causing cell death) inhibiting apoptosis and inversely aiding the promotion of cell survival (Datta *et al.*, 1997). Oestrogen has also been reported to inhibit collagen synthesis and enhance its degradation, resulting in improved vascular compliance. However post menopause, oestrogen levels decline, thereby reducing cardio-protective mechanisms. This reduction in cardio-protective mechanisms has also been attributed to an increase in the androgen: oestrogen ratio. Androgens are known to induce myocyte hypertrophy and enhance collagen synthesis (Xu *et al.*, 2003). It has therefore been suggested that HRT could improve cardiac function in post-menopausal women.

Green *et al.* (1998) reported that in older endurance trained women, cardiac index was increased at peak exercise after oestrogen supplementation. This increase was attributed to a reduction in total peripheral resistance, which contributes to a higher peak stroke volume, causing an increase in peak cardiac output. Conversely, Snabes *et al.* (1996) found that 3 months of oestrogen replacement in sedentary and physically active post-menopausal women did not improve aerobic capacity, cardiac indices of function or blood pressure during maximal exercise. However, other studies have found an increase in aerobic capacity, but attribute it not to an increase in a-vO_2 difference, but due to central factors (Katyal *et al.*, 2003; Spina *et al.*, 1993, 1996a). Moreau *et al.* (2003b) studied 44 sedentary, postmenopausal women who were either oestrogen deficient or on HRT, and a further 14 endurance trained oestrogen-deficient menopausal women, who participated in a 3 month training study. This training protocol employed 60-80 % of maximal heart rate for 40-45 mins/ day, 4-5 days/ week. The results showed that carotid arterial compliance increased in women who regularly exercised aerobically and in women on HRT, compared to their sedentary non-HRT peers. Secondly, 3 months of aerobic exercise re-established carotid arterial compliance in previously sedentary postmenopausal women on HRT. The restoration was to compliance levels which have been observed in pre-menopausal women. Therefore, HRT and regular aerobic exercise was beneficial in preserving carotid arterial compliance. The HRT and exercise induced increases in arterial compliance have been attributed to an increase in nitric oxide bioavailability in the endothelium of the conduit arteries, which increases vascular smooth muscle relaxation in the carotid artery. HRT is also known to lower peripheral sympathetic nerve activity through either alterations within the central nervous system (Weitz *et al.*, 2001) or a change in baroreflex stimulation (Hunt *et al.*, 2001).

4.2 Rationale.

In some studies with normal populations, ageing has been associated with small increases in heart size, caused by left ventricular hypertrophy (Gerstenblith *et al.*, 1977). Ventricular enlargement could be attributed to an increase in myocyte size, compensating for age-related reduction in myocyte number, in an attempt to

maintain stroke volume and cardiac output (Ferrari *et al.*, 2003). Olivetti *et al.* (1995) stated that the size of the female heart did not alter over 20-95 years. In contrast, men's LV mass is reduced due to the progressive loss of myocyte number (~30 %). Also cardiac output is greater and total peripheral resistance lower in women compared to men at the same age. It is not currently known why myocyte loss is considerably greater in men than women as age progresses. Clearly this is an important unanswered question. Ageing also causes a decreased response of the whole circulatory system to β-adrenergic stimulation (Weisfeldt, 1998). However, research has shown that women have a greater ability to cope with haemodynamic overload as age increases and are less affected by cardiovascular events (Olivetti *et al.*, 1995). Most of these studies have used echocardiographic techniques at rest, but no measurements have been taken during exercise. Previous work in our laboratories suggests that a 12 month aerobic exercise programme does not affect cardiac output both at rest and during maximal exercise in women (Sharp *et al.*, 2005). The literature suggests that older men and women's cardiac adaptations to exercise are different. Therefore the aim of this study was to investigate this potential sex-specific difference by subjecting healthy but sedentary, age-matched older men and women (aged 55-65 years) to an aerobic exercise training programme over 30 weeks, measuring $\dot{V}O_{2\,max}$ and cardiac power output (CPO).

4.3 Protocol.

4.3.1 Recruitment.

Sedentary, but healthy women (n = 25) and men (n = 35), between the ages of 55-65 years, were recruited from the local Merseyside region. This recruitment was initiated with adverts in local newspaper, the local radio, and attending local Age Concern events throughout Liverpool. Our research group within the Research Institute for Sport and Exercise Sciences held an Open Evening to explain the project in detail to prospective volunteers and to allow them to familiarise themselves with the equipment and laboratories.

This project was a multi-disciplinary collaboration to maximise the participants and the cardiovascular data which was available. The collaboration included measurements in forearm blood flow (Shellina Skyrme, MPhil thesis) and also balance and co-ordination (Jos Vanrenteghem, Post-Doctoral Fellow).

4.3.2 Medical Screening.

To ascertain participant suitability for the project a medical health questionnaire (appendix 9.1) was completed by each participant. Exclusion criteria included any past history of cardiovascular disease, hypertension (i.e. blood pressure > 160/ 90 mmHg), diabetes, ECG abnormalities, neuromuscular problems limiting exercise, and taking any prescribed medication, including HRT or dietary supplements. Only post-menopausal women were accepted onto the study, which was verified through the cessation of menses for over two years prior to the study. Over 120 questionnaires were received and if participants complied with the inclusion criteria, they then attended a health screening session at the University. Over 25 women and 35 men attended the medical screening. This consisted of a treadmill cardiorespiratory exercise stress test.

Prior to exercise, resting blood pressure was measured in a seated position. Throughout the exercise test, a 12-lead ECG was continually monitored by a qualified cardiologist to detect any participants, who may have underlying heart abnormalities. Participants who had a $\dot{V}O_{2\,max}$ value over the 50th percentile for their age group according to American College of Sports Medicine guidelines (Johnson, 1998), were excluded as the focus of the study was healthy but sedentary individuals. To ensure the exclusion of obese individuals, a Dual Energy X-ray Absorptiometry (DEXA) scan was performed to ascertain percentage body fat. Any potential female participants with a body fat percent over 38 % and male participants over 34 % were excluded (Johnson, 1998). Only 26 men and 12 women met the strict criteria to be included on the study.

4.3.3 Measurements.

The CPX/D system (Medgraphics, St.Pauls, Minnesota, USA) measured breath-by-breath VO_2, VCO_2, $P_{ET}CO_2$, VE and RR. VO_2 and VCO_2 were averaged from 5 breaths. Maximal oxygen consumption was measured using the modified Bruce (1971) protocol. Cardiac outputs were measured at rest and during exercise using the CO_2 re breathing method (see chapter 2 for more details). Blood pressure was taken manually, at rest and during exercise, using manual auscultation. Exercise was undertaken with a mean room temperature of 22 °C ± 0.1 °C and room humidity of 50 ± 10 %. Cardiac power output and cardiac reserve were subsequently calculated accordingly. All these measurements were taken at baseline, 12, 18, 24 and 30 weeks.

4.3.4 Training.

The training programme consisted of a 30-week endurance based exercise programme. Participants were tested at baseline (zero time), 12 weeks, 18 weeks, 24 weeks and 30 weeks to determine $\dot{V}O_{2\,max}$, $\dot{Q}$, MAP, CPO, lean body mass and percent body fat . Exercise was undertaken with a mean room temperature of 22 °C ± 0.1 °C and room humidity of 50 ± 10 %.

Exercise intensity started at individually determined 30 % HRR (heart rate reserve), with step-wise increments every six weeks to 45 %, 60 % and 75 % HRR (Figure 4.2).

Heart rates were obtained at rest and at maximal exercise from the ECG. Percentage heart rate reserve was calculated using the following formula:

$$\% \text{ Heart Rate Reserve (HRR)} = HR_{rest} + (HR_{max} - HR_{rest} \times \frac{\%}{100})$$

(Karvonen *et al.*, 1957)

Participants trained 3 times a week for the first 6 weeks to allow familiarisation of exercise equipment and their re-introduction to exercise, without injury because of their prior long-term sedentary state. Thereafter, the exercise was increased to 5 times per week for the remainder of the programme. During the training, heart rate was measured using a polar heart rate monitor (CardioSport, USA) to establish their exercise intensity levels. Participants trained for 30 minutes in each session, with this consisting of 10 minutes walk/ run on a motorised treadmill, 10 minutes cycling, followed by a further 10 minutes walk/ run. Each participant completed an exercise diary after each session (see appendix 9.2). Participants performed the exercise in the University gym or in local gyms and were supervised by the investigator at least once a week, when diaries were scrutinised to check the programme was being adhered to.

4.3.5 Statistical Analyses.

The Kolmogorov-Smirnov test was used to ascertain normality of distribution of data. Once established, repeated measures analysis of variance was used (ANOVA) between each time point (0, 12, 18, 24, 30 weeks) and between sexes (male and female). Bonferroni was performed after the ANOVA to determine at which time points these differences occurred. Statistics were analysed using SPSS (vs. 14, Chicago, Illinois) and all data presented as mean ± SE.

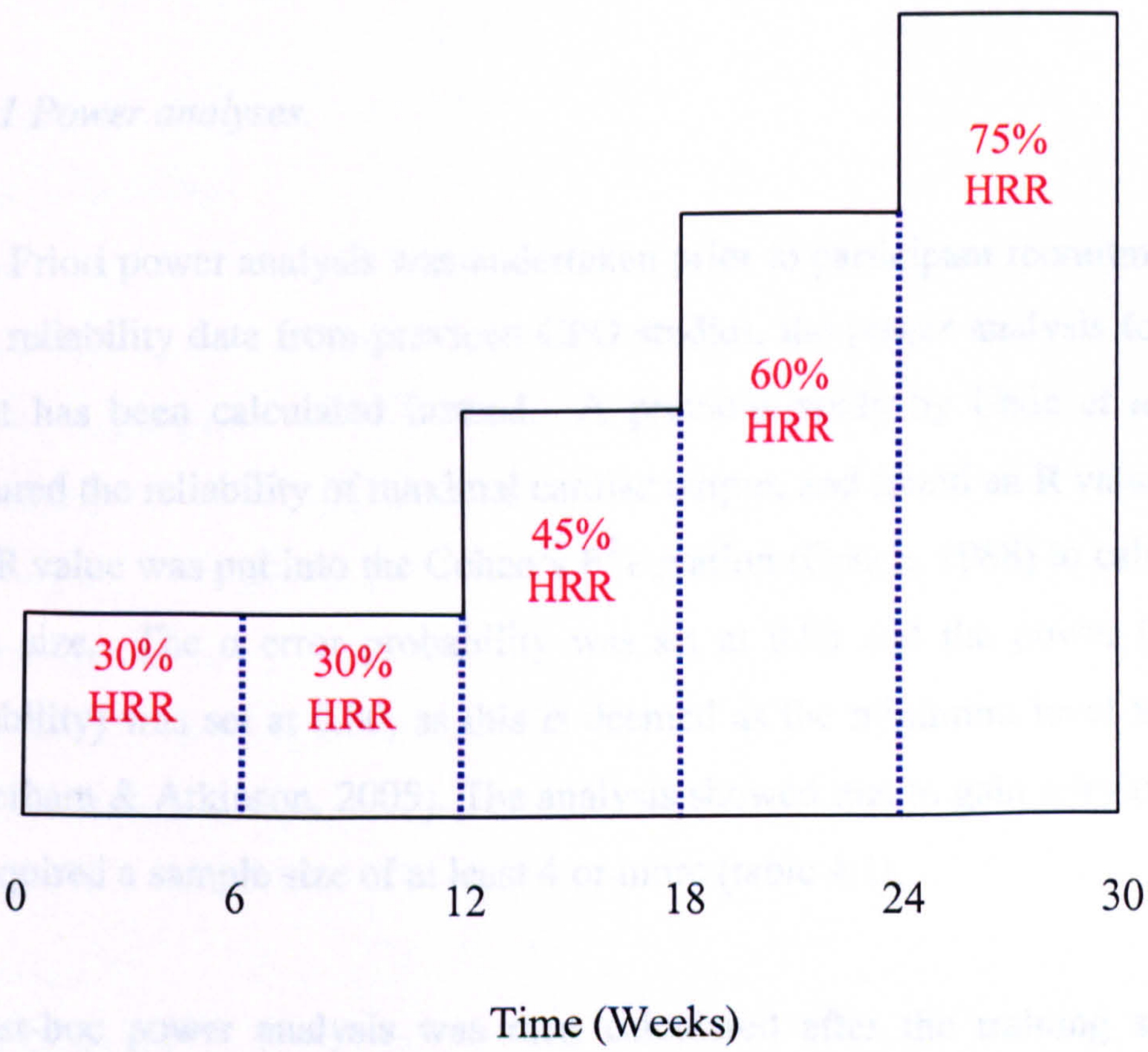


Figure 4.2: Step-wise increases in exercise intensity over 30 weeks of exercise training.
(HRR = heart rate reserve).

For allometric scaled data to lean body mass, regression analysis was performed to ascertain if male and female data could be selected together. If there were no significant differences then the data were merged. Regression analysis was then performed at each time point to generate an unstandardised **b** coefficient value of the log of the physiological variable. If each **b** value was within the standard error range, then the **b** values were averaged to gain one **b** exponent to put into an allometric scaling equation ($y=ax^bE$; Batterham & George, 1998).

4.3.5.1 Power analyses.

An A Priori power analysis was undertaken prior to participant recruitment. Due to no reliability data from previous CPO studies, the power analysis for cardiac output has been calculated instead. A previous study by Cade *et al.* (2004) measured the reliability of maximal cardiac output, and found an R value of 0.88. This R value was put into the Cohen's F^2 equation (Cohen, 1988) to calculate the effect size. The α error probability was set at 0.05 and the power (1-β error probability) was set at 0.80, as this is deemed as the minimum level acceptable (Batterham & Atkinson, 2005). The analysis showed that to gain relevant results, we required a sample size of at least 4 or more (table 4.1).

A post-hoc power analysis was then calculated after the training study was completed. Our results produced an R value for maximal cardiac output of 0.66. This R value was then put into Cohen's F^2 equation (Cohen, 1988) to calculate the effect size F. Total sample size equalled 19, with 2 groups (men and women) and 5 repetitions (0, 12, 18, 24 and 30 weeks). The α error probability was set at 0.05. Using these results, power (1-β error probability) was calculated at 1.0, with a critical F value of 2.50 (table 4.1). This suggests that our participant numbers were sufficient to gain meaningful statistical power.

Table 4.1: A Priori and Post-Hoc power analysis for the 30 week training study.

	A Priori	**Post-Hoc**
Effect size F	1.8297	0.8686
α error probability	0.05	0.05
Power (1-β error probability)	0.80	1.0
Number of groups	2	2
Repetitions	5	5
Correlation among repeated measures	0.5	0.5
Nonsphericity correlation	1	1
Noncentrally parameter λ	133.913	143.333
Critical F	3.838	2.507
Numerator df	4	4
Denominator df	8	68
Total sample size	4	19
Statistics test	ANOVA repeated measures within factors	ANOVA repeated measures within factors
Test family	F value	F value

4.4. Results.

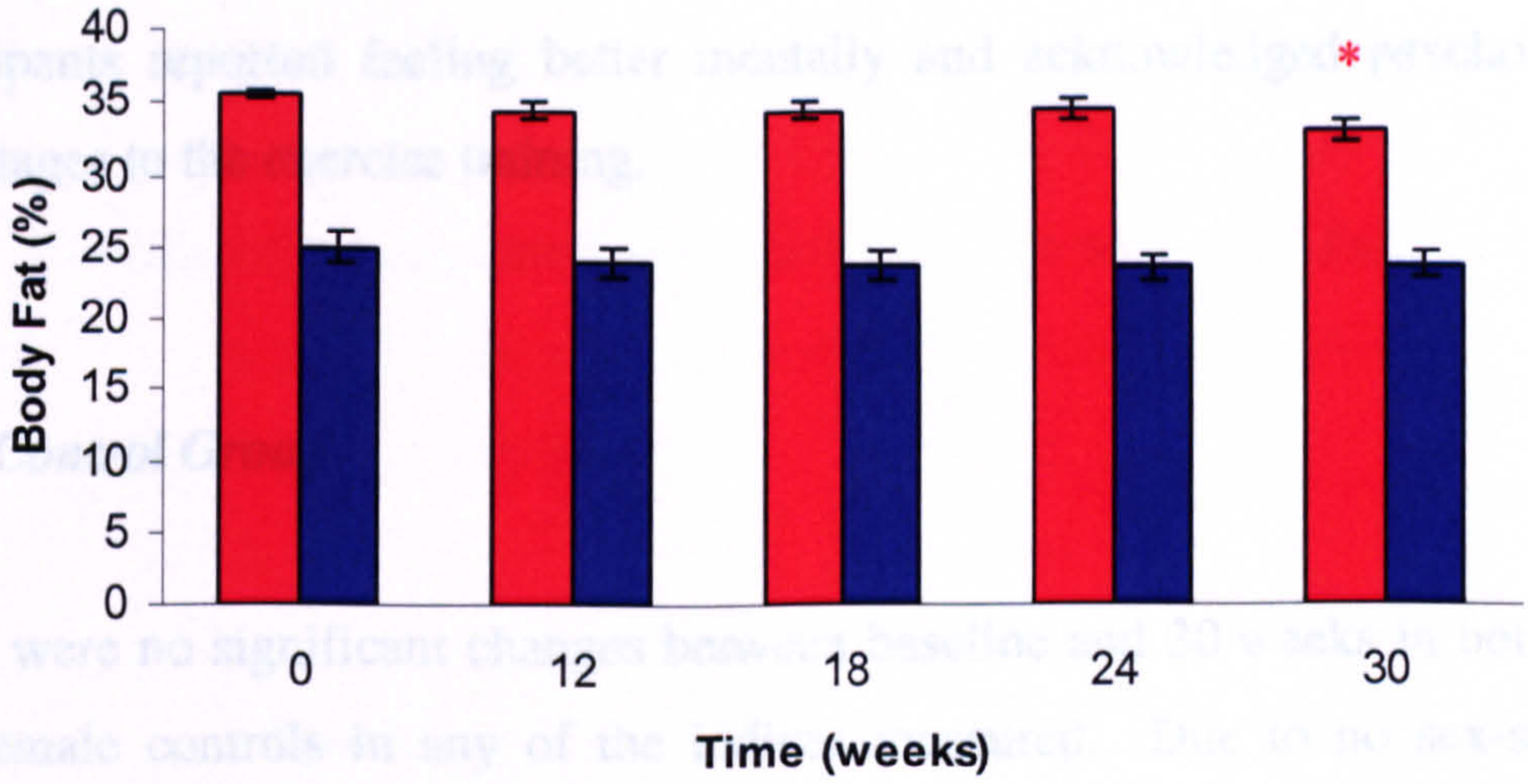
By the end of the 30 weeks of training, 15 men and 4 women had withdrawn from the exercise programme. This was due to the low exercise intensity at the beginning of the programme (contributing to 40 % of the withdrawal rate), aversion of testing procedures, musculoskeletal problems, family issues, and one woman was found to have underlying heart problems during the 3 month exercise stress test.

Eleven health-screened sedentary men (age 60 ± 1 years, body mass 83.5 ± 3.6 kg, body fat 24 ± 2 % and a mean $\dot{V}O_{2\,max}$ of 30.3 ± 0.6 ml.kg.min^{-1}) and 8 women (age 58 ± 1 years, body mass 72.6 ± 3.3 kg, body fat 36 ± 1 % and a mean $\dot{V}O_{2\,max}$ of 23.7 ± 0.6 ml.kg.min^{-1}) completed the training programme, after giving their informed consent to the ethically approved procedures. The final 8 women and 11 men completed the study with an adherence rate of 99 % out of 150 training sessions. Although the exercise training participants acted as their own internal controls, a non-exercising control group was formed. This control group consisted of 6 men (age 59 ± 3 years, body mass 90.6 ± 6.5 kg, $\dot{V}O_{2\,max}$ 35.0 ± 4.0 ml.kg.min^{-1}) and only 2 women (age 60 ± 5 years, body mass 64 ± 2.5 kg, $\dot{V}O_{2\,max}$ of 25.7 ± 1.9 ml.kg.min^{-1}), conforming to the strict selection criteria, and were tested at baseline and 30 weeks. These controls confirmed that no significant changes in any physiological variables occurred over the 30 week period.

4.4.1 The effects of training on body composition.

All participants underwent a DEXA scan at each testing time point to detect changes in body composition with endurance training (Figure 4.3A). Women significantly decreased (P = 0.023) body fat % from 35 % to 33 %. Men also demonstrated a decrease from 25 % to 23 % body fat, but over 30 weeks exercise programme, this was not significantly (P = 0.069) different. As expected, there

A



B

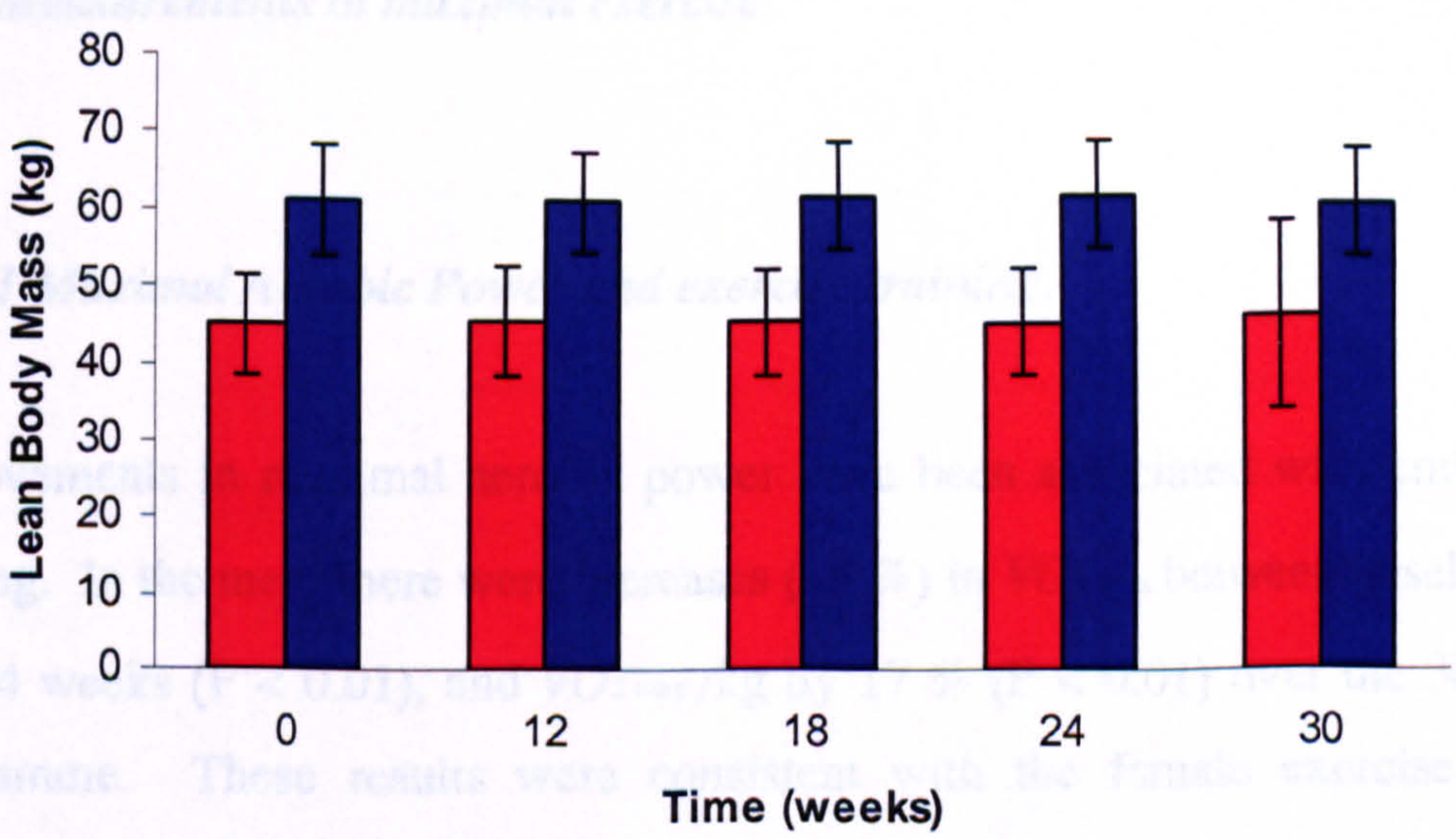


Figure 4.3: Changes in body composition with 30 weeks of exercise training. Values are mean ± SE for **A.** percentage body fat in men (■, n=11) and women (■, n=8) and **B.** lean body mass for the same men (■) and women (■).

was no significant changes (males, P = 0.937; females, P = 0.507) in lean body mass in either sexes (Figure 4.3B). There were also no differences between the control group and the exercise group with either of the described indices over the 30 weeks of exercise training. However, after 6 weeks of exercise training, all participants reported feeling better mentally and acknowledged psychological advantages to the exercise training.

4.4.2 Control Group.

There were no significant changes between baseline and 30 weeks in both male and female controls in any of the indices measured. Due to no sex-specific differences within the control group of the indices measured, data is shown as whole group means (table 4.2).

4.4.3 Measurements at maximal exercise.

4.4.3.1 Maximal Aerobic Power and exercise training.

Improvements in maximal aerobic power have been associated with endurance training. In the men, there were increases (15 %) in $\dot{V}O_{2\,max}$ between baseline, 18 and 24 weeks ($P < 0.01$), and $\dot{V}O_{2\,max}$/kg by 17 % ($P < 0.01$) over the 30 week programme. These results were consistent with the female exercise group showing an increase of 15 % in $\dot{V}O_{2\,max}$ ($P < 0.02$) and 19 % $\dot{V}O_{2\,max}$/kg ($P = 0.000$) between baseline and 30 weeks training (Figure 4.4). Indeed, a progressive increase in $\dot{V}O_{2\,max}$ was observed at all time points, but these only became significant ($P = 0.018$; $P = 0.025$) after 18 weeks in men, and women. These results suggest that 12 weeks of exercise training at a low intensity does not sufficiently improve $\dot{V}O_{2\,max}$, compared to a longer exercise duration and higher intensity. Maximal oxygen consumption was higher in men compared to women throughout the exercise training. However, when both men and women were allometrically scaled to lean body mass, it was no longer significantly

Table 4.2: Data for non-exercised control men and women at baseline and 30 weeks.

Variables	Baseline	30 weeks	P value
At Exercise			
$\dot{V}O_{2\,max}$	32.7 ± 7.3	31.6 ± 7.1	0.779
SBP max	199 ± 28	203 ± 21	0.316
DBP max	74 ± 12	73 ± 10	0.450
MAP max	125 ± 17	126 ± 12	0.557
$\dot{Q}$ max	20.8 ± 4.7	20.5 ± 4.8	0.710
CPO max	5.78 ± 1.43	5.72 ± 1.16	0.974
At Rest			
SBP rest	135 ± 20	131 ± 13	0.091
DBP rest	83 ± 5	83 ± 5	0.128
MAP rest	104 ± 10	103 ± 8	0.088
$\dot{Q}$ rest	4.1 ± 0.9	4.2 ± 0.9	0.826
CPO rest	0.94 ± 0.19	0.95 ± 0.23	0.519

Values are mean ± SE for both men (n=6) and women (n=2).

different (P = 0.113) between sexes, but showed similar improvement with the exercise training (Figure 4.4). Maximal a-vO_2 difference increased in men by 9 % with training, but was not significantly different from baseline (P = 0.164). A similar increase in a-vO_2 difference (10 %) was found in women, but this was significantly different from baseline (P < 0.002; Figure 4.5). Exercise duration was also increased by 15 % (P = 0.000) from 10.59 ± 0.36 minutes at baseline to 12.45 ± 0.27 minutes in men and by 12 % (P = 0.033) from 9.08 ± 0.6 minutes at baseline to 10.36 ± 0.43 minutes in women after 30 weeks training, during the $\dot{V}O_{2\,max}$ exercise test (Figure 4.6).

Increases in $\dot{V}O_{2\,max}$ have been attributed to an increase in a-vO_2 difference in women, but also an increase in maximal cardiac output has been attributed in men with exercise training. This was true whether $\dot{V}O_{2\,max}$ was scaled for LBM or not. Therefore, to ascertain sex-specific differences with exercise training, CPO was measured incorporating both cardiac output ($\dot{Q}$) and mean arterial pressure (MAP).

4.4.3.2 Cardiac function during maximal exercise.

4.4.3.2.1 Maximal Cardiac output ($\dot{Q}$).

The female exercise group showed no improvement in maximal cardiac output (P = 0.119). Similarly, maximal cardiac output did not change (P = 0.182) with exercise training in the men. Consistent with these findings, the determination of maximal stroke volume and maximal heart rate showed no changes with endurance exercise in either sex (Figure 4.7).

4.4.3.2.2 Maximal blood pressure.

Peak diastolic blood pressure decreased by 9 % (P < 0.02) between baseline and 30 weeks in the male training group (Figure 4.8). Therefore, there was a

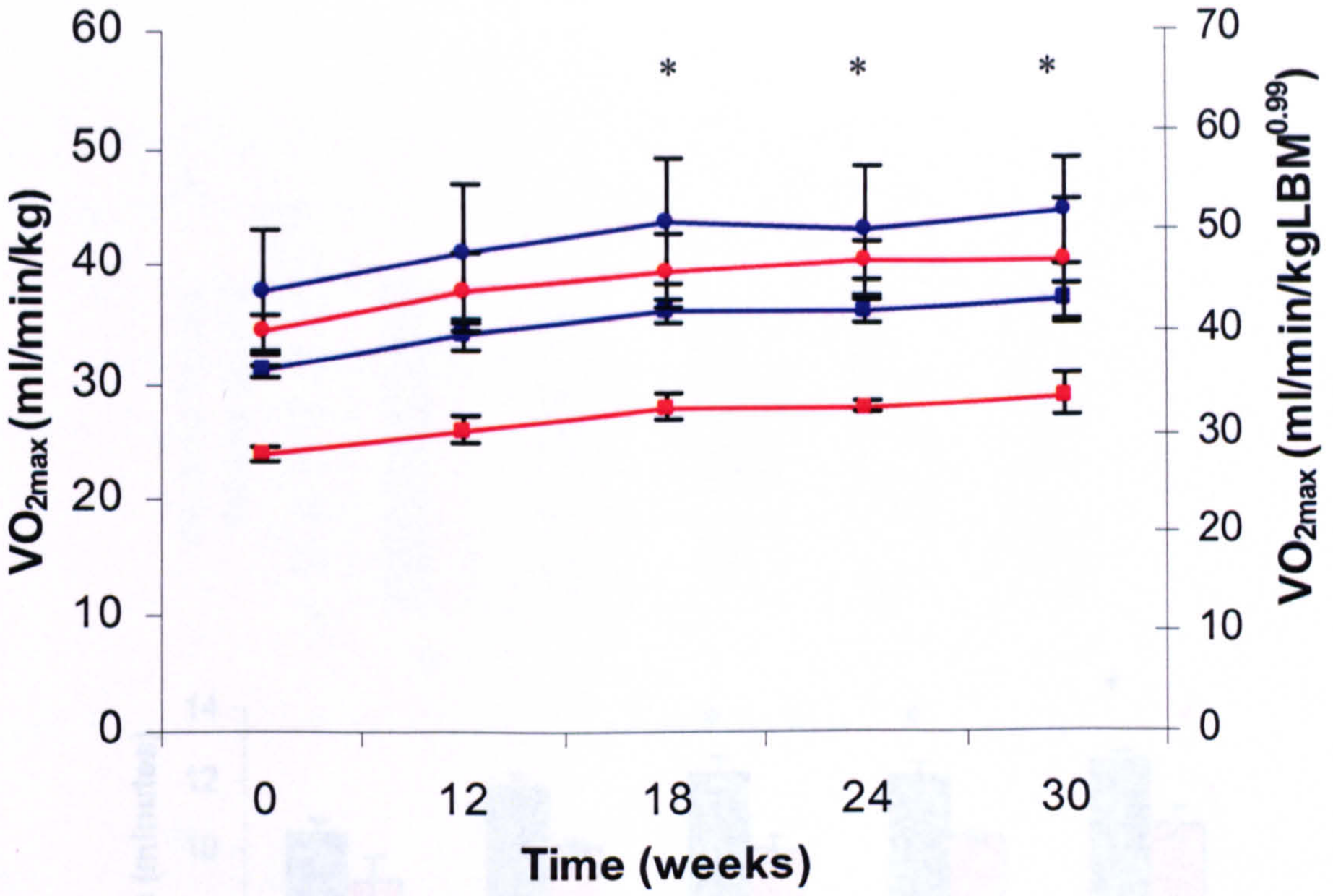


Figure 4.4: Training-induced changes in $\dot{V}O_{2\,max}$.

Data were means ± SE for men (▬) and women (▬), before (■), and after allometrically scaling to LBM (●), over 30 weeks of exercise training.

*P<0.05 statistically significant from pre-exercise values in both sexes.

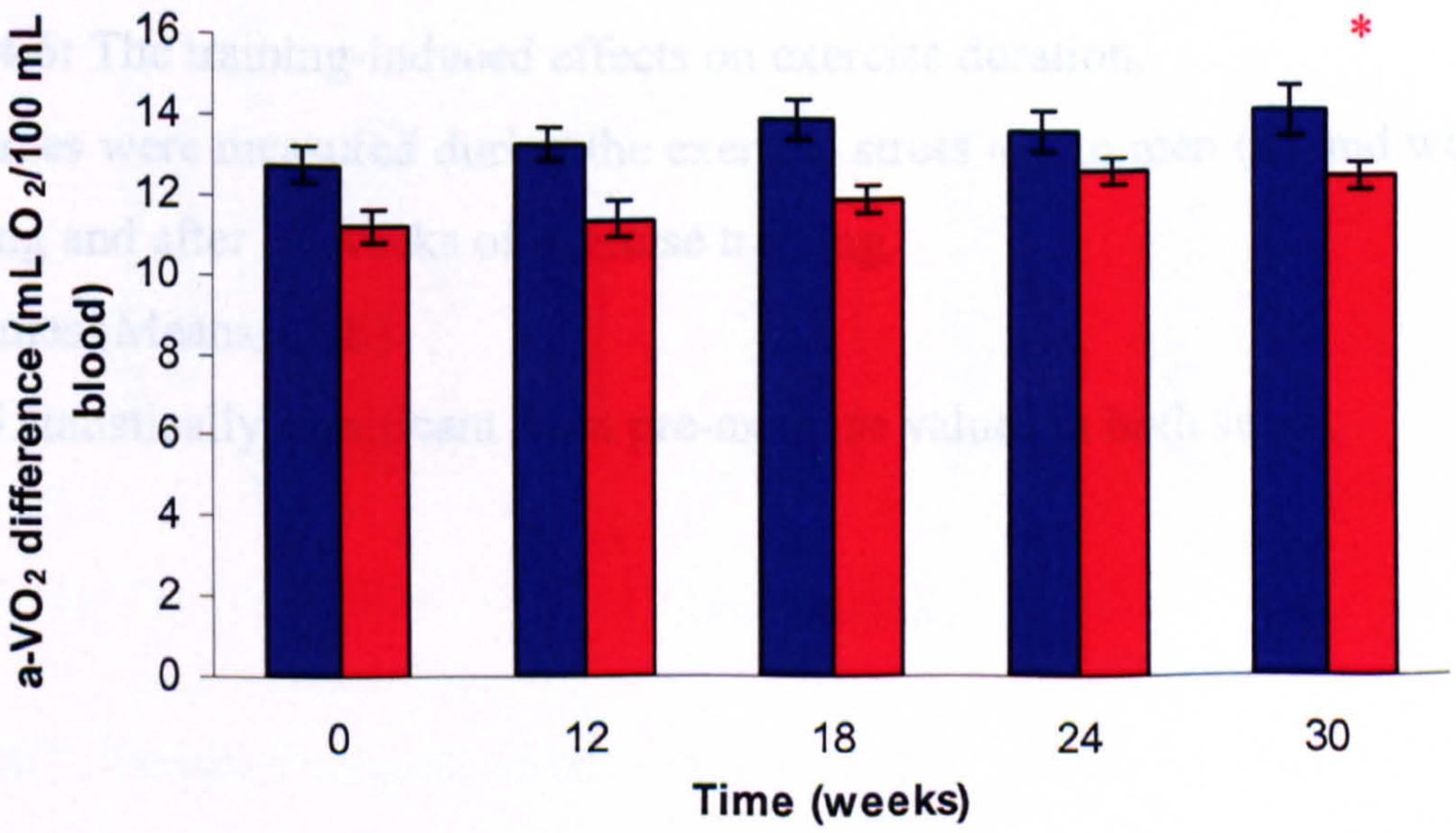


Figure 4.5: Training-induced changes in a-vO_2 differences.

Data were means ± SE for men (■), and women (■), over 30 weeks of exercise training.

*P<0.05 statistically significant from pre-exercise values in both sexes.

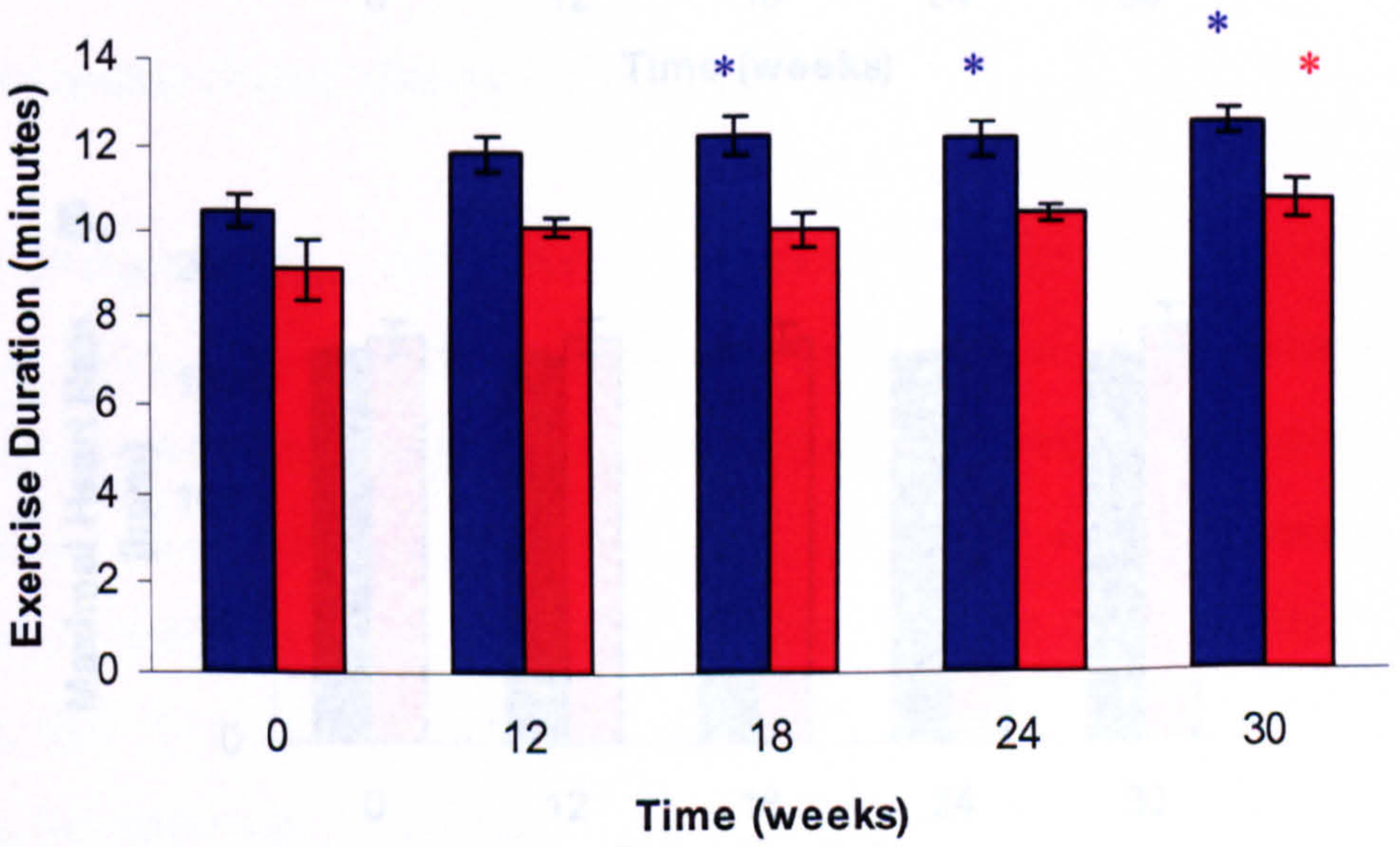


Figure 4.6: The training-induced effects on exercise duration.

These times were measured during the exercise stress test in men (■) and women (■) during and after 30 weeks of exercise training.

These times (Means ± SE).

*P<0.05 statistically significant from pre-exercise values in both sexes.

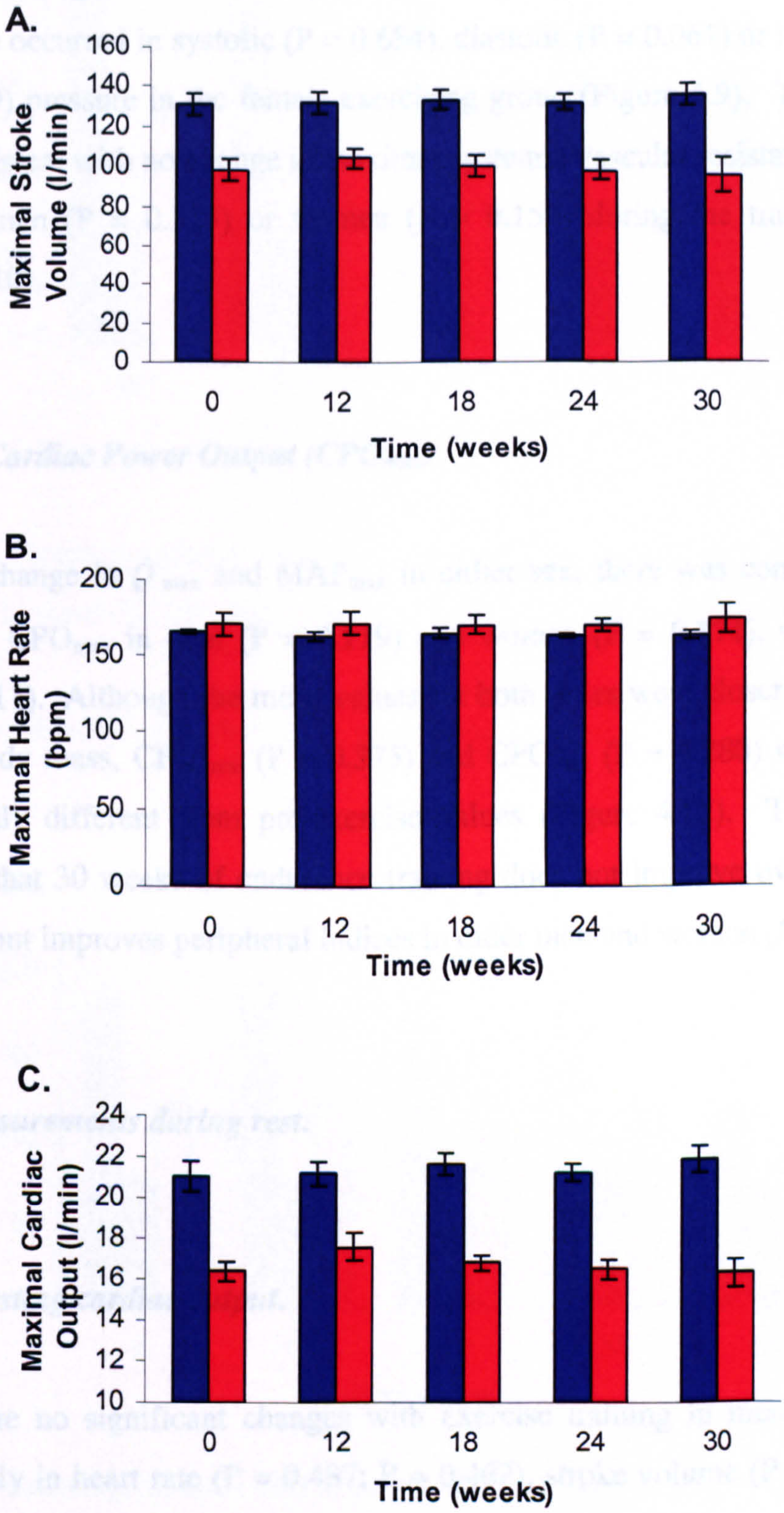


Figure 4.7: Training-induced effects on maximal stroke volume (A), heart rate (B) and cardiac output (C).

Data are means ± SE for both men (■) and women (■).

decrease in MAP_{max} (5 %, P = 0.210) between baseline and 12 weeks, but this was not sustained throughout the entire programme, mainly due to non significant changes in maximal systolic blood pressure (P = 0.229; Figure 4.8). No change occurred in systolic (P = 0.654), diastolic (P = 0.061) or mean arterial (P = 0.809) pressure in the female exercising group (Figure 4.9). These results were consistent with no change in maximal systemic vascular resistance (SVR_{max}) in either men (P = 0.315) or women (P = 0.159) during the training period (Figure 4.10).

4.4.3.2.3 Cardiac Power Output (CPO_{max}).

With no change in $\dot{Q}_{max}$ and MAP_{max} in either sex, there was conclusively no change in CPO_{max} in men (P = 0.119) and women (P = 0.174), with training (Figure 4.11). Although the mean values for both sexes were closer with scaling to lean body mass, CPO_{rest} (P = 0.375) and CPO_{max} (P = 0.280) were still not significantly different from pre-exercise values (Figure 4.12). This provides evidence that 30 weeks of endurance training does not improve overall cardiac function, but improves peripheral indices in older men and women (Figure 4.5).

4.4.4 Measurements during rest.

4.4.4.1 Resting cardiac output.

There were no significant changes with exercise training in men and women respectively in heart rate (P = 0.487; P = 0.462), stroke volume (P = 0.317; P = 0.896) and hence cardiac output at rest (P = 0.380, P = 0.590; Figure 4.13).

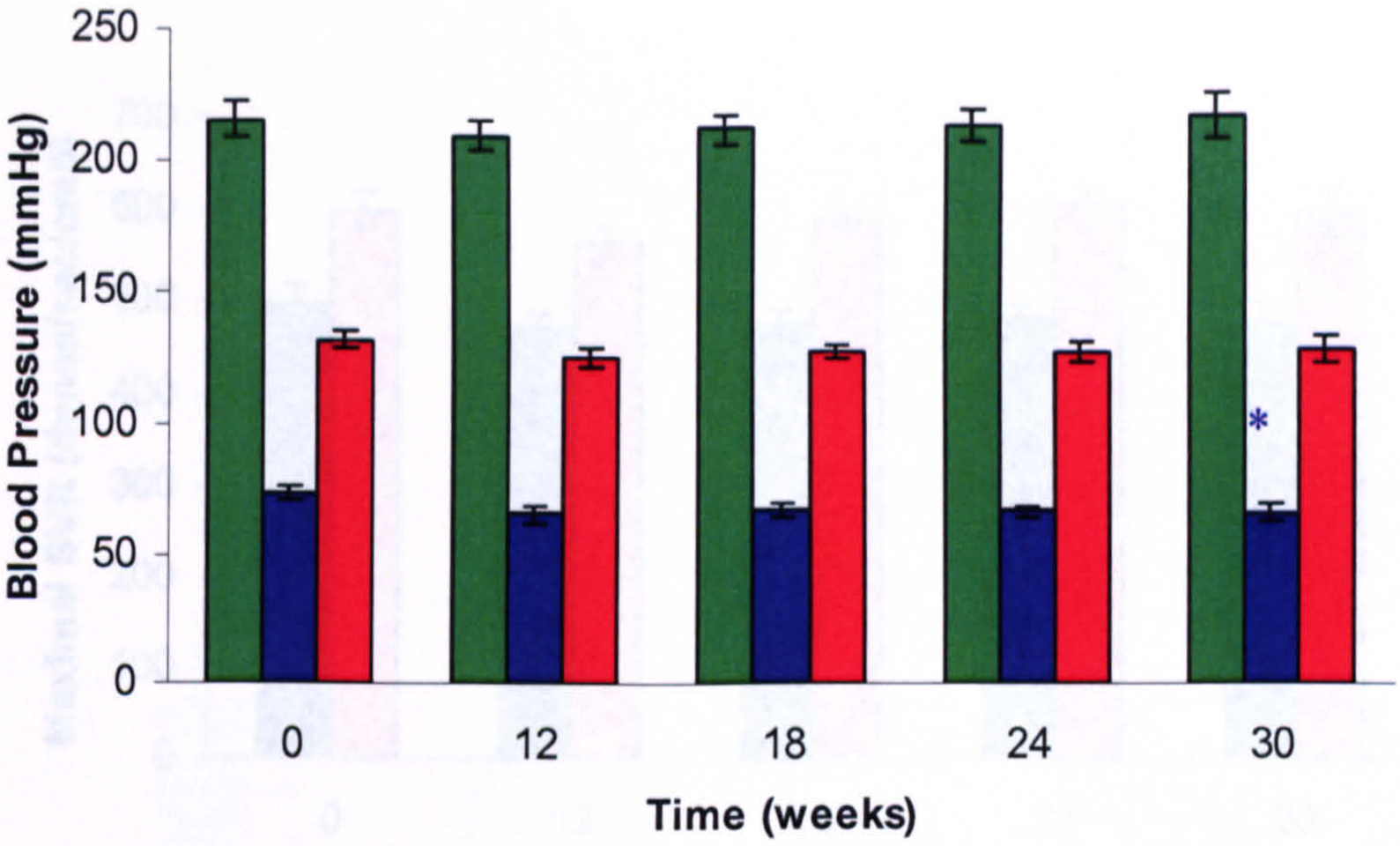


Figure 4.8: Training-induced changes in maximal blood pressure in men.

Systolic (■), diastolic (■) and mean (■) arterial blood pressure are given as means ± SE.

*P<0.05 statistically significant from pre-exercise values.

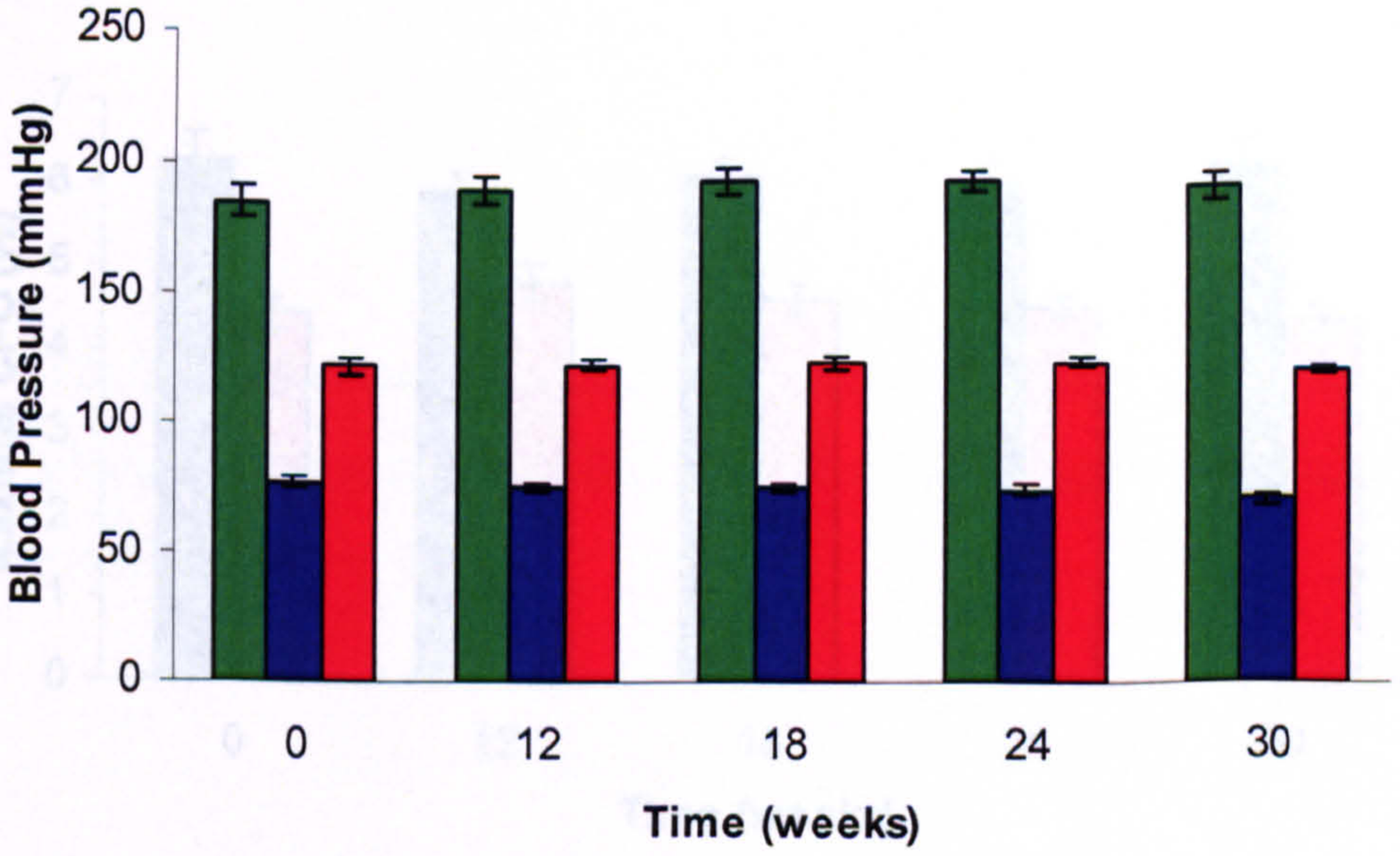


Figure 4.9: Training-induced effects on maximal blood pressure in women.

Systolic (■), diastolic (■) and mean (■) arterial blood pressure are presented as means ± SE.

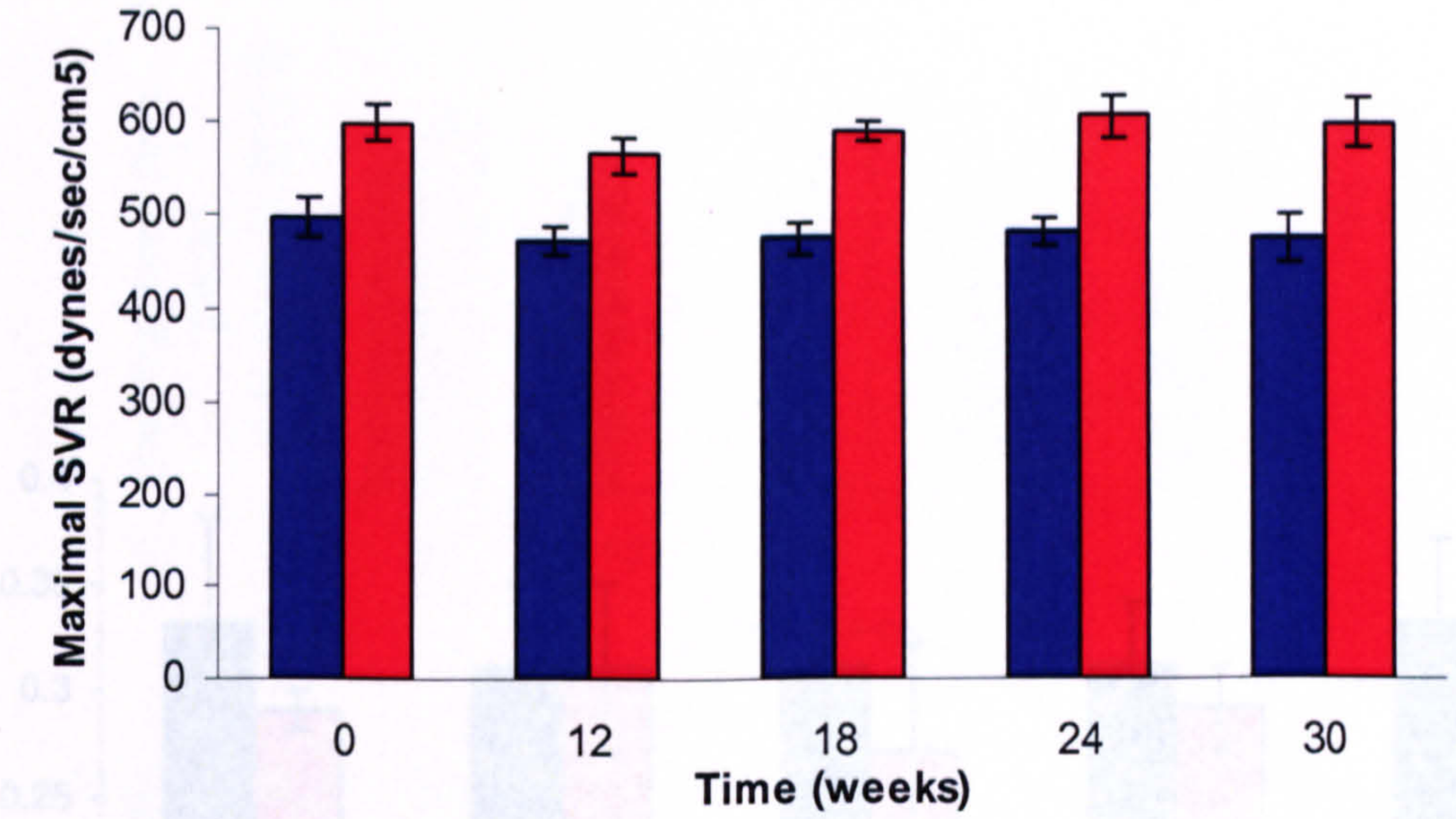


Figure 4.10: Training induced effects on maximal SVR.

Values shown as means ± SE for both men (n=11, ■) and women (n=8, ■).

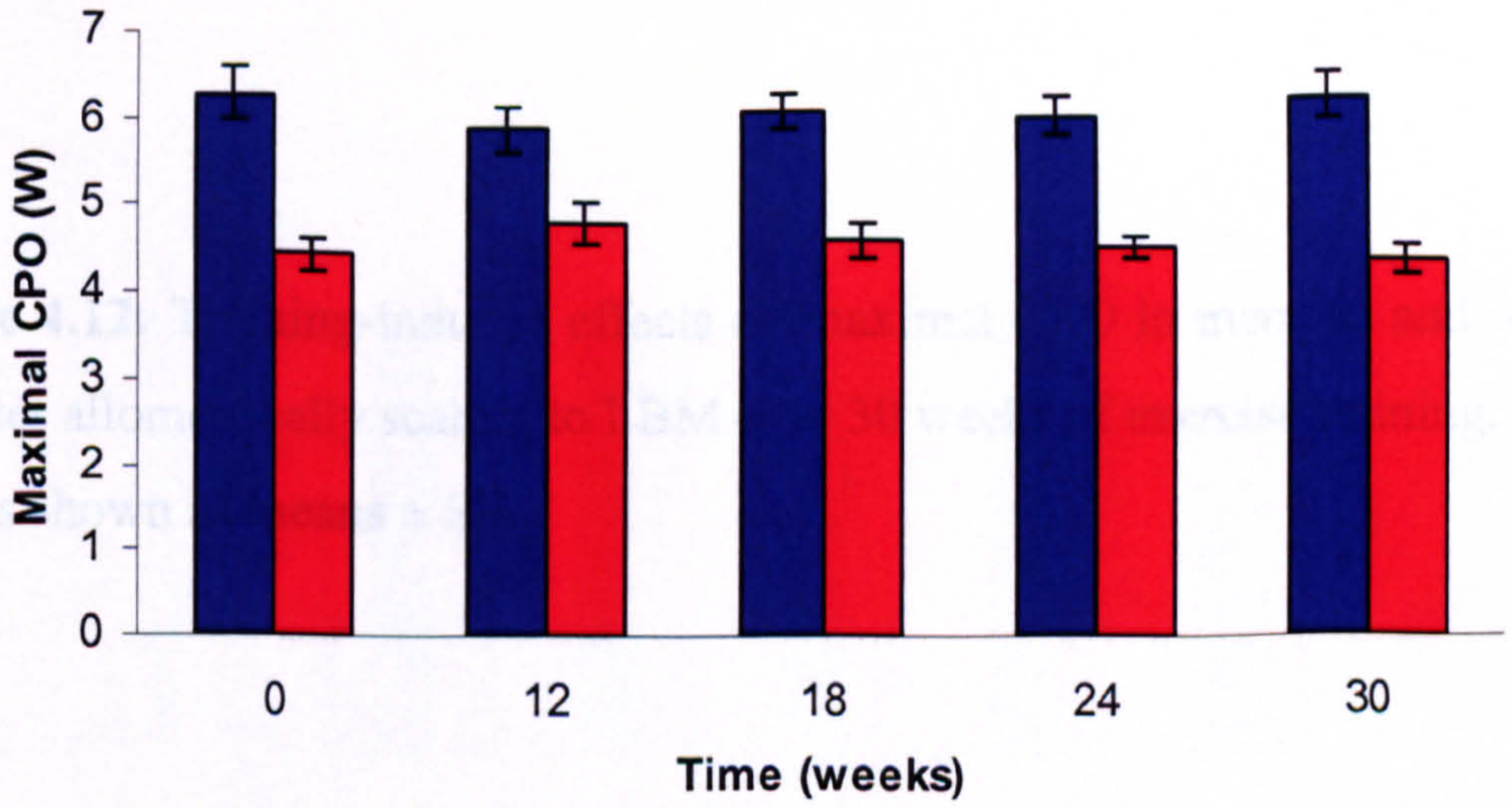


Figure 4.11: Training induced effects on Cardiac Power Output.

Values shown as means ± SE for both men (n=11, ■) and women (n=8, ■).

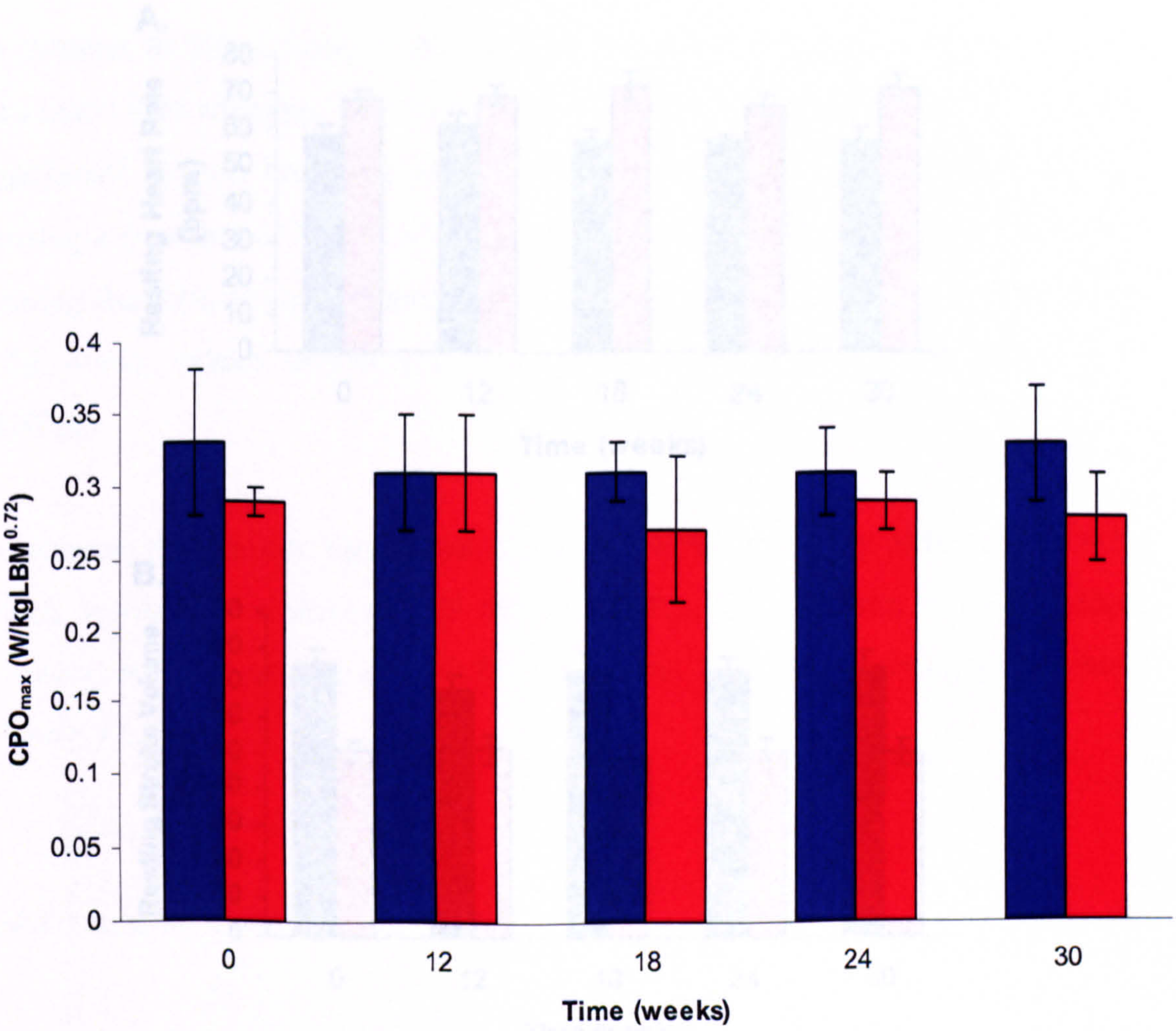


Figure 4.12: Training-induced effects on maximal CPO in men (■) and women (■) after allometrically scaling to LBM over 30 weeks of exercise training. Values shown as means ± SE.

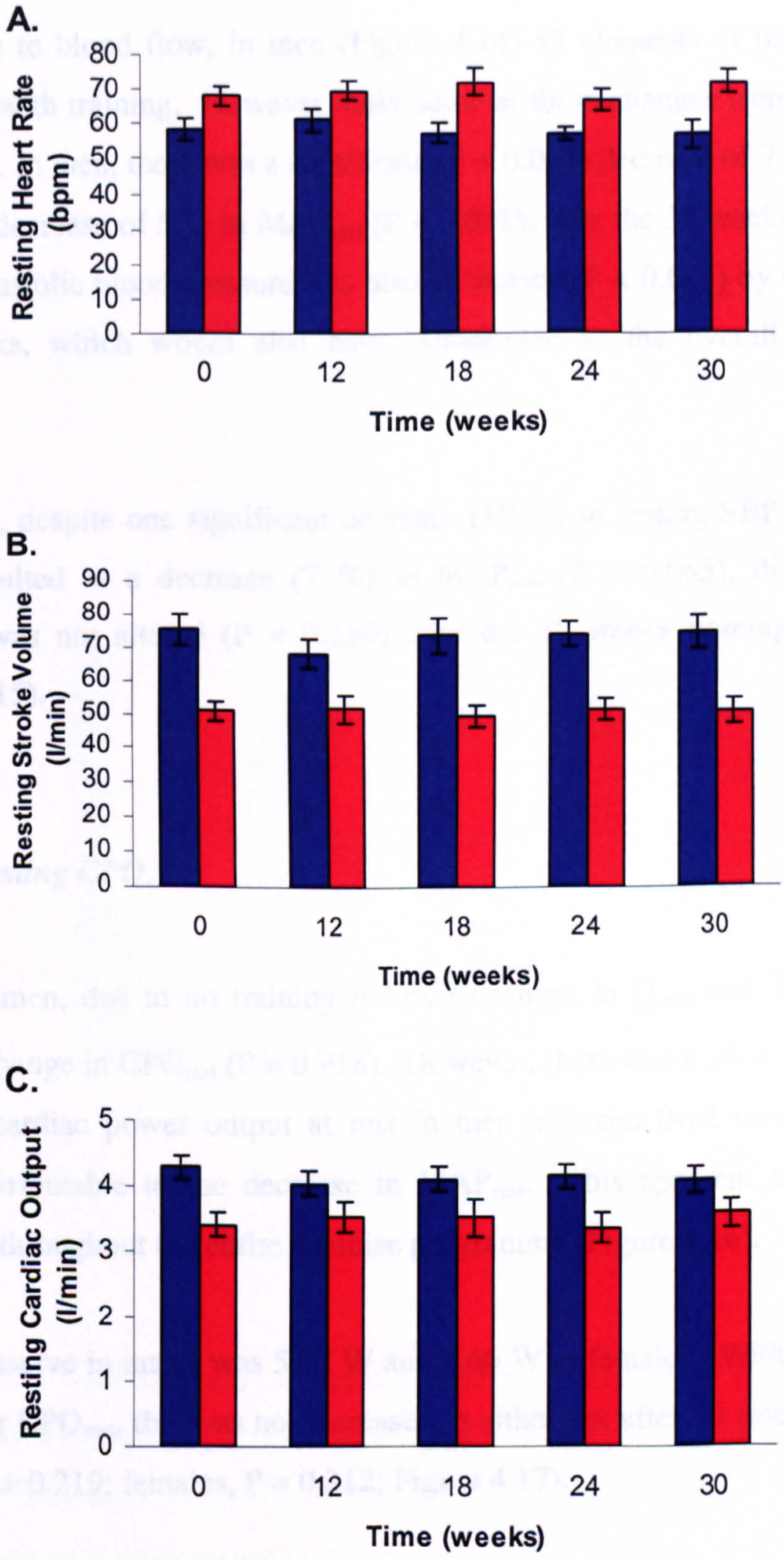


Figure 4.13: Training-induced effects on resting heart rate, resting stroke volume and resting cardiac output.

Means ± SE values for both men (■) and women (■).

4.4.4.2 Resting blood pressure.

In contrast to blood flow, in men (Figure 4.14) all elements of blood pressure decreased with training. However, only some of these changes were statistically significant. In men, there was a significant ($P < 0.001$) decrease of 7 % in SBP_{rest}, causing a decrease of 5 % in MAP_{rest} ($P < 0.001$), over the 30 weeks of exercise. Resting diastolic blood pressure was also decreased ($P < 0.001$) by 6 % between 0-12 weeks, which would also have contributed to the overall decrease in MAP_{rest}.

In women, despite one significant decrease (10 %) in resting SBP ($P = 0.042$), which resulted in a decrease (7 %) in MAP_{rest} ($P = 0.045$), diastolic blood pressure was not altered ($P = 0.210$) over the 30 weeks training programme (Figure 4.15).

4.4.4.3 Resting CPO.

In the women, due to no training induced changes in $\dot{Q}_{rest}$ and MAP_{rest}, there were no change in CPO_{rest} ($P = 0.918$). However, there was a 10 % decrease ($P < 0.05$) in cardiac power output at rest in men between 0-12 weeks, with this mainly attributable to the decrease in MAP_{rest}. This apparent trend was not sustained throughout the entire exercise programme (Figure 4.16).

Cardiac reserve in males was 5.27 W and 3.66 W in females. With no change in CPO_{max} or CPO_{rest}, this was not increased in either sex after 30 weeks of training (males, $P = 0.219$; females, $P = 0.212$; Figure 4.17).

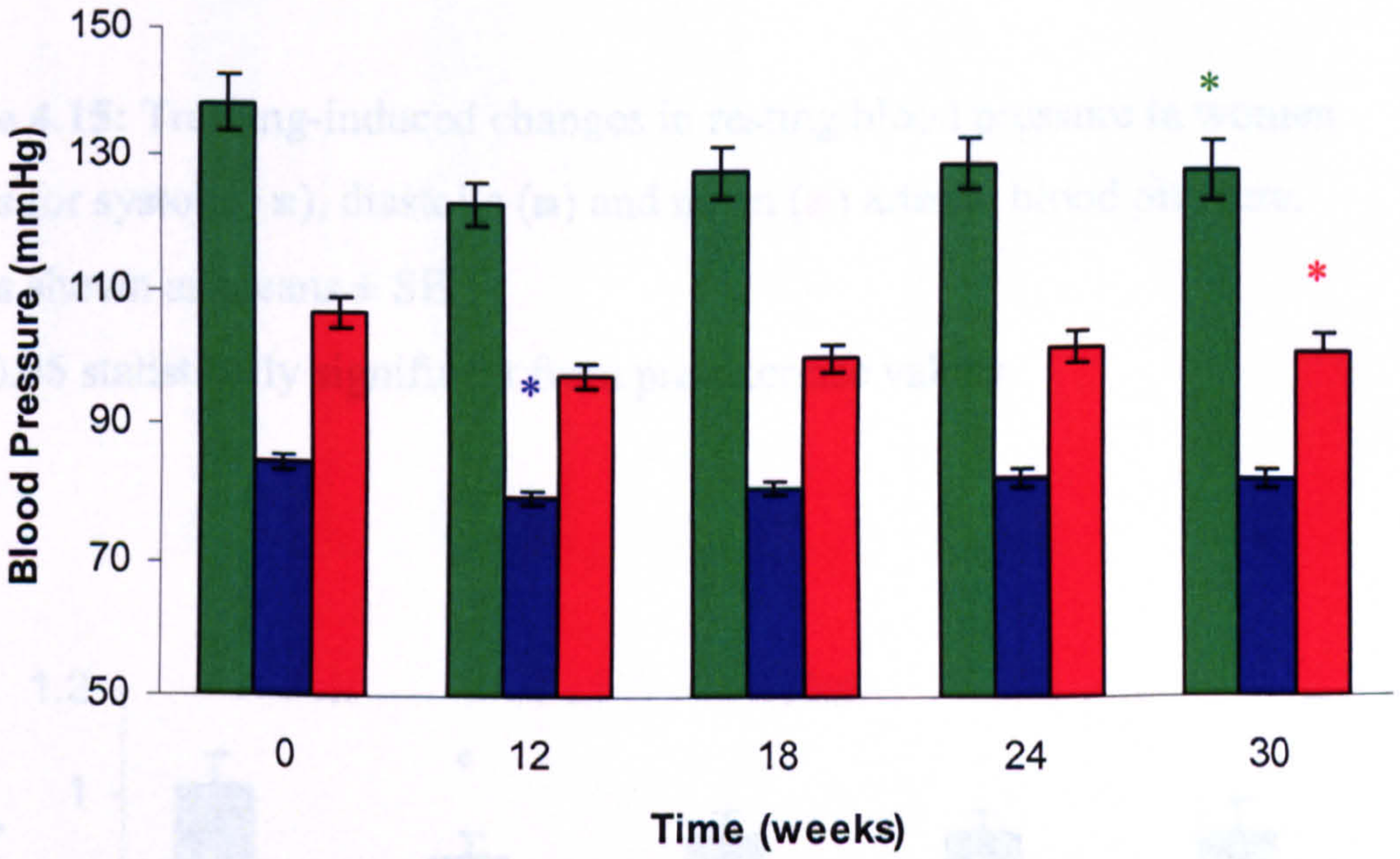


Figure 4.14: Training-induced changes in resting blood pressure in men. Values for systolic (■), diastolic (■) and mean arterial (■) blood pressure. Values shown as means ± SE.

*P<0.05 statistically significant from pre-exercise values.

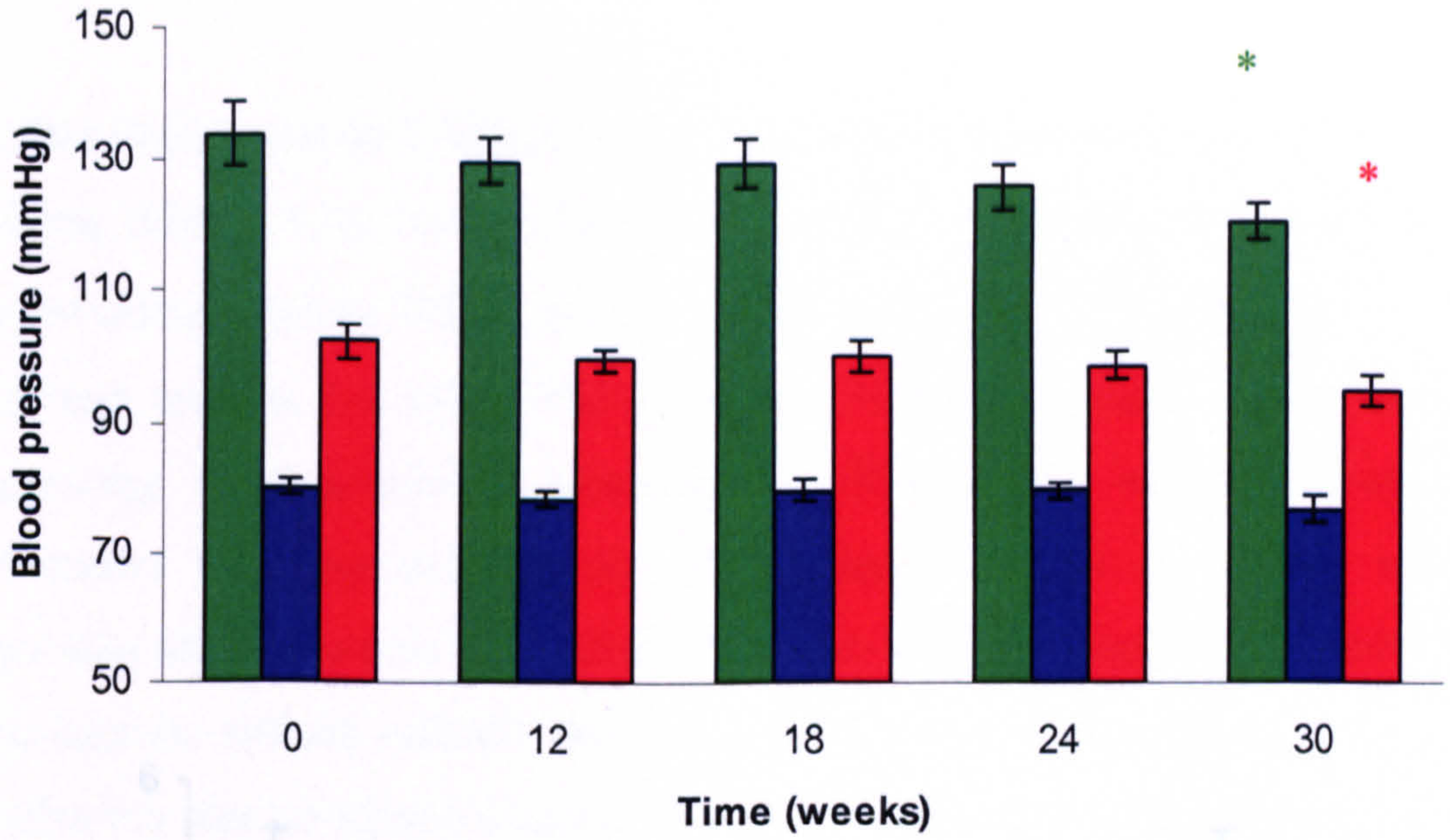


Figure 4.15: Training-induced changes in resting blood pressure in women.
Values for systolic (■), diastolic (■) and mean (■) arterial blood pressure.
Values shown as means ± SE.
*P < 0.05 statistically significant from pre-exercise values.

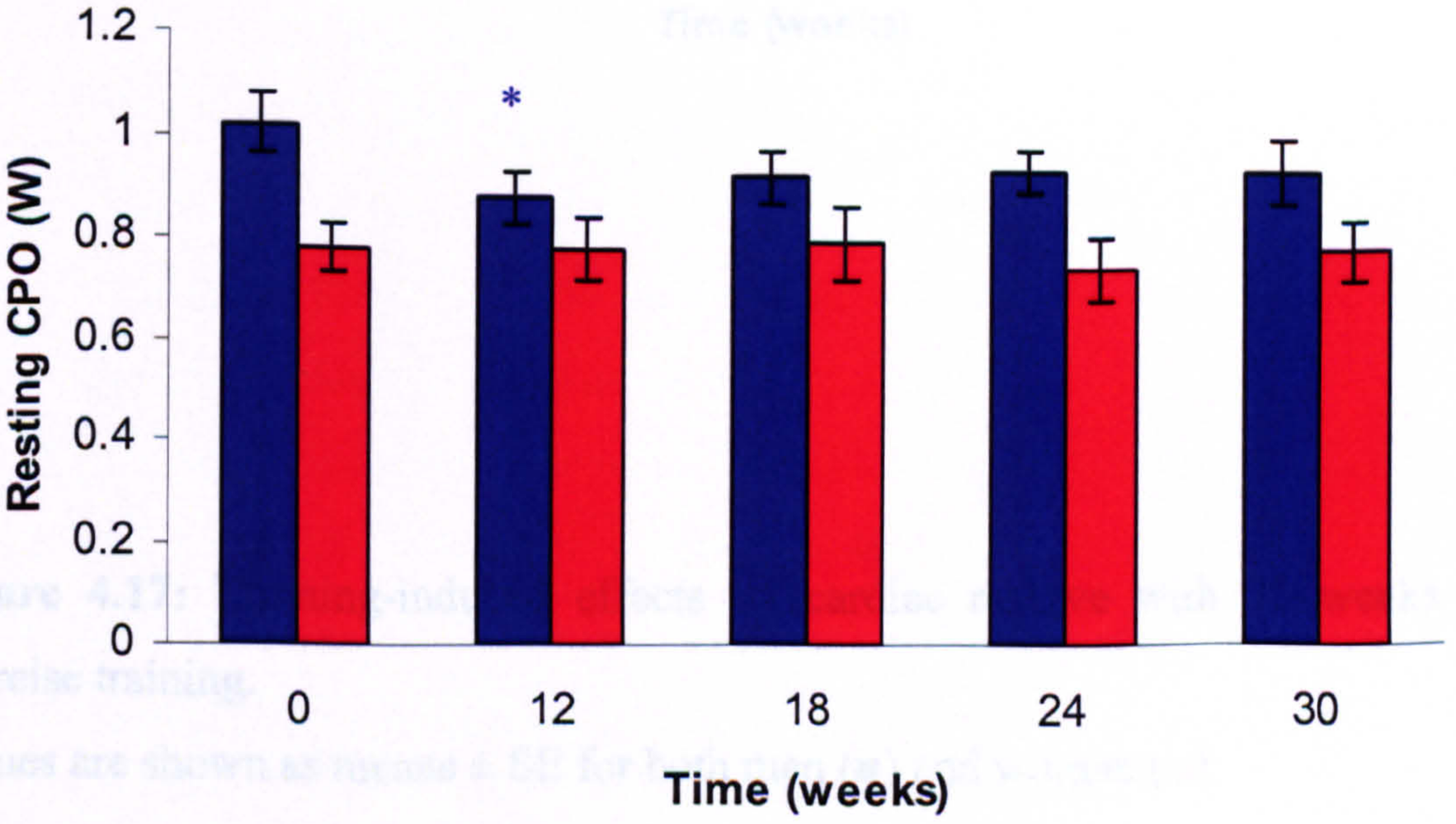


Figure 4.16: Training-induced changes in resting CPO.
Values shown as means ± SE for men (■) and women (■).
*P < 0.05 statistically significant from pre-exercise values.

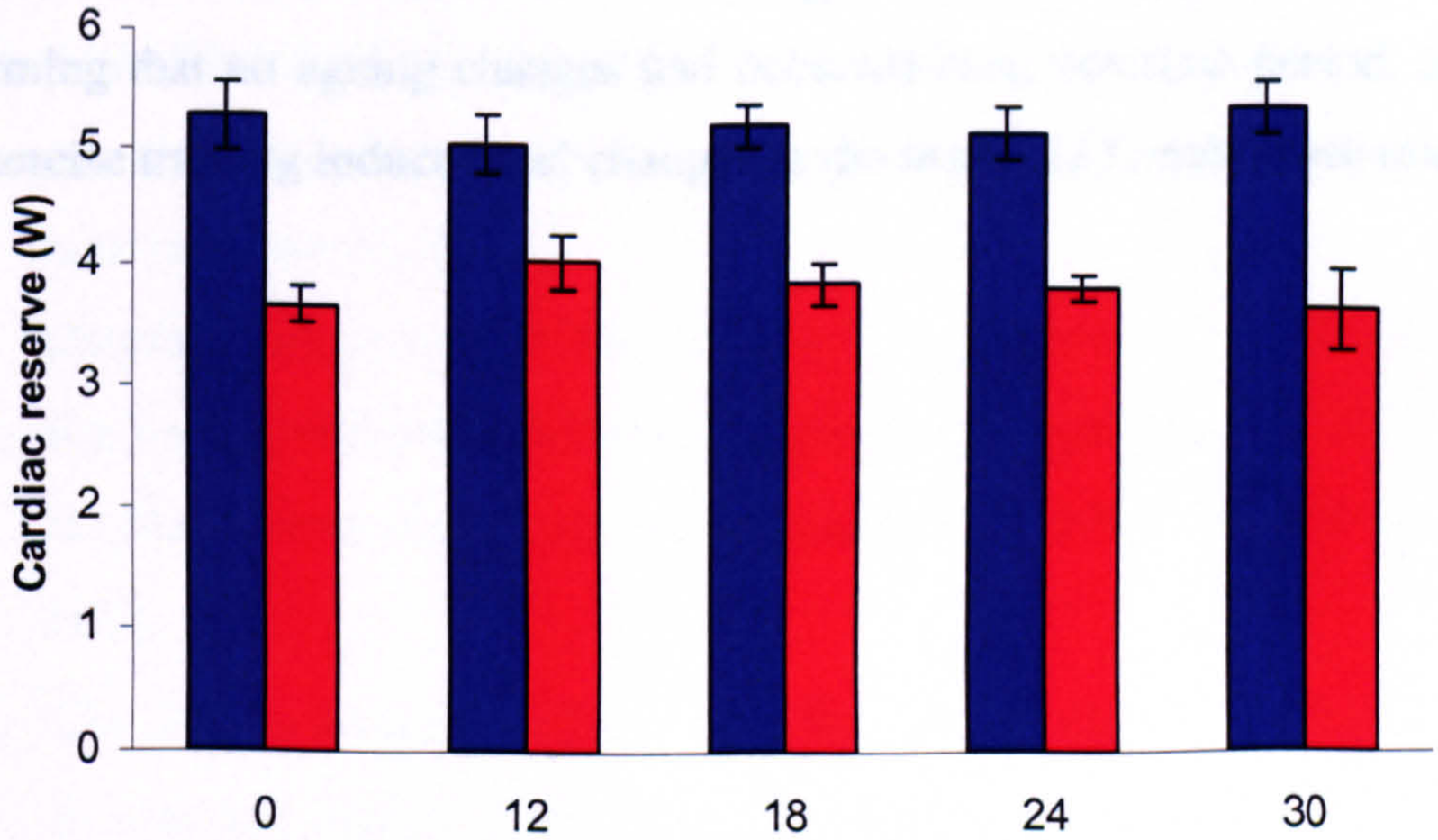


Figure 4.17: Training-induced effects on cardiac reserve with 30 weeks of exercise training.

Values are shown as means ± SE for both men (■) and women (■).

4.4.5 Summary.

Similar improvements in $\dot{V}O_{2\,max}$ in men and women were seen after 30 weeks of training (Figure 4.4), leading to improvements in VO_2 reserve capacity. The improvements seen in $\dot{V}O_{2\,max}$ were due to an increase in a-vO_2 difference in both men and women, but there was no change in central function with training, suggesting only peripheral adaptations. There were neither sex-specific differences nor improvements in cardiac power output or cardiac reserve capacities after 30 weeks of endurance exercise training (Figures 4.11 and 4.17). Non-exercise trained controls were unchanged between baseline and 30 weeks, confirming that no ageing changes had occurred over this time period, and that the exercise training induced real changes in the male and female participants.

4.5. Discussion.

Although $\dot{V}O_{2\,max}$ has been found to increase after many exercise programmes, no one has previously measured overall cardiac function (CPO). Instead, they have measured changes in cardiac output, but this is only one of the two functions of the heart, i.e. flow and pressure generation.

4.5.1 Key findings.

1. After 30 weeks of exercise training, involving progressive increases in exercise intensity, there were increases in aerobic capacities ($\dot{V}O_{2\,max}$) in both men and women.
2. Despite this outcome, overall cardiac function was not affected in either sex by the endurance training programme, suggesting that CPO_{max} and cardiac reserve are not altered with exercise intensities lower than 75 % HRR.
3. The improvements in $\dot{V}O_{2\,max}$ were due to increases in a-vO_2 differences as maximal cardiac output was not affected with training in either sex. Since lean body masses were also not altered, the improvement in a-vO_2 difference must be attributed to a greater oxygen extraction and utilisation by more oxidative muscles.
4. As such, there were no discernable sex-specific differences with 30 weeks of endurance exercise training.
5. No significant changes were found in any of the physiological variables measured in the sedentary, non-trained control groups (Table 4.2) over the 30 weeks of the study. These observations confirm that the results found were indicative of a training response and not an ageing process.

4.5.2 Control group.

The control group exhibited no alterations in $\dot{V}O_{2\,max}$, blood pressure or cardiac function, at both rest and maximal exercise, over the duration of 30 weeks.

Therefore, the effects observed were due to the response to 30 weeks exercise training, not in response to the ageing.

4.5.3 The effect of training on cardiovascular function when measured at rest.

Previous studies have found that at rest, a reduction in blood pressure occurs after exercise training (Perini *et al.*, 2002). The reduction of SBP_{rest} and DBP_{rest} in the males can be attributed to a decrease in total peripheral resistance (Perini *et al.*, 2002). With a lowering of both SBP_{rest} and DBP_{rest}, MAP_{rest} was also significantly reduced by 5 % over the 30 weeks training in men.

In contrast, DBP_{rest} was not significantly changed in women with training. In fact, females showed a consistently greater decrease of 9.4 % in SBP in women (Figure 4.15), compared to 5.4 % in men after 30 weeks training. These changes in arterial pressure show that the ageing process, with regards to arterial compliance, can be, at least partially, reversed with an aerobic exercise programme.

At rest, heart rate, stroke volume and thus cardiac output remained unchanged in both sexes post-training (Figure 4.13). A training-induced decrease in resting heart rate has been observed in young (Kanakis, 1982; Upton, 1984), but not in older people (Cunningham *et al.*, 1987; Meredith *et al.*, 1989; Schocken *et al.*, 1983), which is consistent with our findings. No other measurements of cardiac function at rest were affected by the 30-week training, which is largely consistent with existing findings within the literature (Carroll *et al.*, 2007; Marshall *et al.*, 2001; Perini *et al.*, 2002). Therefore, these results conclude that there are no advantageous health benefits in regards to resting variables, after training in older individuals.

4.5.4 The effect of exercise training on maximal cardiovascular function.

Blumenthal *et al.* (1991) found that 12 weeks of aerobic exercise improved $\dot{V}O_{2\,max}$ by 10-15 % in men, after starting at 50 % HRR and increasing the intensity with step-wise increments up to 70 % HRR after week 4. A further 12 weeks of exercise at 70 % HRR showed a modest additional improvement of 1-6 % in peak aerobic power, with an increase of 18 % from baseline after 14 months. These results are comparable to this present study (Figure 4.4) as similar significant increases in $\dot{V}O_{2\,max}$ were seen after 12 weeks (9 % in men, 14 % in women). Although there was an increase in $\dot{V}O_{2\,max}$ throughout the 30 weeks, the degree of improvement between each 6 week interval declined as the training progressed. Asikainen *et al.* (2002) studied the effects on older post-menopausal women with a walking programme for 24 weeks. Results showed that there was a dose-response relationship between $\dot{V}O_{2\,max}$ improvement and exercise intensity, with a notable increase in $\dot{V}O_{2\,max}$, even with a low dose of 45 % $\dot{V}O_{2\,max}$. However, it is interesting to note in our study, that the women improved their $\dot{V}O_{2\,max}$ quicker with a shorter training time than the men (Figure 4.4). However after 30 weeks, changes in the males and females were similar.

Our study found similar increases in $\dot{V}O_{2\,max}$ compared to other studies with 11 % (Cunningham *et al.*, 1987) and 10 % (Niinimaa & Shephard, 1978) improvements in $\dot{V}O_{2\,max}$ after 12 months of aerobic training. However, other studies (Barry *et al.*, 1966; Seals *et al.*, 1984; Sidney & Shephard, 1978) have reported greater increases in $\dot{V}O_{2\,max}$ with training, which has been attributed to higher intensities of training and varying modalities of exercise, i.e. bike, walking, jogging. The collective decision from the majority of studies state that the greatest improvements in $\dot{V}O_{2\,max}$ (18-28 %) have been found in training programmes >26 weeks, with training being performed >3 days per week, 40 min/day, progressing to intensities of ~80-90 % of HR_{max} (Hagberg *et al.*, 1989a, 1989b; Seals *et al.*, 1984). Hagberg *et al.* (1989a) found that 12 months endurance training could improve $\dot{V}O_{2\,max}$ by 30 % in men and women aged 70-79 years old, concluding there is a similar potential for older individuals to enhance their aerobic power.

In this study, the increases in $\dot{V}O_{2\,max}$ in both sexes can be attributed to increases in a-vO_2 differences as there were no discernable changes in cardiac output. Various studies have shown an improvement in maximal cardiac output with exercise training in men (Kilbom & Astrand, 1971; Ogawa *et al.*, 1992; Stratton *et al.*, 1994). Spina *et al.* (1993) suggested that two-thirds of the increase in $\dot{V}O_{2\,max}$ in men was due to an increase in cardiac output and one-third due to a-vO_2 difference. In women, results suggested that the increase in $\dot{V}O_{2\,max}$ was solely due to an increase in a-vO_2 difference, which is consistent with the results of this study.

Men have been shown to adapt to endurance exercise by enhancing their systolic and diastolic function during exercise (Levy *et al.*, 1993; Spina *et al.*, 1996a; Stratton *et al.*, 1994), such that an improvement in $\dot{V}O_{2\,max}$ with training in men, was attributed to an enhancement of left ventricular systolic performance and greater stroke volumes during exercise. The increase in SV_{pcak} could be mediated by left ventricular wall enlargement and an increase in myocardial contractility (Ehsani *et al.*, 1991). The latter also suggests an increase in inotropic response to β-adrenergic stimulation (Spina *et al.*, 1998). It has been well established that there is no known effect on maximal heart rate as a consequence of exercise training (Hagberg & Graves, 1989; Meredith *et al.*, 1989; Seals *et al.*, 1984). Hence, the increase in maximal cardiac output in men after training must be attributed to an increase in maximal stroke volume. However, this was not apparent from our results in men or women (Figure 4.7). However, our results in regards to the female training group adapting only peripherally, are supported by the literature. Post-menopausal women who participated in aerobic training programmes of over 4 months duration have been shown to elicit no change in maximal cardiac output, stroke volume, ejection fraction, left ventricular diastolic or systolic volumes post training (Carroll *et al.*, 1997; Park *et al.*, 2003). This lack of adaptation to training was attributed to oestrogen deficiency. However, a recent study has shown that hormone replacement therapy has no effect on cardiac output during maximal exercise (McCole *et al.*, 1999). Spina *et al.* (2000) concluded that increases in aerobic power were not due to central adaptations, but rather to the periphery in post-menopausal women.

This was concluded from the absence of left ventricular hypertrophy and β-adrenergic-mediated augmentation of left ventricular diastolic filling and systolic function in trained women.

Resting sympathetic nervous activity is enhanced with age, resulting in an over-activation of cardiac and vascular β-adrenergic receptors and consequently a desensitisation of β-adrenergic cells. Therefore, due to the decreased response to β-adrenergic stimulation, a reduction in maximal heart rate occurs with age. Various studies have also shown a decline in parasympathetic activity with ageing due to a decline in heart rate variability and baroreflex sensitivity (Byrne *et al.*, 1996; Craft *et al.*, 1995; Flucklager *et al.*, 1999). Pichot *et al.* (2005) found that after 14-weeks of intensive interval training in older men, vagal modulation (heart rate variability and cardiac baroreflex activity) improved during exercise. This has also been apparent with post-menopausal women with endurance training over 8 weeks (Jurca *et al.*, 2004). These findings have however been contradicted, and suggestions have emerged, which propose that training decreases maximal heart rate (Stein *et al.*, 1999). However, a recent meta-analysis has suggested that the age-related decrease in maximal heart rate with endurance training is less in older adults, thus maintaining fitness can attenuate the decline of parasympathetic control over heart rate with normal ageing, and also allows a better heart rate recovery post-exercise (Giallauria *et al.*, 2005). No such vagal modulation was seen in this study as maximal heart rates were unaltered in either sex with training (Figure 4.7B).

When exercising, young participants reduce their DBP_{max} in association with the exercise intensity. In older individuals, a decreased vasodilatory capacity and hence increased vasoconstriction causes an increase in DBP_{max} during exercise. Our results showed a decrease in maximal systolic and diastolic blood pressure in men (Figure 4.8). This could be attributed to a decrease in total peripheral resistance at maximum exercise, therefore suggesting an increase in compliance throughout the vasculature with time (Iwasaki *et al.*, 2003). One explanation is a decrease in maximal arterial pressures could arise from a reduced level of sympathetic activation produced at the same workload after training. Perini *et al.* (2002) trained men at a low exercise intensity and then increased the exercise

intensity incrementally. Results showed a reduction in arterial pressures after low intensity training, but at higher intensities (50-60 % of maximal work loads) found no significant differences in arterial pressures. They suggested that older men are limited in their performance due to a lack of adaptation in cardiac function and a higher sympathetic activation, which subsequently maintains arterial pressure at maximal exercise intensities. Therefore, a low intensity-training programme is sufficient to reduce maximal arterial pressure, which may explain why a significant reduction in DBP_{max} was only seen from 0-12 weeks.

Due to the reduction in DBP_{max}, MAP_{max} was marginally reduced. However, as there was no change in cardiac output, CPO_{max} remained unchanged in the men. In women, there were no apparent changes in either maximal blood pressure or $\dot{Q}_{max}$. Hence, CPO_{max} was unchanged. Only one previous study has assessed the impact of endurance training on cardiac power output. This study showed an increase in CPO_{max} by 16 % in older participants (aged 50-60 years old), through an increased maximal stoke volume (11 %), with 8 weeks of endurance training (Marshall *et al.*, 2001). However, caution is needed as the exercise training was home-based (unsupervised), and the work rate was set at a higher $\dot{V}O_{2\,max}$ (75-80 %) compared to our study. Our results therefore suggest only a peripheral adaptation of enhanced oxygen extraction from the bloodstream in response to this exercise training protocol for both men and women.

4.5.5 Peripheral Blood Flow.

This study was a multi-purpose study and included measurements of blood flow in the same women throughout the training period by a fellow colleague (Shellina Skyrme, MPhil). Measurements included Doppler ultrasound to assess cutaneous blood flow, plethesmography to assess vascular conductance and Acetylcholine (Ach) and Sodium Nitroprusside (SNP) infusions to assess endothelial-dependant and independent vasodilation respectively. All measurements were conducted on the forearm.

After just 12 weeks of endurance training, the women showed an increase of 67 % in maximal cutaneous blood flow and 78 % in maximal cutaneous vascular conductance. No changes were seen in either endothelial-dependant or independent vasodilation. These results suggest that the increase in $\dot{V}O_{2\,max}$ was due to the decrease in vascular resistance and an increase in resistance arteriole capillary blood flow. Therefore, peripheral benefits can be gained through low-intensity exercise training. Arterial compliance has also been found to improve in men after 14 weeks of endurance training (Tanaka et al.,.2000) and increases in maximal cutaneous blood flow after just 4 weeks of endurance training (Ho et al., 1997). These studies suggest that both older men and women acquire similar improvements in vascular conductance and cutaneous blood flow after 3 months of endurance training.

Maximal cutaneous blood flow and vascular conductance continued to increase up to 30 weeks of training in women. At 30 weeks, an increase in endothelial-dependant vasodilation was seen with in response to 3.2 mC of Ach. Therefore, a longer training period is required to improve NO synthesis. Also over 30 weeks of training are possibly needed to elicit structural remodelling of the cutaneous vasculature, to compensate for the age-related decline, as there was no improvement in endothelial-independent vasodilation occurred after 30 weeks of training. Cross-sectional studies in men have shown that endurance trained men have a 50 % greater endothelial-dependent vasoreactivity than their sedentary counterparts (DeSouza et al., 2000). Consistent with our results, Bergholm *et al.* (1999) reported that 3 months of high intensity running reduced endothelial-dependent function, but not endothelial-independent function. Therefore, due to our training programme eliciting no effect on cardiac function, these additional results support the findings that $\dot{V}O_{2\,max}$ was improved as a result of peripheral mechanisms.

Within muscles, ageing is generally associated with a shift in fibre types from type IIa to more type IIx fibres, identified through changes in myosin heavy chain expressions. This conversion results in a diminished oxidative capacity and lower fatigue resistance (Conley et al., 2000). Although we did not directly measure changes within the muscles, the increases in $\dot{V}O_{2\,max}$ were attributed to

increases in oxygen extraction (a-$\dot{v}O_2$ difference) at the muscles. Hence, we might expect that the exercise training had apparently reversed the ageing process by retaining more oxidative fibres. Some studies have however shown no changes in fibre-type distributions or capillary-to-fibre ratios with endurance training in older men (Aniansson *et al.*, 1981; Denis *et al.*, 1986; Orlander & Aniannson, 1980). In addition, Suominen *et al.* (1977a) found increases of only 5-15 % in mitochondrial respiratory capacity (as measured by malate dehydrogenase levels) in both older men and women as a result of endurance training. However, these previous studies used participants who were trained and at a low intensity work rate, except for Denis *et al.* (1986) who used a more strenuous programme. More short-term strenuous exercise programmes have shown increases of 45% in malate dehydrogenase and succinate dehydrogenase (Souminen *et al.*, 1977b) and an increase in muscle respiratory capacity of 41 % (Meredith *et al.*, 1989). Coggan *et al.* (1992) endurance trained 60- 70 year old men and women for 9-12 months. The results showed that type I fibres did not change, but there were decreases in type IIx fibres and increases in type IIa ratios. This suggests that endurance training can reverse the age-associated changes in muscle fibre types. Capillary density also increased by 21 % in response to training, and indicated a 25-30 % increase in capillary-to-fibre ratio. Interestingly, there were no statistical interaction between training and sex, suggesting that both older men and women adapt in a similar manner to endurance training, which is consistent with our results. Therefore, the increase in a-$\dot{v}O_2$ differences, within our study, can probably be attributed to an increase in arterial compliance and peripheral blood flow and probably an increase in capillary-to-fibre ratio and oxidative metabolism at the exercised muscles.

4.5.6 Limitations.

4.5.6.1 Participants.

The ageing process has now been postulated to involve a variety of multi-factorial processes. 'Normal or healthy ageing' has been defined as senescence

in the absence of disease. However, ageing in older populations rarely occurs without disease and therefore normal ageing becomes a more atypical phenomenon. Our rigorous selection criteria enabled us to study a non-diseased population. This was ascertained through health questionnaires, resting and exercise ECG traces, a stress exercise test and blood pressure measurements at the initial screening sessions. Although our rigorous selection criteria may not be representative of the general population, it allowed us to delineate the ageing process from the development of diseases within that particular age group.

Due to the limitations set by the selection criteria, a large proportion of the population who were aged between 55-65 years old, living within the Merseyside region, were automatically excluded. This meant that participant recruitment was difficult and around 150 applicants were rejected by the health/activity questionnaire. We also found that participants falsified their information within the health questionnaire so they would be accepted on to the training programme. However, these cases were detected and excluded through interviews, participant interaction (i.e. informal interviews) and our 2 day physiological screening process. Around 30 applicants were then excluded at the physiological screening stage due to unknown health problems, but mainly related to high blood pressure.

With a large number of participants rejected from the study, it was difficult to achieve high participant numbers for the programme. Also, retention of participants on the programme was difficult due to the lengthy longitudinal nature of the study. Due to lower anticipated participant numbers, a second recruitment drive was initiated 3 months after the start of the training programme. This second participant recruitment drive failed due to our inability to acquire crucial medical supervision, as our replacement cardiologist withdrew at the last-minute. For health and safety reasons when dealing with this older age group, we ensured that there was always a medically qualified practitioner in attendance at each exercise testing session to interpret the ECG output and effect resuscitation if ever required. Due to the participants performing three maximal cardio respiratory tests at each resting time, part-time medical cover could not have sustained another intake of participants. However, power calculations for this study suggest that the participant numbers gained were adequate.

4.5.6.2 Supervision of training.

At the start of the exercise programme, all participants were supervised for all sessions over the first 2 weeks. This was to ensure that all participants comprehensively understood the exercise regime and the use of equipment. However, due to large initial participant numbers and limited exercise facilities, collaboration was initiated with Liverpool Council Lifestyle Gyms'. To fully supervise every participant throughout the training programme would have meant the employment of gym staff to fulfil this role, in order to allow the researchers to concentrate on participant testing. Unfortunately due to financial constraints this was not possible. To remedy the reduced supervision of participants, exercise diaries (see appendix 9.2) were distributed to participants and were completed at each training session. Participants also attended the University gym at least once a week, at which time the exercise diary was checked. If there were any diaries that were either incomplete or looked falsified, the participant was asked to attend more training sessions at the University. Although essential, diaries are open to falsification and hence are less reliable than fully supervised training sessions.

4.5.6.3 Measurement Techniques.

All cardiovascular measurements made within this study were non-invasive. Although the measurements made were reliable, invasive techniques still remain the gold standard. However, invasive techniques such as heart cathertisation are potentially dangerous with exercise and not allowed by our ethics committee. Therefore, the most appropriate non-invasive substitute was used (for full reliability and validity of our measurement techniques see chapter 3).

4.5.6.4 Blood volume and haemoglobin content.

Maximal aerobic capacity can be affected by high blood volumes i.e. high haemoglobin content (Convertino & Luwig, 2000). Therefore, high $\dot{V}O_{2\,max}$ measurements may have been due to increased haemoglobin content, resulting in an increased O_2 carrying capacity, causing false results. Consequently, some individuals may have been accepted or rejected onto the programme exhibiting higher $\dot{V}O_{2\,max}$ values. Endurance training has been shown to increase blood volume, hence increasing $\dot{V}O_{2\,max}$ and SV_{max}. However, neither men nor women showed a change in $\dot{Q}_{max}$ or SV_{max} with 30 weeks of endurance training, signifying a lack of cardiac adaptation with exercise training, and implying that appreciable blood volume expansion did not occur with training. Cardiac power output also did not change with training, which incorporates both preload and afterload; although only a direct measure of blood volume would have supported this assumption.

4.5.7 Summary.

In conclusion, our 30-week aerobic training programme increased $\dot{V}O_{2\,max}$ in both men and women, with no apparent sex differences. Hence, the main effect was an increase in a-vO_2 differences and not a central cardiac adaptation. The increase in a-vO_2 difference can be attributed to an increase in limb muscle oxidative capacity, as muscle mass did not increase with training as determined by measuring LBM with DEXA. Exercise prescription must therefore be carefully considered in regards to improving cardiac function. However, a low intensity exercise programme will give health benefits (e.g. countering atherosclerosis), to both men and women, but particularly in respect to improving peripheral blood flow. We can conclude that aerobic exercise reaps greater health benefits, compared to doing no exercise (control group). In contrast, if aerobic exercise training is to affect central cardiac performance, and hence CPO and cardiac reserve, the intensity of that exercise must be greater than the 75 %

HRR as used here, or conducted over a longer duration to increase exercise volume.

4.5.8 Future Research.

The data from this study can be interpreted and used in two ways. Firstly, a higher intensity of exercise may be required to improve cardiac function in both men and women. Makrides *et al.* (1990) showed that a short-term, high-intensity exercise programme in older men improved peak cardiac output by 30 %, using the CO_2-rebreathing manoeuvre. Improvements in cardiac function with high intensity exercise have also been seen in patients with reduced ventricular function (Myers *et al.*, 1999) and intense swim training in older populations (Martin *et al.*, 1987). Therefore, future research using a high-intensity (>75 % HRR) exercise programme may exhibit an improvement in central function. However, high intensity exercise programmes can elicit huge strains on the cardiovascular system and could cause medical complications. To reduce this, interval training would be an appropriate training mode. Interval training for 12 weeks has resulted in an increase in $\dot{V}O_{2\,max}$ of 20 % with older men and women (Spina *et al.*, 1996). There have been few studies investigating the effects of interval training on cardiac function in older men and women. However, Makrides *et al.* (1990) stated that high-intensity interval training elicits similar cardiovascular changes in both young and old men. This suggests that interval training prescribed to younger participants may be a safer health option, but still produce the same trends as you would find in older participants.

Secondly, older populations may not be able to improve their overall cardiac function, and only enhance their peripheral mechanisms and blood flow. To overcome this, it may be necessary to commence exercise at a younger age to counter the effects of ageing on the heart. Studies have already shown that long-term endurance athletes have a greater cardiac function than their sedentary counterparts (Seals *et al.*, 1994), but they still undergo the ageing decline in function at the same rate (Ogawa *et al.*, 1992). Therefore, although exercise at an older age is still beneficial, overall cardiac function may be more difficult to improve by exercise training.

Chapter 5

Study 3 - The Effects of Interval and Continuous Exercise Training on Cardiac Function in Young Men and Women.

5.1 Background.

5.1.1 Aerobic power and oxygen uptake after interval and endurance-based exercise training.

Training-induced adaptations, in regards to cardiovascular and aerobic fitness, have been extensively studied using endurance-based exercise training. Maximal oxygen consumption ($\dot{V}O_{2\,max}$) has been recognised as the fundamental component in determining physical fitness (Astrand & Rodahl, 1986) and success in aerobic endurance sports (Saltin, 1990). The magnitude of the increase in aerobic capacity is dependent on the initial fitness status of the participants, frequency and duration of the training sessions (Pollock, 1977; Wenger & Bell, 1986).

Continuous endurance exercise at a fixed intensity (continuous training) is defined as exercise of at least 20 minutes in duration, with a heart rate elevated 60-80 % of its maximum. With the majority of studies showing improvements in maximal oxygen consumption ($\dot{V}O_{2\,max}$) after endurance training, the optimal exercise volumes and intensities are still under debate. However more recent findings from the literature suggest that the largest increase in $\dot{V}O_{2\,max}$ is gained through a high-intensity training protocol. Carter *et al.* (1999) trained 16 physical activity students for 6 weeks (3-5 sessions per week, 20-30 minutes duration) at their lactate threshold running speed. The results showed an improvement of 10 % in $\dot{V}O_{2\,max}$, which is consistent with other short-term endurance studies which have found improvements of between 5-10 % (Billat *et al.*, 1999; Franch *et al.*, 1998; Gaesser *et al.*, 1984; Gibbons *et al.*, 1983; Mier *et al.*, 1997; Spina *et al.*, 1996b; Weston *et al.*, 1997). Hickson *et al.* (1981) showed an improvement of 23 % in $\dot{V}O_{2\,max}$ after 9 weeks of endurance training, with over one-half of this increase (14 %) occurring after just 3 weeks of training. This rapid increase in $\dot{V}O_{2\,max}$ after short-term training has been partly attributed to hypervolemia, causing an increase in maximal stroke volume (Convertino, 1991; Green *et al.*, 1991). Long-term training programmes have found a stabilisation in $\dot{V}O_{2\,max}$ during exercise training, due to improvements in exercise

economy and lactate threshold (Jones, 1998; Martin *et al.*, 1986; Rusko, 1992). Therefore, short-term endurance training could be equally as beneficial as long-term endurance training in relation to improvements in $\dot{V}O_{2\,max}$.

More recently, studies have shown that the modality of exercise can affect the degree of improvement in relation to aerobic fitness. Interval training has been shown to increase aerobic fitness and cardiovascular function in heart failure patients (Wisloff *et al.*, 2007), athletes (Helgerud *et al.*, 2007) and elderly people (Billat, 2001). Interval training consists of high-intensity exercise (exercise phase) interspersed with rest periods (rest phase). Seiler and Hetlelid (2005) stated that when prescribing interval training, 5 variables need to be considered: interval intensity, interval duration, exercise intensity of recovery, duration of recovery, and total work duration. The variability of these indices can manipulate different responses regarding aerobic and anaerobic energy capacities.

Khnuttgen *et al.* (1973) found that 3 minute high-intensity exercise phases were more effective in enhancing aerobic power than 15 second exercise phases of an interval training protocol. This effect was also noticed with 2.5 minute exercise phases being more effective than 1 minute exercise phases in improving aerobic fitness (Roskamm *et al.*, 1967). These results suggest that longer exercise phases induce a greater improvement in aerobic capacity. However, the intensity of those exercise phases can affect the level of improvement. Smith & Wenger (1981) showed an exercise phase of 3 minutes, with a low heart rate (140 bpm), did not improve aerobic performance.

To directly compare aerobic improvement: intensity ratios, Esfarjani & Laursen (2007) developed two high-intensity training programmes. The first group of moderately trained male runners completed 5-8 intervals at v_{VO2max} (minimum speed needed to reach $\dot{V}O_{2\,max}$) with a 1:1 work: recovery ratio. The second completed 7-12, 30 second bouts at 130 % v_{VO2max}, separated by 4.5 minutes recovery periods. Both groups trained twice a week for 10 weeks. The results showed that $\dot{V}O_{2\,max}$ significantly increased from baseline in both groups after training, but group 2 were not significantly different from the control group who had performed four, 60 minute low-intensity (75 % v_{VO2max}) bouts a week. These

results therefore suggest that a shorter work: recovery ratio time elicits a greater improvement in $\dot{V}O_{2\,max}$ in moderately trained men. Laursen *et al.* (2002) also noted a greater improvement in $\dot{V}O_{2\,max}$ training at 60 % T_{max} (the point at which v_{VO2max} can be sustained), compared to supramaximal high-intensity exercise in trained cyclists. Similarly, Franch *et al.* (1998) found a 6 % increase in $\dot{V}O_{2\,max}$ after long interval training (6 x 4 min at 16.5 kmh-1, 2 min recovery) compared to a 3 % improvement after short interval training (30 x 15s at 20.5 kmh-1, 15s recovery). These results suggest that high-intensity interval programmes, which require substantially more oxygen delivery, will induce greater improvements in aerobic capacity.

Due to the high-intensity nature of interval training, lactic acid builds up in the muscles during the exercise phase. Studies have shown that active rest periods facilitate faster lactic acid removal compared to passive rest periods (Hermansen & Stensvolo, 1972; Belcastro & Bones, 1975) regardless of training status (Oosthuyse & Carter, 1999). Active rest periods can be defined as a rest period involving a lower intensity exercise compared to the intensity completed during the exercise phase. Whereas passive rest periods involve complete rest between exercise phases. Active rest periods also allow athletes to tolerate a heavier work rate for a longer period of time (Billat, 2001; Billat *et al.*, 2000). Seiler and Hetlelid (2005) showed that increasing recovery time from 1 to 2 minutes increased average running velocity by 2 %, but when recovery time was increased to 4 minutes, there were no additional increases in work intensity. Therefore, this suggests that varying rest periods of 1 to 4 minutes have little impact on sprint performance.

Peak oxygen consumption after sprint training is often (Harmer *et al.*, 2000; MacDougall *et al.*, 1998; McKenna *et al.*, 1997), but not always (Allemeier *et al.*, 1994; Hellsten-Westing *et al.*, 1993; Stathis *et al.*, 1994), increased. However, the majority of studies still consider that $\dot{V}O_{2\,max}$ does not differ between continuous endurance and interval exercise, and that similar increases in $\dot{V}O_{2\,max}$ can be found regardless of pre-training fitness levels (Bhambhani & Singh, 1985; Branch *et al.*, 2000; Gregory, 1979). Conversely, Thomas *et al.* (1984) concluded that in untrained men and women, interval training at 90 %

HR_{max} may be more beneficial than continuous training at 75 % HR_{max}. This outcome has also been affirmed in patients with coronary artery disease after high-intensity interval training at 80-90 % $\dot{V}O_{2\,max}$ was compared to continuous low-intensity exercise of 50-60 % $\dot{V}O_{2\,max}$. This therefore suggests that the training intensities of interval and continuous exercise programmes could provide different cardiovascular benefits. To examine this, Helgerud *et al.* (2006) examined 40 moderately trained (engaged in endurance training and leisure-time physical activity at least three times per week) males undertaking 4 different exercise protocols. The results showed that the 15/15 interval running group (15 seconds of running at 90-95 % HR_{max} followed by 15 seconds of active rest at 70 % HR_{max}) and the 4 x 4 min group (4 minutes of running at 90-95 % HR_{max} followed by 4 minutes active rest at 70 % HR_{max}) significantly improved their $\dot{V}O_{2\,max}$. However, the longer slower distance runners (70 % HR_{max}), and a lactate threshold group (85 % HR_{max}), did not improve their $\dot{V}O_{2\,max}$ after 8 weeks. The authors concluded that interval training with longer exercise phases is recommended to greatly improve $\dot{V}O_{2\,max}$. This result was also found by Izumi *et al.* (1996), who found that 6 weeks of aerobic training at 70 % $\dot{V}O_{2\,max}$ did not improve $\dot{V}O_{2\,max}$ or anaerobic capacity in moderately trained males. However, high-intensity interval training (20 seconds at 170 % $\dot{V}O_{2\,max}$ with 10 seconds rest) improved aerobic capacity by 7 $ml.kg^{-1}.min^{-1}$ and anaerobic capacity by 28 %. The improvements in anaerobic capacity after interval training have been previously reported (Medbo & Burgers, 1990), with some studies on a treadmill showing a longer exercise time to reach anaerobic thresholds and a longer sprint time when compared to continuous endurance training (Sokmen *et al.*, 2002).

In regards to sex differences in $\dot{V}O_{2\,max}$ after training, studies have shown that men and women had the same improvements in $\dot{V}O_{2\,max}$ (~15 %) after 7 weeks of interval and continuous training (Eddy *et al.*, 1977). This was also seen by Fox *et al.* (1975), who showed an improvement in $\dot{V}O_{2\,max}$ of 15 % in both men and women after interval training. However, Lesmes *et al.* (1978) compared two types of supramaximal interval training programmes (i.e. high-intensity short distance versus high-intensity long distance) in women over 8 weeks on $\dot{V}O_{2\,max}$. The results showed that the improvements in $\dot{V}O_{2\,max}$ were the same after both

protocols. The equal improvements in $\dot{V}O_{2\,max}$ between the interval and continuous training protocols in women were also observed by Cunningham *et al.* (1979) who found an increase of 21 % in $\dot{V}O_{2\,max}$ after continuous training and 23 % after interval training. Therefore, it is apparent that changes in $\dot{V}O_{2\,max}$ in women, are independent of frequency, distance and intensity. In contrast, Fox *et al.* (1975) had previously shown that for men, training intensity, rather than frequency or distance, was the most important factor in improvement in $\dot{V}O_{2\,max}$ after interval training. These results therefore suggest potential sex-specific differences in regards to interval training.

However, ensuring similar work loads between training modalities can be difficult to implement. Gorostiaga *et al.* (1991) were first to compare interval and continuous training at the same maintained work loads. The interval group trained for 30 minutes with 30 seconds work interspersed with 30 seconds of rest. The continuous group trained at 50 % of the lowest work rate requiring full utilisation of maximum O_2 uptake for 30 minutes. Both groups trained 3 days per week for 8 weeks using cycle ergometers. The results showed similar improvements in oxygen uptake, but these increases were associated with different metabolic processes. The interval exercise training improvement in O_2 uptake was attributed to a high utilisation of ATP from anaerobic respiration during the work periods, therefore causing depletion a of ATP and PC stores (Essen *et al.*, 1977). During the rest intervals, lactic acid removal was accelerated through oxidation and/or glycogen synthesis in the muscle (McLane and Holloszy, 1979; Essen & Kaijser, 1978). After the continuous protocol, ATP was produced via aerobic mechanisms which were associated with lipid metabolism (Newsholme 1984) with very little anaerobic glycolysis (Essen *et al.*, 1977). Overend *et al.* (1992) also concluded that interval training was not advantageous over continuous training in regards to aerobic power, when using the same average power output in untrained males. Conversely, Daussin *et al.* (2007) compared 8 weeks of interval and continuous training, which were matched for energy expenditure and duration. The results showed that interval training induced a greater improvement in $\dot{V}O_{2\,max}$ (34 %) than continuous training (11 %). The reduced improvement after continuous exercise was attributed to a lower exercise intensity, compared to other studies who found

greater improvements after higher exercise intensities (Beere *et al.*, 1999; McGuire *et al.*, 2001; Stratton *et al.*, 1994). Even though exercise intensity has been equalised between exercise modalities, there are still discrepancies between studies. These differences in results could be related to the indices chosen (i.e. running distance, calorie expenditure, aerobic capacity) to equalise the work loads between training protocols.

5.1.2 Cardiac function after interval and continuous exercise training.

The increase in $\dot{V}O_{2\,max}$ after endurance exercise training in young men has previously been attributed to equal increases in maximal cardiac output ($\dot{Q}_{max}$) and arteriovenous oxygen difference (a-vO_2 difference; Saltin *et al.*, 1967). Green *et al.* (1987) observed that after short-term endurance training, red cell count decreased and therefore it was concluded that to ensure the maintenance of oxygen transport, an increase in $\dot{Q}_{max}$ was also needed.

Goodman *et al.* (2005) found that $\dot{Q}_{max}$ was elevated after 6 days of continuous endurance exercise for 2 hours a day. This elevation after training was attributed to an optimised oxygen carrying capacity. Conversely, Wilmore *et al.* (2001) found in young men and women no change in maximal $\dot{Q}$, stroke volume (SV_{max}), heart rate (HR_{max}), or a-vO_2 difference after 20 weeks of endurance training starting at 55 % $\dot{V}O_{2\,max}$ 30 minutes per session, and increasing to 75 % $\dot{V}O_{2\,max}$ for 50 minutes per session.

At maximal exercise, various studies have shown a decrease in HR_{max} of 5-10 beats/min after short-term endurance training (Brorson *et al.*, 1976; Convertino, 1983; Costill *et al.*, 1988; Mier *et al.*, 1997; Saldanha *et al.*, 1997) and 6-8 beats/min after long-term aerobic training (Sadaniantz *et al.*, 1996; Spina *et al.*, 1993). The decrease in HR_{max} after training has been attributed to numerous factors. Exercise training has shown to diminished sympathetic input (Cousineau *et al.*, 1977; Smith *et al.*, 1989; Winder *et al.*, 1978), with decreases in circulating catecholamine levels. However, Kjaer *et al.* (1986) compared

catecholamine levels between trained and untrained individuals at maximal exercise. Their results showed that at maximal exercise, trained individuals showed higher adrenaline and nor-adrenaline levels than non-trained, which were related to a higher skeletal muscle power output. Therefore, it is unlikely that a diminished catecholamine response decreases HR_{max} after training, since catecholamine levels were not blunted at maximal exercise in trained participants.

Training also causes the expansion of plasma volume (Selby & Eichner, 1994), which is both frequency and intensity dependent. For example, elite runners can expand their plasma volume by 1000 ml whereas a novice jogger increases their plasma volume by 300 ml during a single endurance exercise bout (Convertino, 1991). Therefore, the expansion of blood volume (hypervolemia) has been linked with the improvement in cardiorespiratory function after endurance exercise, by increasing cardiac preload, ventricular filling and ultimately stroke volume, allowing for a decreased maximal heart rate (Kanstrup & Ekblom, 1982; Krip *et al.*, 1997). Convertino, (1983) found that after an 8-day training regimen with fit males, plasma volumes and HR_{max} were negatively related ($r = 0.82$). With the connection between vascular volumes and cardiac function (Gledhill *et al.*, 1994; Krip *et al.*, 1997; Warburton *et al.*, 1998, 1999, 2000), blood volume plays an important role on left ventricular function after training. A greater increase in blood volume has also been observed after high-intensity, short-term training (Green *et al.*, 1984; Richardson *et al.*, 1996). Warburton *et al.* (2004) found that after both interval and continuous training, 30 % variances in the changes in left ventricular function, were attributed to increases in blood volume. However caution should be stressed when regarding this assumption, as there were no elevations in volume-regulatory hormones, e.g. Angiotensin II, aldosterone, vasopressin or atrial naturetic peptide, after both training modalities. Lastly, exercise training using animals has shown a reduction in cardiac β-receptors due to a down-regulation in receptor synthesis, especially with regards to the synthesis of receptors in the right atrium (Hammond *et al.*, 1987, 1988; Werle *et al.*, 1990), whilst concomitantly increasing receptor affinity (Takeda *et al.*, 1985). This decrease in synthesis of adrenergic receptors could therefore explain the decrease in maximal heart rate after training.

With a variety of training-induced changes on cardiac function, recent studies have focussed on which training modality is more suitable for enhancing cardiac output and thus cardiac function. Helgerud *et al.* (2007) tested 55 young recreationally active males who were divided into three groups. Firstly, a continuous training group ran at 70 % HR_{max} for 45 minutes. The second training group performed 47 repetitions of 15 second intervals at 90-95 % HR_{max} with a 15 second active resting period at 70 % HR_{max}. A third group trained 4 x 4 minute intervals at 90-95 % HR_{max} with 4 minutes of active resting at 70 % HR_{max}. All groups trained 3 days a week for 8 weeks in total. The results showed that there were no changes in $\dot{Q}_{max}$, SV_{max} or $\dot{V}O_{2max}$ within the continuous group. However, there were significant increases in $\dot{Q}_{max}$ (9 %), SV_{max} (9 %), and $\dot{V}O_{2max}$ (5 - 7 %) after the 15/15 seconds interval training and 4 x 4 min interval training. It was therefore concluded that the latter increases in $\dot{V}O_{2max}$ were due to an increase in cardiac output. However, a recent study showed that interval training 3 times per week, over 8 weeks, improved central and peripheral functions in middle-aged men and women. This increase (10 %) in cardiac output was attributed to a rise in maximal stroke volume and heart rate. The same study also trained a continuous exercise group, equating the same workload as the interval group. The results showed that there were no effects on central cardiac function, but an increase in $\dot{V}O_{2max}$, suggesting a greater oxygen extraction at the muscle (Daussin *et al.*, 2007). A similar interval training study for 12 weeks and using sedentary males aged 20-30 years, showed that VO_2 peak increased by 28 %. This increase was attributed to equal contributions of a-vO_2 difference (14 %) and $\dot{Q}_{max}$ (14 %). The increase in $\dot{Q}_{max}$ was attributed to equal increases in SV_{max} (9 %) and HR_{max} (5 %; Makrides *et al.*, 1990). After 12 weeks of interval swim training, Martin *et al.* (1987) also showed an increase (7 %) in peak cardiac output, resulting from an improvement in peak stroke volume (10 %). The increase in peak VO_2 was the result of equal contributions from both peak cardiac output and peak a-vO_2 difference. Therefore, these studies suggest that interval training increases $\dot{V}O_{2max}$ through an increase in both $\dot{Q}_{max}$ and a-vO_2 difference, whereas continuous training has no effect on cardiac function.

Endurance training has been found to enhance both diastolic filling rate and left ventricular emptying, determined by HR (Gledhill *et al.*, 1994). Ferguson *et al.* (2001) compared young sedentary women to young endurance trained women. The results showed that the endurance trained women had a higher $\dot{V}O_{2\,max}$, maximal a-vO_2 difference, and $\dot{Q}_{max}$ (due to an increase in SV_{max} despite a lower HR_{max}). It is generally believed that at lower work rates SV is influenced by the Frank-Starling mechanism, whereas at higher work rates, myocardial contractility is more important. However, Ferguson *et al.* (2001) showed that beyond sub maximal work rates, differences in total peripheral resistance did not decrease, but SV still continued to rise, suggesting that there was an increase in preload and thus the enhancement of the Frank Starling mechanism, rather than an increase in contractility. Warburton *et al.* (2004) found that 6 weeks of both continuous and interval training improved left ventricular diastolic filling, cardiac output and stroke volume. This improvement in diastolic function occurred with little improvement in myocardial contractility. Therefore, SV appears to increase after both interval and continuous exercise due mainly to the utilisation of the Frank-Starling mechanism.

Prolonged steady-state exercise has shown a reduction in left ventricular systolic function and diastolic filling. Conversely, Rodrigues *et al.* (2006) found that after 6 months of endurance exercise (60 minutes a day, 3 days a week increasing in intensity up to 85-90 % max heart rate) peak VO_2 increased by 14.5 %, but there was little change in LV diameter and volume. Doppler measurements also showed that there were no changes in stroke volume or LV ejection fraction after training. However, left ventricular ejection fraction has been known to increase after short-term high intensity interval exercise (Foster *et al.*, 1995, 1997, 1999 Upton *et al.*, 1980). This has been associated with a loss of the Frank Starling reinforcement of ventricular function, causing the maintenance of SV and $\dot{Q}$ through a decline in LV afterload. To examine LV function after interval training, George *et al.* (2004) examined LV function in 19 male rugby and football players, before and after their competitive games. The results showed that LV systolic function was not depressed after the game and LV contractility was unaltered. However, the ratio of early and atrial filling velocities (E:A) were reduced after the game, which was attributed to an increase in heart rate, but the

relationship between heart rate and diastolic filling still remains unclear. Echocardiographic studies can be useful in determining cardiac structures after exercise, but the measurements are taken at rest and not during exercise, and therefore are a poor determinant of cardiac function.

However, LV ejection fraction has been shown to be a very poor indicator of cardiac function, let alone overall cardiac function, after exercise training (Crawford *et al.*, 1985). As previously stated, cardiac power output (CPO) incorporates both blood flow and blood pressure, therefore giving a better indicator of overall cardiac function. Using this technique, Marshall *et al.* (2001) studied 9 middle-aged men and women undertaking 8 weeks of home-based endurance exercise at 75-80 % $\dot{V}O_{2\,max}$. The results showed an improvement of 9 % in $\dot{V}O_{2\,max}$, a 16 % increase in CPO_{peak} and a 21 % increase in cardiac reserve. With no change in mean arterial pressure after training, the enhancement in overall cardiac function was attributed to an 11 % increase in SV_{peak}, and hence $\dot{Q}_{max}$. No other known studies have examined the effects of exercise training in healthy men and women using CPO, despite the fact that CPO provides a better indicator of overall cardiac function.

5.1.3 Sex-specific differences in cardiac function after interval and continuous exercise training.

The limitations to some of the stated studies are that there are no differentiations between the two sexes within studies, therefore often making the assumption that men and women respond the same to exercise training. Women have shown similar cardiovascular responses to men to training, but generally do not reach the same magnitude of $VO_{2\,max}$ as men (Kohrt *et al.*, 1991; Raven *et al.*, 1972; Saltin & Astrand, 1967; Tanaka & Seals, 1997). The differences in women adapting to training have been attributed to their sex hormones, i.e: oestrogen and progesterone. Also, female characteristics such as a difference in metabolism, autonomic control (greater parasympathetic control), smaller lean body mass, blood volume and heart size, may affect training-induced adaptations compared to males (Mitchell *et al.*, 1992; Spina, 1999).

There is still some debate as to how women adapt to exercise training, with regard to central and peripheral functions. Cunningham *et al.* (1979) found that cardiac output was not affected by 12 weeks of interval or continuous training in young women. Consequently, they attributed an increase in $\dot{V}O_{2\,max}$ to peripheral adaptations involving oxygen extraction. Alternatively it is believed that cardiac adaptations only occur at higher exercise intensities. Kemi *et al.* (2005) trained 24 female rats for 1 hour, 5 days a week using an interval programme with either a high interval intensity (85-90 % $\dot{V}O_{2\,max}$) or a more moderate interval intensity (65-70 % $\dot{V}O_{2\,max}$) for 8 minutes, interspersed by 2 minute rest periods. The results showed that the higher intensity interval intensity had a larger effect on cardiac hypertrophy, cardiomyocyte contractility, Ca^{+} handling and $\dot{V}O_{2\,max}$. However, vascular endothelial function was not strongly correlated to an increase in $\dot{V}O_{2\,max}$, which suggested no increase in arterial conductance to allow an increase in peripheral blood flow. Peak VO_2 has been known to increase with high-intensity interval training after 8 weeks, significantly improving VO_{2pcak} in young women, via an increase in amplitude in the primary phase of VO_2 (phase gained before lactate threshold; Duffield *et al.*, 2006). This suggests a reduction in anaerobic metabolism post training compared to the same absolute work-load prior to training. Few studies have focussed on men and women simultaneously with regards to interval training. The previously discussed studies in women do not consider the comparison between interval and continuous exercise and do not mention menstrual status.

Oestrogen has been found to vasodilate arterial smooth muscle, alter sympathetic tone and effect fluid balance (Altemus *et al.*, 2001; Lebrun *et al.*, 2001). Few studies have observed cardiovascular variations within the normal menstrual cycle. Several studies have reported elevations in resting heart rate during the luteal phase (Hassan *et al.*, 1990; Kaplan *et al.*, 1990; Kelleher *et al.*, 1986; Manhem & Jern, 1994), however in contrast, some studies have shown that menstrual cycle phases have no effect on resting heart rate (Girdler *et al.*, 1993; Sato *et al.*, 1998). Similar discrepancies regarding variations in blood pressure during the menstrual cycle have also been apparent. Freedman (1974) showed that just after ovulation, both systolic and diastolic blood pressures increased. In

contrast, Dunne *et al.* (1991) and Kelleher *et al.* (1986) observed a larger increase in systolic blood pressure at the onset of menstruation, when compared to other phases. Greenberg *et al.* (1985) also found that systolic blood pressure was higher in the luteal phase. More recent studies have shown that there were no changes in systolic, diastolic or mean arterial pressures during both follicular and luteal phases (Beek *et al.*, 1996; Lewandowski *et al.*, 1998). However, these studies only took single measurements during the two phases. Moran *et al.* (2000) studied 26 women and showed that the follicular phase caused decreases in heart rate, systolic pressure and provided a faster recovery rate after exercise. Therefore, it was concluded that exercise training during the follicular phase could induce greater exercise recovery.

The combined oestrogen-progesterone pill has been reported to increase $\dot{Q}$ (Walters & Lim, 1970). However in contrast, Littler *et al.* (1974) found that women taking the combined contraceptive pill did not affect $\dot{Q}_{max}$ during exercise. However, these results did show that $\dot{Q}$ was higher in the oral contraceptive group, compared to a control female group at baseline. The higher $\dot{Q}_{max}$ was attributed to an increase in SV_{max}. Therefore although baseline $\dot{Q}_{max}$ is higher in women taking the contraceptive pill, this does not affect $\dot{Q}_{max}$ during exercise. These results suggest that women taking the combined contraceptive pill would be more ideal to use during training studies.

5.1.4 Detraining.

Some of the previously mentioned studies, have compared both interval and continuous exercise training at the same work-loads, and also used different participants for each training mode. Therefore, the issue raised is why the same participants are not used in both training regimes, so that direct comparisons can be made, and thus limiting biological variations. The discrepancy seems to be related to the detraining of participants back to previous pre-exercise levels before the second training modality is undertaken.

Detraining has been defined as the partial, or complete loss, of training-induced physiological, anatomical and performance adaptations, due to a reduction or cessation of training (Mujika & Padilla, 2000). Maximal oxygen consumption has been shown to decline between 4–6 % over 2-4 weeks in individuals who recently trained for 4–8 weeks, resulting in the return to pre-training $\dot{V}O_{2\,max}$ values (Klausen *et al.*, 1981; Shoemaker *et al.*, 1998; Wang *et al.*, 1997; Wibom *et al.*, 1992). This initial decrease in $\dot{V}O_{2\,max}$ was be attributed to a decrease in $\dot{Q}_{max}$, as a-vO_2 difference was shown not to decline after 56 days of ceasing training (Coyle *et al.*, 1984). Coyle *et al.* (1984) also found that endurance trained individuals showed a decrease in $\dot{Q}_{max}$ (8 %) after 21 days since training. This decrease in $\dot{Q}_{max}$ was due to a decline in SV_{max}. Reductions of 10 – 17 % in SV_{max} have been reported after 12-21 days of detraining (Coyle *et al.*, 1984, 1986; Martin *et al.*, 1986), in association with a 12 % reduction in left ventricular end diastolic dimension (Martin *et al.*, 1986). Wang *et al.* (1997) showed that the decreases in HR_{max} and HR_{rest} after 12 weeks of training reverted back to pre-training levels after 8 weeks of training. Therefore, the increase in HR_{max} does not seem to counterbalance the decrease in SV_{max}, thus lowering $\dot{Q}_{max}$ with detraining. Four weeks of detraining was also found to completely reverse systolic and diastolic pressures which had been decreased previously through the 8 weeks of endurance training. These results suggest that cardiac indices decline faster during detraining than the improvement rate gained through exercise training. Consequently, detraining periods of the same duration as the training period should be adequate enough to allow cardiac variables to return to pre-training values.

5.2 Rationale.

There are no known studies that have examined the effects of interval and continuous exercise training (with the same maintained work-loads between groups) in both men and women. Recent literature does suggest that women may adapt peripherally to interval training, whereas men adapt both centrally and peripherally. Therefore, it is important to study both sexes simultaneously to

ascertain the sex-specific differences after interval and continuous exercise training.

The aims of this study were to:

- To establish whether interval and continuous exercise training has the same or different effects on aerobic power and cardiac function.
- To determine if there are any sex-specific differences in cardiac function in response to interval and continuous exercise training.

5.3 Protocol.

5.3.1 Recruitment.

Recreationally active females (n = 18) and males (n = 22), between the ages of 18-24 years, were recruited from the student body of the University. This recruitment was initiated with presentations in lectures and leaflets. We then held a meeting to explain the project in detail to prospective volunteers and allowed them to familiarise themselves with the equipment and laboratories.

5.3.2 Health Screening.

To ascertain subject suitability for the project a medical health questionnaire was completed by each subject (appendix 9.1). Exclusion criteria included any past history of cardiovascular disease, hypertension, neuromuscular problems limiting exercise, and taking any dietary supplements. All female participants were taking the combined contraceptive pill. If subjects met with the inclusion criteria, they then attended a health screening session at the University. Following the initial screening via the health questionnaire, over 15 women and 19 men attended the medical screening. This consisted of a treadmill exercise stress test. Prior to exercise, resting blood pressure was measured in a seated position. Throughout the exercise test, a 12-lead ECG was continually monitored to detect any underlying heart abnormalities. To ensure the exclusion of obese individuals, BMI and a Dual Energy X-ray Absorptiometry (DEXA) scan were used to ascertain percentage body fat. Any female subjects with a body fat percentage over 30 % and male subjects over 26 % were excluded as being obese, according to the ACSM guidelines for the age group. The study was approved by LJMU Ethic's Committee and subjects gave their informed consent. At the end of the screening, only 16 men and 12 women met the strict criteria to be included on the study.

5.3.3 $\dot{V}O_{2\,max}$ test.

Each subject performed a $\dot{V}O_{2\,max}$ test to volitional exhaustion before, and after 6 weeks of both continuous and interval training. This test was performed on a treadmill (Cosmos, Nussdorf-Traustein, Germany) using the ACSM (2003) protocol to ascertain subject endurance capacity. The treadmill started at a speed of 6 kph with a 0 % incline, and increased in speed by 2 kph every 2 minutes, up to a maximum of 16 kph, and thereafter, 2 % increments in gradient were added every minute. This protocol was used to gain linear increases in VO_2 and heart rate relative to workload to avoid fluctuations in these variables, which had become apparent in the unmodified version (Bruce, 1971). Heart rate was determined during exercise from the 12-lead ECG. All subjects were told not to support themselves on the treadmill bar and were verbally encouraged until volitional exhaustion was reached.

To gain accurate values for maximal oxygen consumption, the following 3 criteria must be met:

1. Respiratory exchange ratio > 1.1 at maximal exercise.
2. Heart rate > 95 % of age predicted maximum heart rate (220 – age).
3. Plateau in VO_2 occurs (i.e. < 150ml/ min increase), despite further increases in workload.

(Johnson, 1998)

5.3.4 Measurements of Cardiac function.

The CPX/D system (Medgraphics, St. Pauls, Minnesota, USA) measured breath-by-breath VO_2, VCO_2, $P_{ET}CO_2$, VE and RR. VO_2 and VCO_2 were averaged from 5 breaths. Cardiac outputs were measured at rest and during exercise using the CO_2 re-breathing method (see chapter 2 for more details). Blood pressure was taken manually, at rest and during exercise, using manual auscultation. Exercise was undertaken with a mean room temperature of 22 °C ± 0.1 °C and room humidity of 50 ± 10 %. Cardiac power output and cardiac reserve were

subsequently calculated. All these measurements were taken before and after each exercise programme.

5.3.5 Exercise training.

Each subject participated in both interval and continuous training programmes, with a 6 week detraining period in between. Each exercise programme was performed for 6 weeks, training 3 times a week, with at least one rest day between training sessions. Each individual's average heart rate and the distance covered on the treadmill were recorded during each session (appendix 9.2). Subjects were randomly assigned to which training method they completed first. Subjects were fully supervised throughout both training protocols.

5.3.5.1 Interval training.

The interval training consisted of a one minute bout at 90-100 % VO_{2max}, then 4 minutes at 50 % VO_{2max} on a treadmill (Cosmos, Nussdorf-Traustein, Germany). This interval set was repeated 6 times over 30 minutes.

5.3.5.2 Continuous training.

Continuous training consisted of a work rate of 70 % $\dot{V}O_{2\,max}$ on a treadmill (Cosmos, Nussdorf-Traustein, Germany), covering the same distance as when interval training. Therefore, the exercise time varied between 28 - 34 minutes depending on the individual and their fitness level.

5.3.6 Calculations.

The individualised treadmill speed given to each subject was calculated during the $\dot{V}O_{2\,max}$ test. The treadmill speed that the subject was running at was recorded when attaining 50 % $\dot{V}O_{2\,max}$, 70 % $\dot{V}O_{2\,max}$ and 100 % of their $\dot{V}O_{2\,max}$ during the exercise stress test. These speeds were then used throughout both the interval and continuous training programmes.

5.3.7 Statistical Analyses.

The Kolmogorov-Smirnov test was used to ascertain normality of distribution for the data. Once established, repeated measures analysis of variance was used (ANOVA) between baseline and post training values (i.e. within each subject) and between sexes (i.e. between subject factors). Statistics were analysed using SPSS (vs. 14, Chicago, Illinois) and all data presented as Mean ± SE.

For allometric scaled data to lean body mass, regression analysis was performed to ascertain if data from males and females could be selected together. If there were no significant differences, then the data were merged. Regression analysis was then performed at baseline and post training to generate an unstandardised **b** coefficient value of the log of the physiological variable. If each **b** value was within the standard error range, then the **b** values were averaged to gain one **b** exponent to put into an allometric scaling equation ($y = ax^bE$; Batterham & George, 1998).

5.4.8 Power calculations.

An a Priori power calculation was undertaken prior to subject recruitment. Due to no reliability data from previous CPO studies being available, the power analysis for cardiac output has been calculated instead. A previous study by Cade *et al.* (2004) measured the reliability of maximal cardiac output, and found an R value of 0.88. This R value was put into the Cohen's F^2 equation (Cohen,

1988) to calculate the effect size. The α error probability was set at 0.05 and the power (1-β error probability) was set at 0.80, as this is deemed as the minimum level acceptable (Batterham & Atkinson, 2005). The analysis showed that to gain statistically testable results, we required a sample size of 4 or more (Table 5.1).

A post-hoc power analysis was then calculated after the training study was completed. Our results produced an R value for maximal cardiac output of 0.68. This R value was then put into Cohen's F^2 equation (Cohen, 1988) to calculate the effect size F. Total sample size equalled 2, with 2 groups (men and women) and 2 repetitions (0 and 6 weeks). The α error probability was set at 0.05. Using these results, power (1-β error probability) was calculated at 1.0, with a critical F value of 4.38 (Table 5.1). This suggests that our subject numbers of 21 were sufficient to produce statistically significant results.

Table 5.1: A Priori and Post-Hoc power analysis for the 6 week training study.

	A Priori	**Post-Hoc**
Effect size F	1.83	0.92
α error probability	0.05	0.05
Power (1-β error probability)	0.80	1.0
Number of groups	2	2
Repetitions	2	2
Correlation among repeated measures	0.5	0.5
Nonsphericity correlation	1	1
Noncentrally parameter λ	53.57	71.56
Critical F	18.5	4.38
Numerator df	1	1
Denominator df	2	19
Total sample size	4	21
Statistics test	ANOVA repeated measures within factors	ANOVA repeated measures within factors
Test family	F value	F value

5.4 Results.

By the end of the two exercise programmes, 3 men and 4 women had withdrawn. The reasons for dropping-out included time commitments, aversion to testing procedures and musculoskeletal injuries. Thirteen health-screened sedentary men (age 20 ± 2 years, body mass 75.7 ± 2.6 kg, and body fat 17 ± 1 %) and 8 women (age 20 ± 1 years, body mass 58.7 ± 3.5 kg, and body fat 25 ± 2 %) completed the training programme after giving their informed consent to the ethically approved procedures. The adherence rate to the training sessions were 98 % out of 36 sessions over both training modalities. A control group, as such, were not used in this study, due to the exercise training subjects acting as their own internal controls between training modalities. During each training session, participants recorded the distance covered on the treadmill and their average heart rates (appendix 9.2). Participants CV between interval and continuous training showed an average CV for distance covered of 1 % in men and 2 % in women, and an average CV for heart rate of 6 % in men and 8 % in women throughout the six weeks of training. These results suggest that both the interval and continuous training protocols for work loads were well matched.

5.4.1 The effects of training on body composition.

All subjects underwent a DEXA scan before and after both training programmes to detect possible changes in body composition and calculated lean body mass (LBM) to permit Allometric scaling (Batterham & George, 1998). Men decreased their percentage of body fat with both interval (from 17.3 % ± 1.1 % to 16.2 % ± 1.1 %; P = 0.001) and continuous (from17.6 % ± 1.4 % to 16.8 % ± 1.5 %; P = 0.013) exercise training protocols. No significant changes were apparent in the women, with either interval (P = 0.515) or continuous (P = 0.052) training. Supporting the decrease in percentage body fat in men, there were decreases in hip: waist ratios for the interval training (from 0.89 ± 0.02 to 0.83 ± 0.01; P = 0.001) but an increase (from 0.90 ± 0.02 to 0.84 ± 0.01; P = 0.015) after continuous training in men. Supporting the lack of change in percentage body fat in females, there were no changes in hip: waist ratios, after either interval (P =

0.078) or continuous (P = 0.407) training. As anticipated, there were no changes in lean body mass in either males or females after either interval (P = 0.084) or continuous (P = 0.800) exercise (Figures 5.1 and 5.2).

5.4.2 Maximal Aerobic Power and exercise training.

Maximal oxygen consumptions were higher at baseline in men, compared to women, and throughout the exercise training programmes. However, when allometrically scaled to lean body mass, their $\dot{V}O_{2\,max}$ values were not significantly different between sexes (P = 0.212), but both sexes still showed similar improvements with exercise training (Figure 5.3).

In men after 6 weeks of interval exercise training, average $\dot{V}O_{2\,max}$ increased by 5 % (P = 0.004) from 52.3 ± 1.6 ml kg^{-1} min^{-1} to 54.8 ± 1.7 ml kg^{-1} min^{-1}(Figure 5.3). After continuous training, $\dot{V}O_{2\,max}$ increased by 9 % (P = 0.004; from 49.9 ± 0.9 ml kg^{-1} min^{-1} to 54.7 ± 1.2 ml kg^{-1} min^{-1}: Figure 5.4). In women, interval exercise training increased average $\dot{V}O_{2\,max}$ by 7 % (P = 0.001) from 40.2 ± 1.2 ml kg^{-1} min^{-1} to 43.5 ± 1.2 ml kg^{-1} min^{-1} (Figure 5.3), and by 11 % (P = 0.025) from 40.9 ± 0.9 ml kg^{-1} min^{-1} to 46.0 ± 1.7 ml kg^{-1} min^{-1} after continuous training (Figure 5.4).

These improvements in aerobic capacities after interval training, were associated with significant increases in a-vO_2 differences of 4 % in men (P = 0.018) and 5 % women (P = 0.034) after interval and continuous training (Figure 5.5).

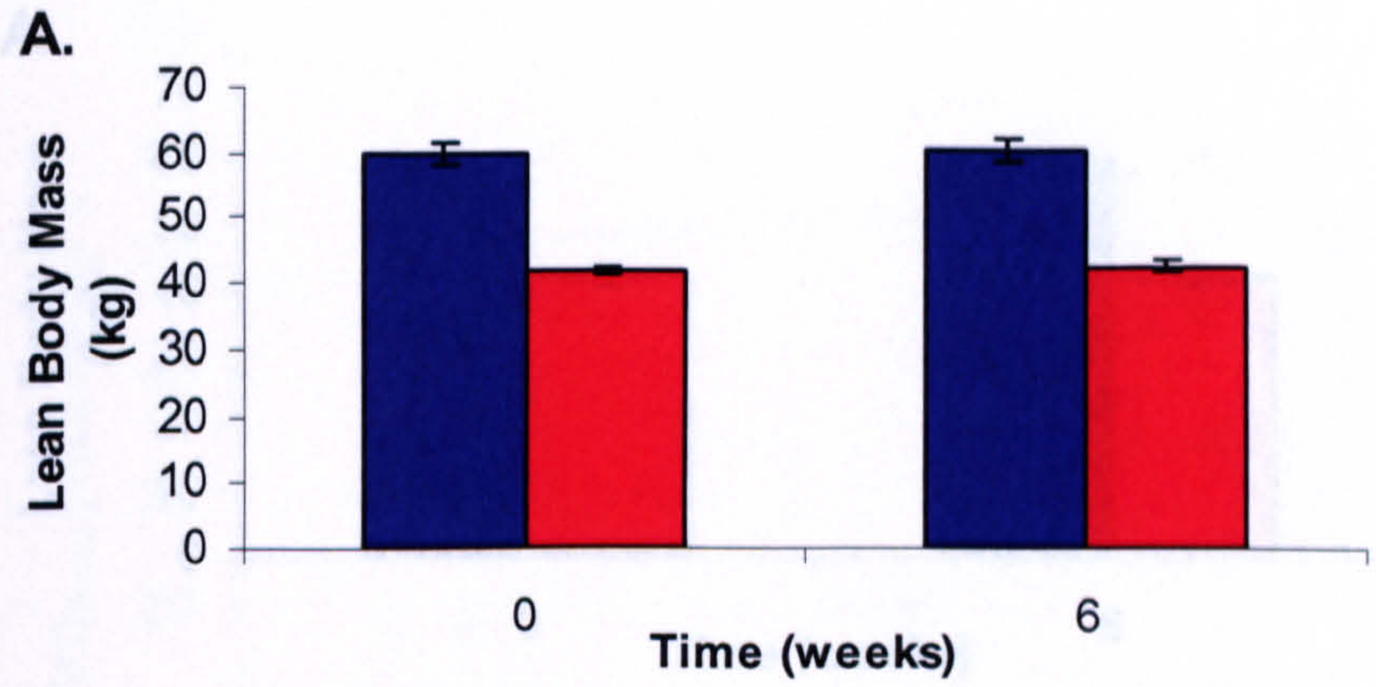


Figure 5.1: Body compositional changes of % body fat (B), lean body mass (A), and hip: waist ratios (C) before (0) and after (6) interval exercise training.
Data are means ± SE for both men (n=13 ■) and women (n=8 ■).
*P < 0.05 statistically significant from pre-exercise training values (0) in both sexes.

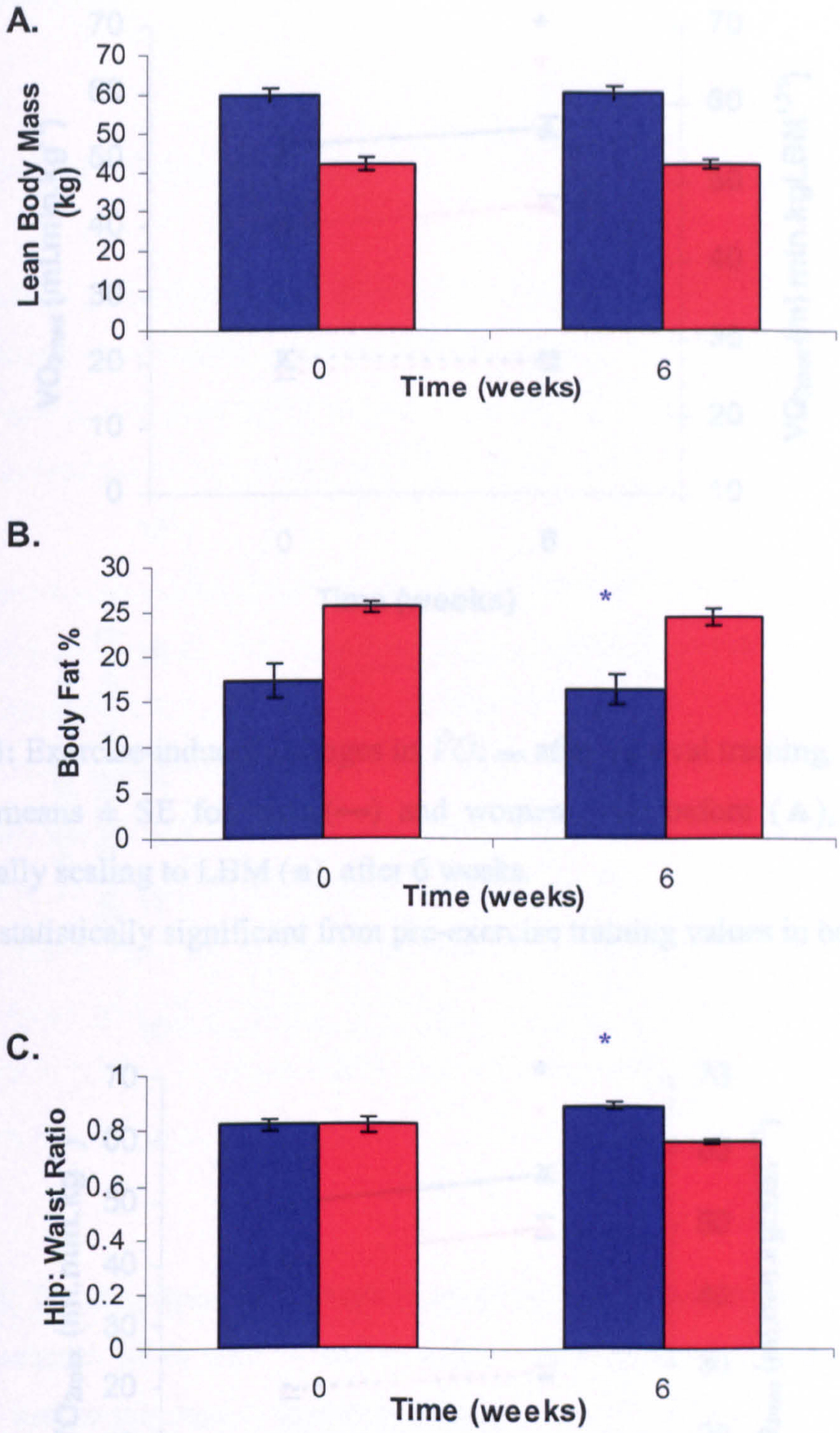


Figure 5.2: Body compositional changes of % body fat (B), lean body mass (A), and hip: waist ratios (C) before (0) and after (6) continuous exercise training. Data are means ± SE for both men (n = 13 ■) and women (n = 8 ■). *P<0.05 statistically significant from pre-exercise training values (0) in both sexes.

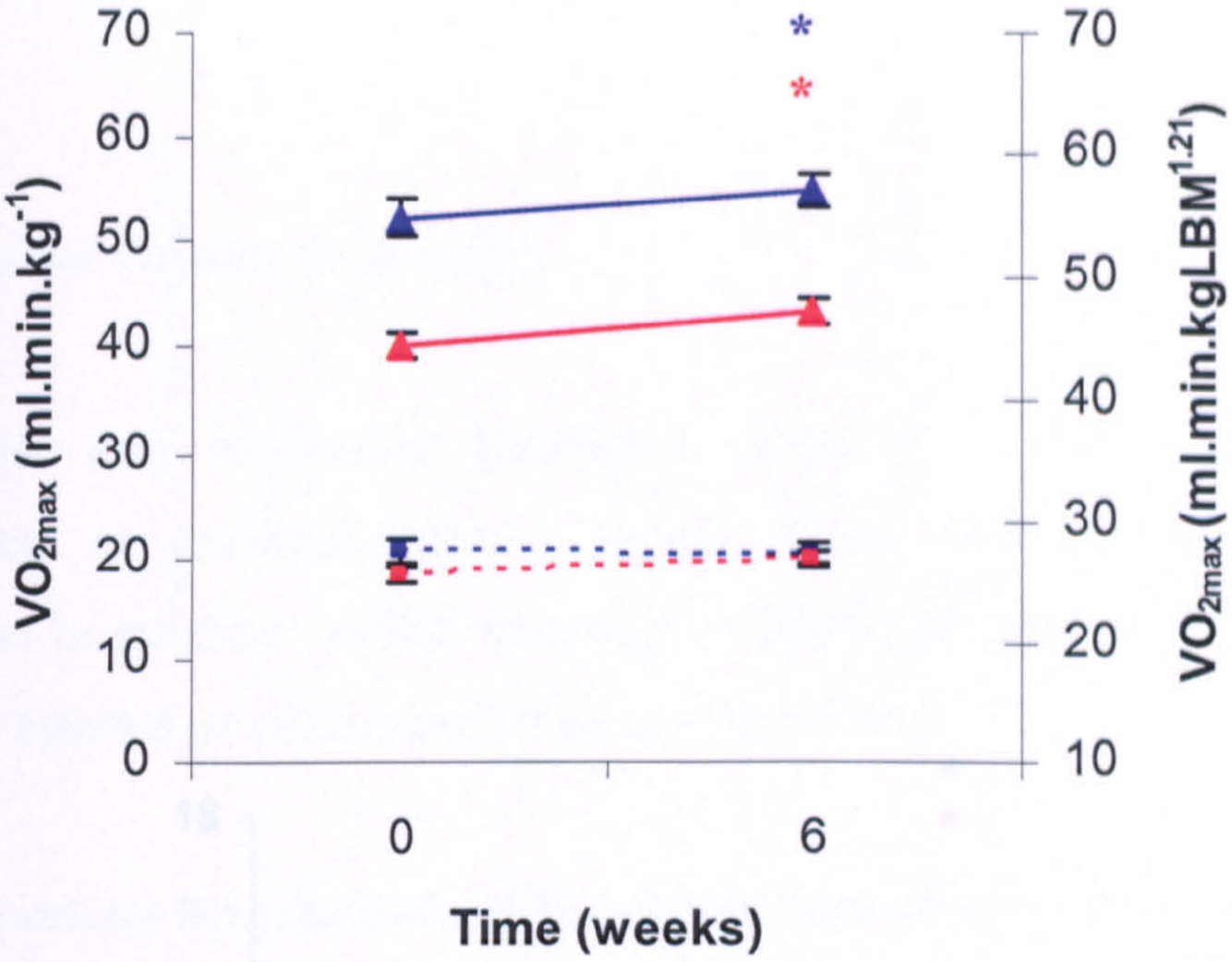


Figure 5.3: Exercise-induced changes in $\dot{V}O_{2\,max}$ after interval training.

Data are means ± SE for men (—) and women (—), before (▲), and after allometrically scaling to LBM (■), after 6 weeks.

*P < 0.05 statistically significant from pre-exercise training values in both sexes.

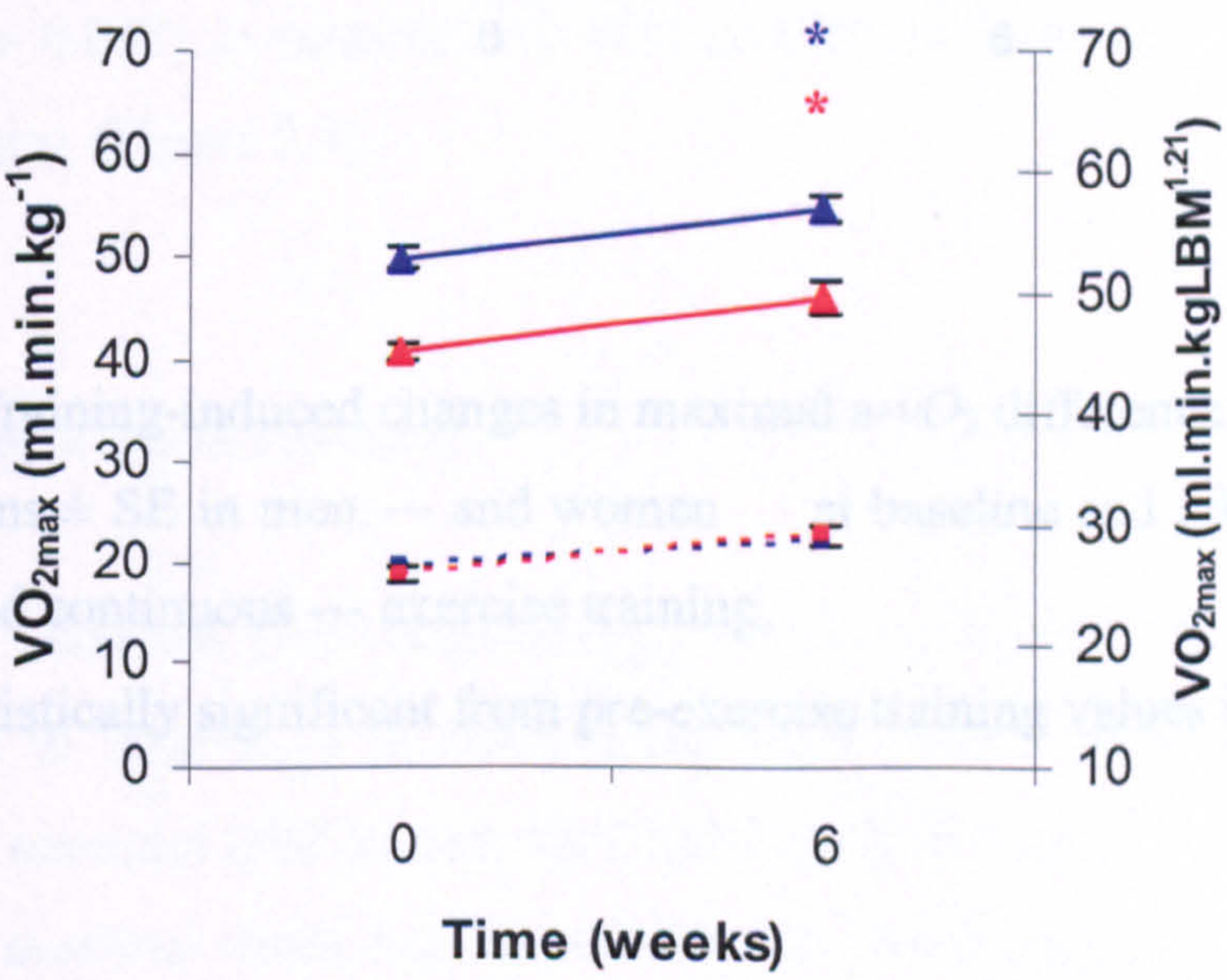


Figure 5.4: Exercise-induced changes in $\dot{V}O_{2\,max}$ after continuous training.

Data are means ± SE for men (—) and women (—), before (▲), and after allometrically scaling to LBM (■), after 6 weeks.

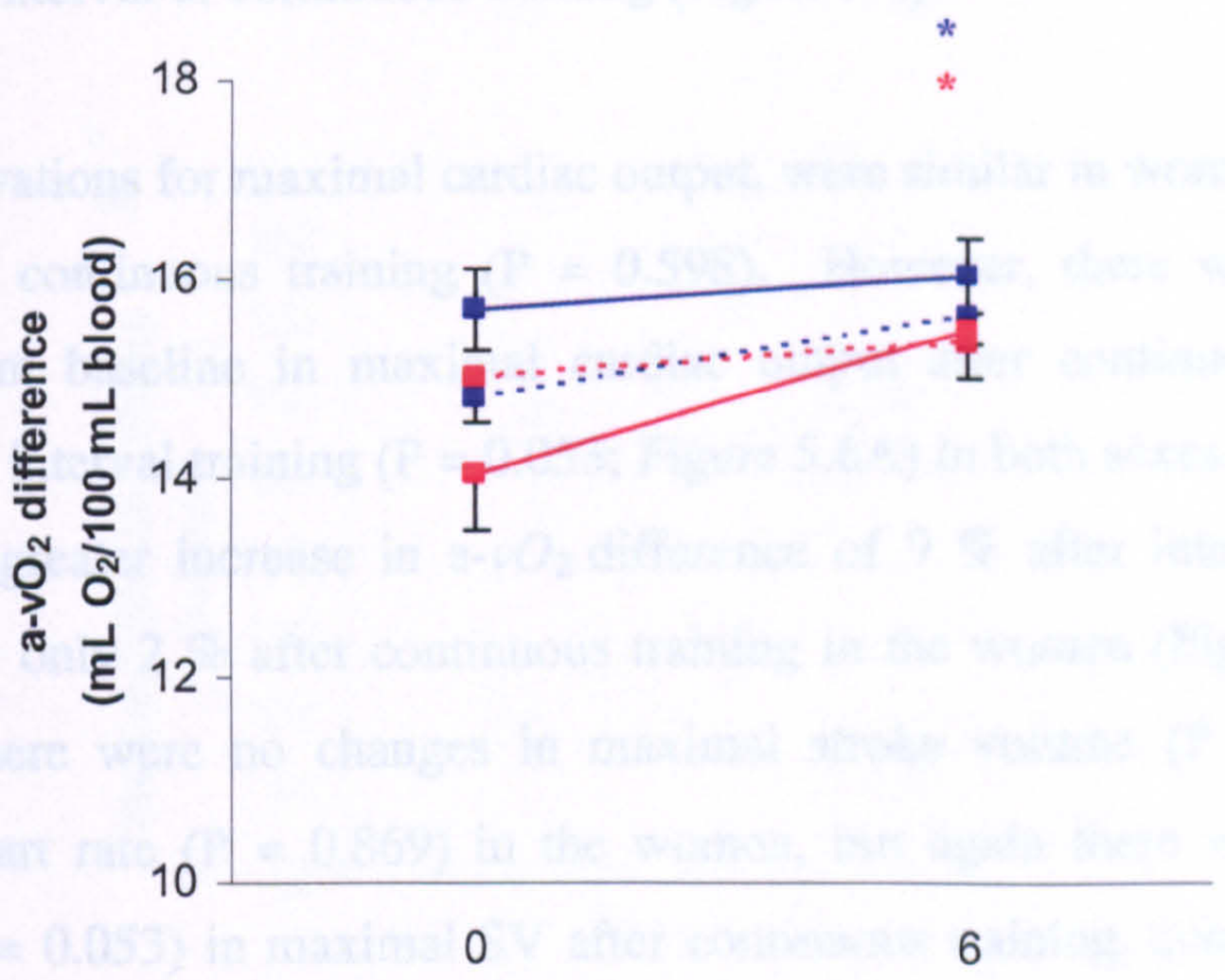


Figure 5.5: Training-induced changes in maximal a-vO_2 difference.
Data are means ± SE in men — and women — at baseline and after 6 weeks of interval — and continuous --- exercise training.
*P < 0.05 statistically significant from pre-exercise training values in both sexes.

5.4.3 Cardiac Function during maximal exercise.

5.4.3.1 Maximal Cardiac Output (Q).

Both interval and continuous training in men (P = 0.344) produced no improvements in maximal cardiac output, which was associated with no improvement in maximal stroke volume (P = 0.641) or maximal heart rate (P = 0.515) after interval or continuous training (Figure 5.6).

These observations for maximal cardiac output, were similar in women after both interval and continuous training (P = 0.598). However, there was a greater increase from baseline in maximal cardiac output after continuous training, compared to interval training (P = 0.053; Figure 5.6A) in both sexes. This would explain the greater increase in a-νO_2 difference of 9 % after interval training compared to only 2 % after continuous training in the women (Figure 5.5). As expected, there were no changes in maximal stroke volume (P = 0.788) or maximal heart rate (P = 0.869) in the women, but again there was a greater increase (P = 0.053) in maximal SV after continuous training, compared to the interval training (Figure 5.6).

5.4.3.2 Maximal Blood Pressure.

In the men, maximal SVR decreased (P = 0.045) slightly after both training protocols (3 % interval, 4 % continuous; Figure 5.8). Although, there were decreases in maximal DBP in men after training, (8 % interval, 10 % continuous; P = 0.000), maximal mean arterial pressure did not change with either training protocols (P = 0.222) due to only a slight increase in maximal SBP in men (P = 0.160; Figure 5.7).

Conversely, maximal SVR in the women did not change (2%) after interval training (P=0.211). However, there was a significant decrease (P=0.044) of 8% after continuous training (Figure 5.8). However, there were no changes in

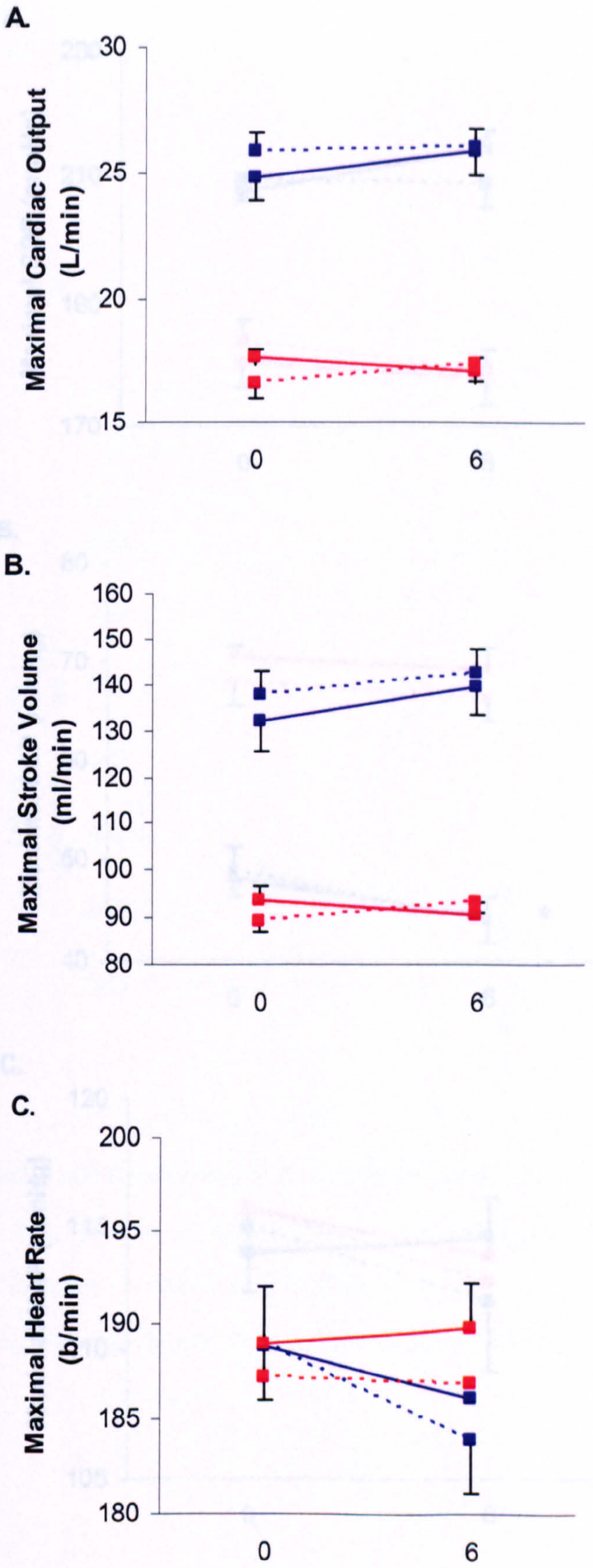


Figure 5.6: Training-induced effects on maximal cardiac output (A), maximal stroke volume (B) and maximal heart rate (C).

Data are means ± SE for both men — and women — at baseline and after 6 weeks of interval — and continuous --- exercise training.

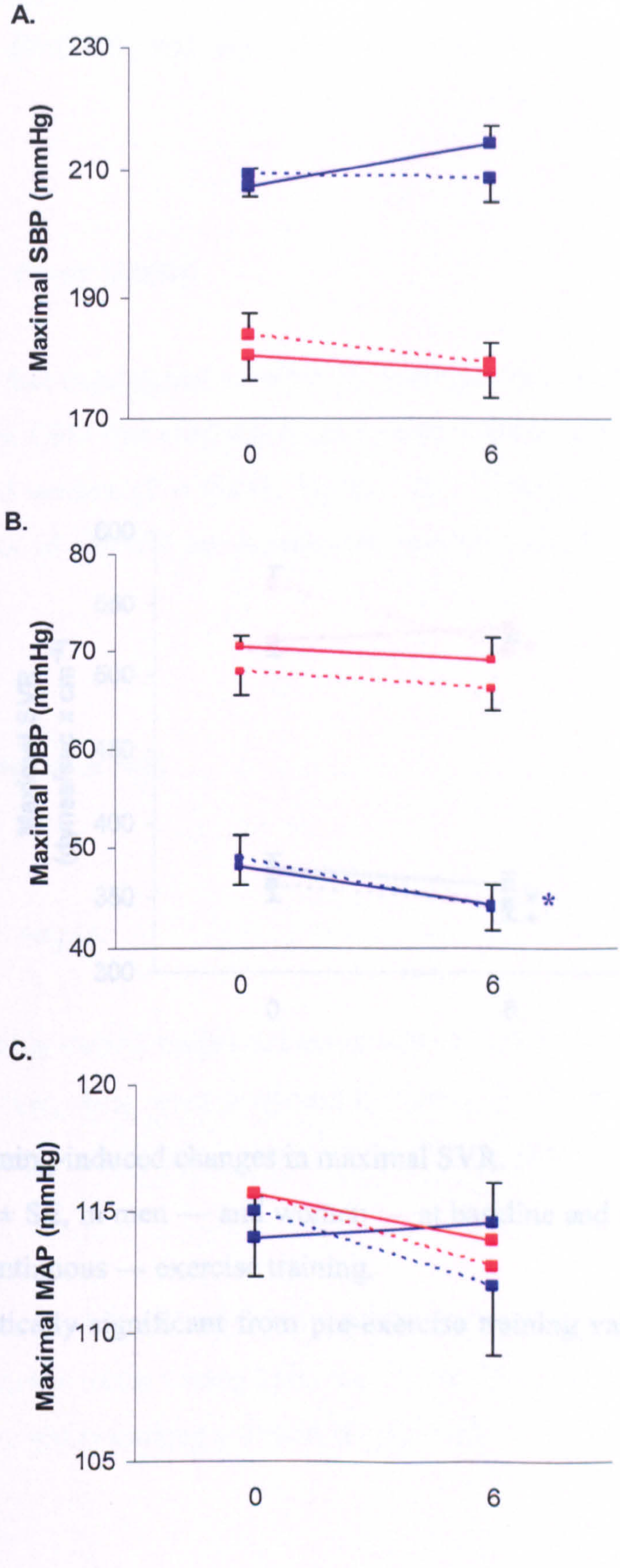


Figure 5.7: Training-induced changes in maximal SBP (A), maximal DBP (B) and maximal MAP (C).

Data are means ± SE in men — and women — at baseline and after 6 weeks of interval — and continuous --- exercise training.

*P < 0.05 statistically significant from pre-exercise training values in both sexes.

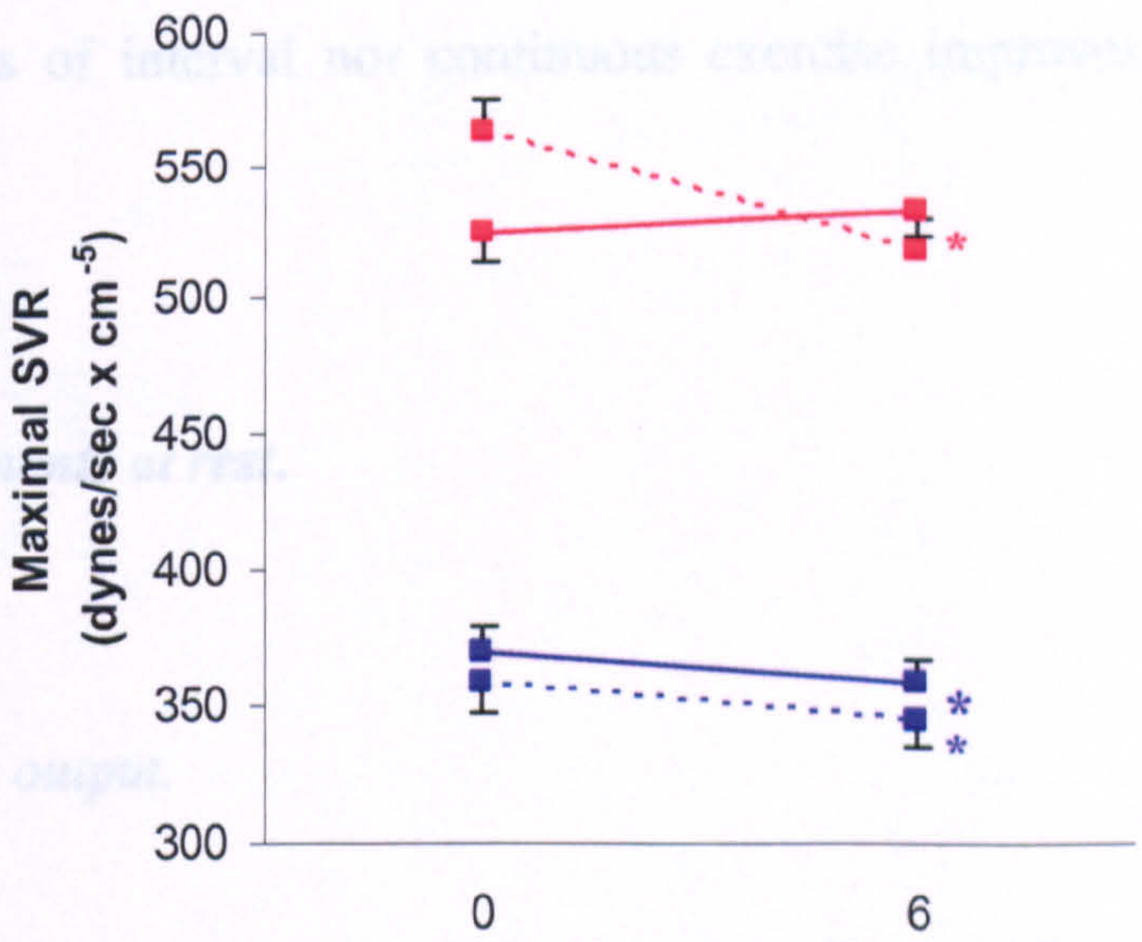


Figure 5.8: Training-induced changes in maximal SVR.

Data are means ± SE, in men — and women — at baseline and after 6 weeks of interval — or continuous --- exercise training.

*P < 0.05 statistically significant from pre-exercise training values in the both sexes.

maximal SBP (P=0.306) or maximal DBP (P=0.080), and so no change in maximal MAP (P=0.203) was noted after each protocol for either sex (Figure 5.7).

5.4.3.3 Cardiac Power Output.

Due to the fact that training had no effect on maximal MAP and maximal cardiac output, maximal CPO was unchanged after either training protocol in both men (P = 0.465) and women (P = 0.453; Figure 5.9). This provides evidence that neither 6 weeks of interval nor continuous exercise improves overall cardiac function.

5.4.4 Measurements at rest.

5.4.4.1 Cardiac output.

In the men, resting cardiac output did not change (P = 0.367) with either training protocol. However, there were decreases in resting heart rate (P = 0.003; 7 % interval, 9 % continuous), but no changes in resting stroke volume (P = 0.186; Figure 5.10).

There were also no changes in resting cardiac output (P = 0.160) in the women due to no changes in either resting heart rate (P = 0.391) or resting stroke volume (P = 0.097) after either training protocol (Figure 5.10).

5.4.4.2 Blood pressure.

There were significant decreases in resting SVR (P = 0.043) in the women, with the interaction plot also showing larger decreases after interval training (P = 0.041; 12 %; Figure 5.12) compared to continuous training (4 %). However,

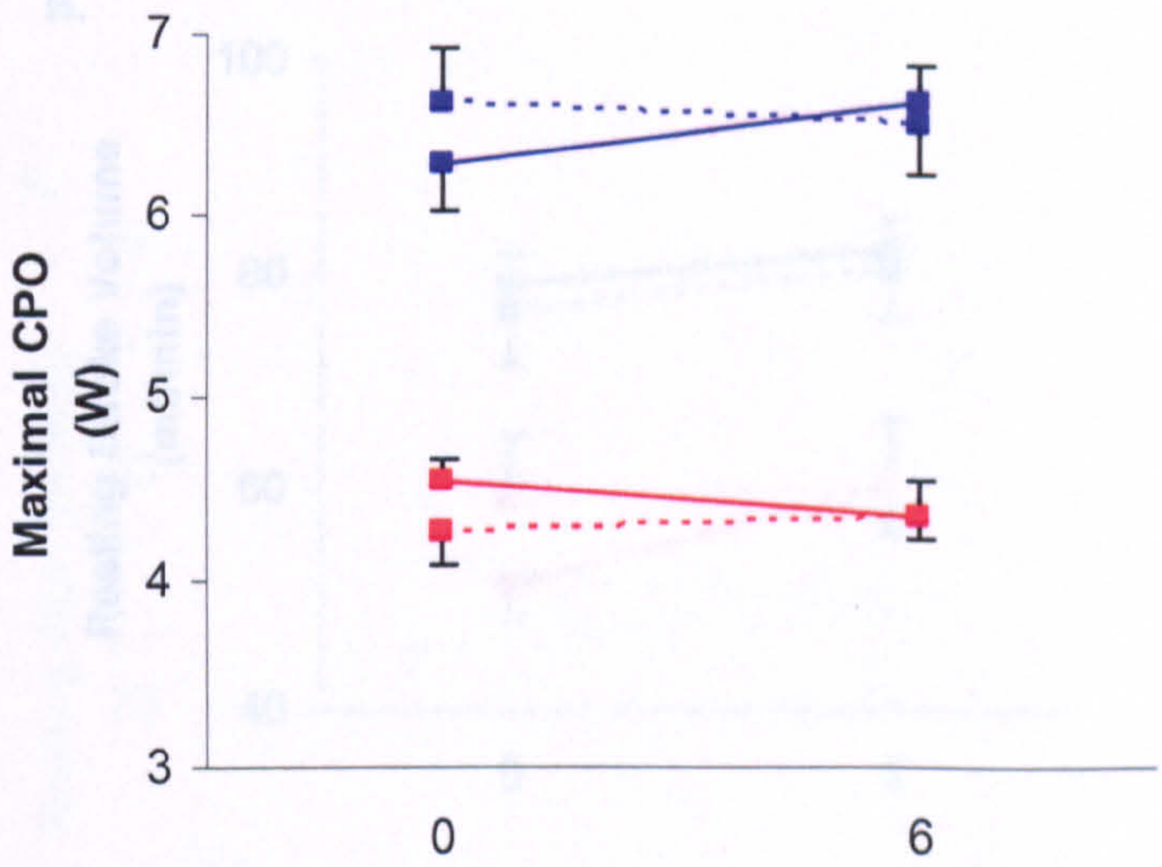


Figure 5.9: Training-induced effects on maximal CPO.

Data are means ± SE, in men — and women — at baseline and after 6 weeks of interval — and continuous --- exercise training.

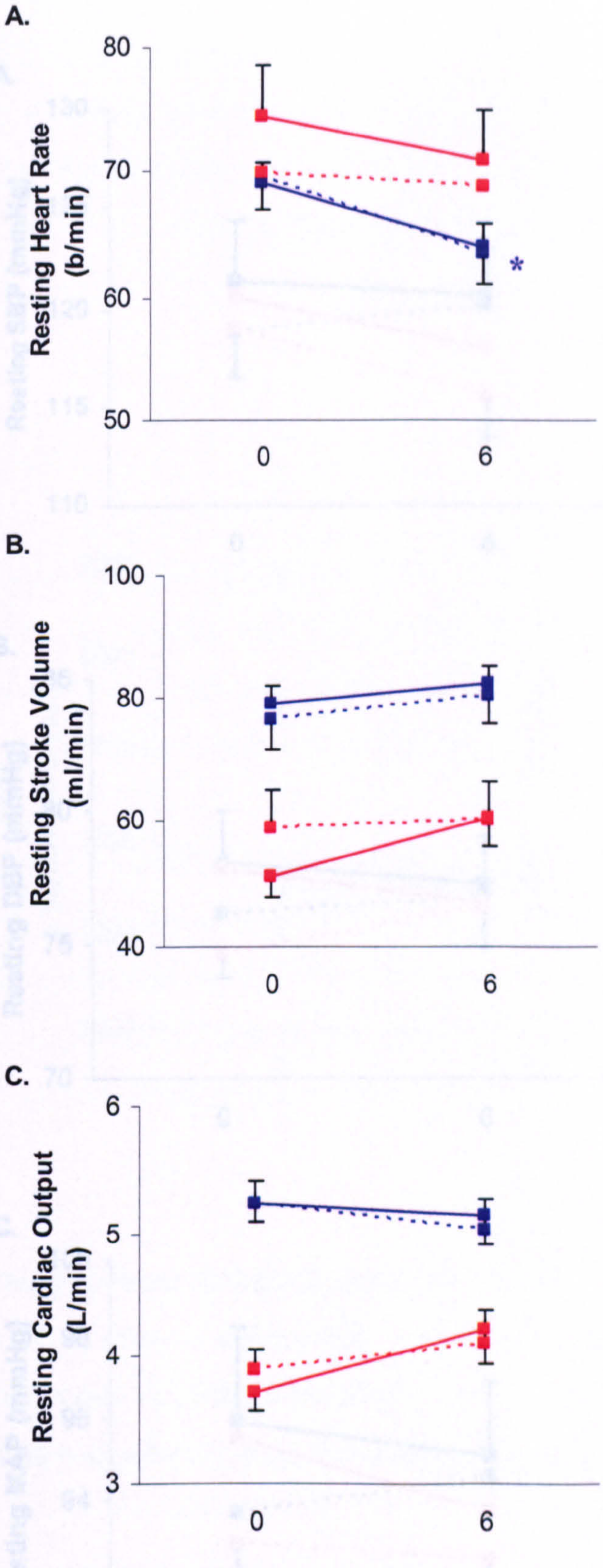


Figure 5.10: Training-induced changes in resting heart rate (A), resting stroke volume (B) and resting cardiac output (C).

Data are means ± SE, in men — and women — at baseline and after 6 weeks of interval — and continuous --- exercise training.

*P < 0.05 statistically significant from pre-exercise training values (0) in both sexes.

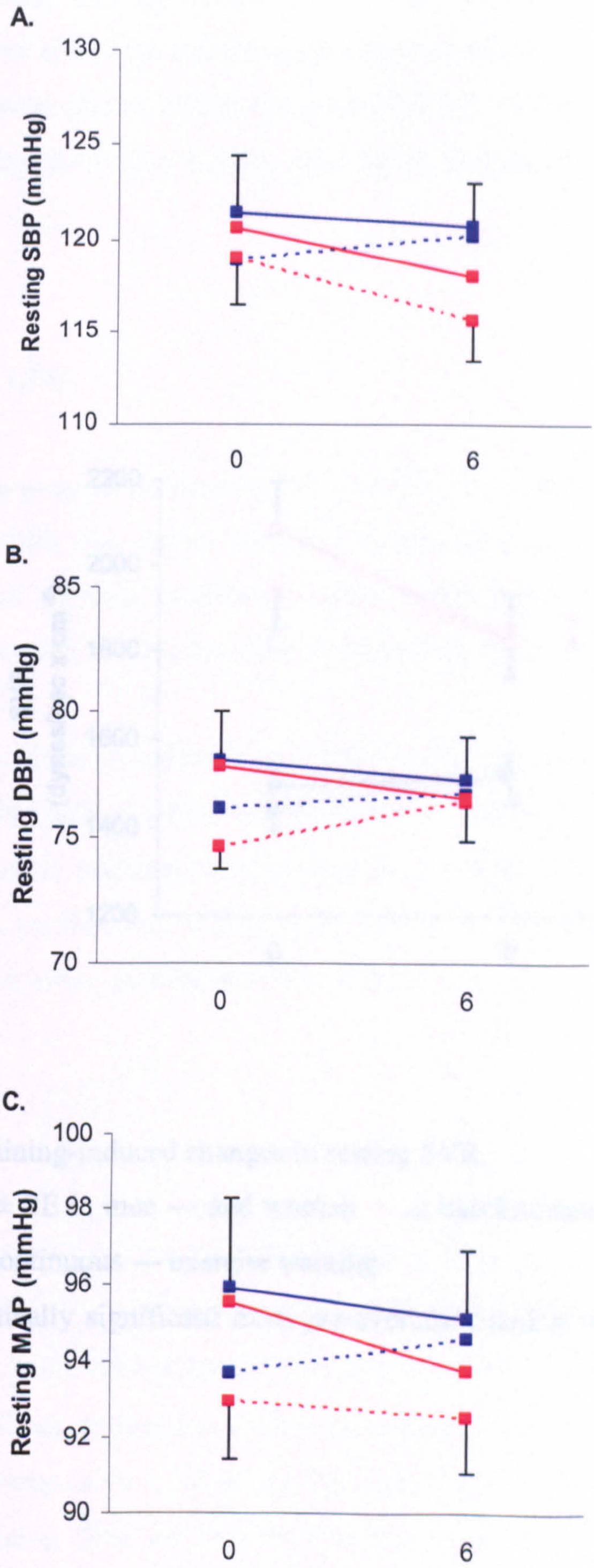


Figure 5.11: Training-induced effects on resting SBP (A), resting DBP (B) and resting MAP (C).

Data are means ± SE, in men — and women — at baseline and after 6 weeks of interval — and continuous --- exercise training.

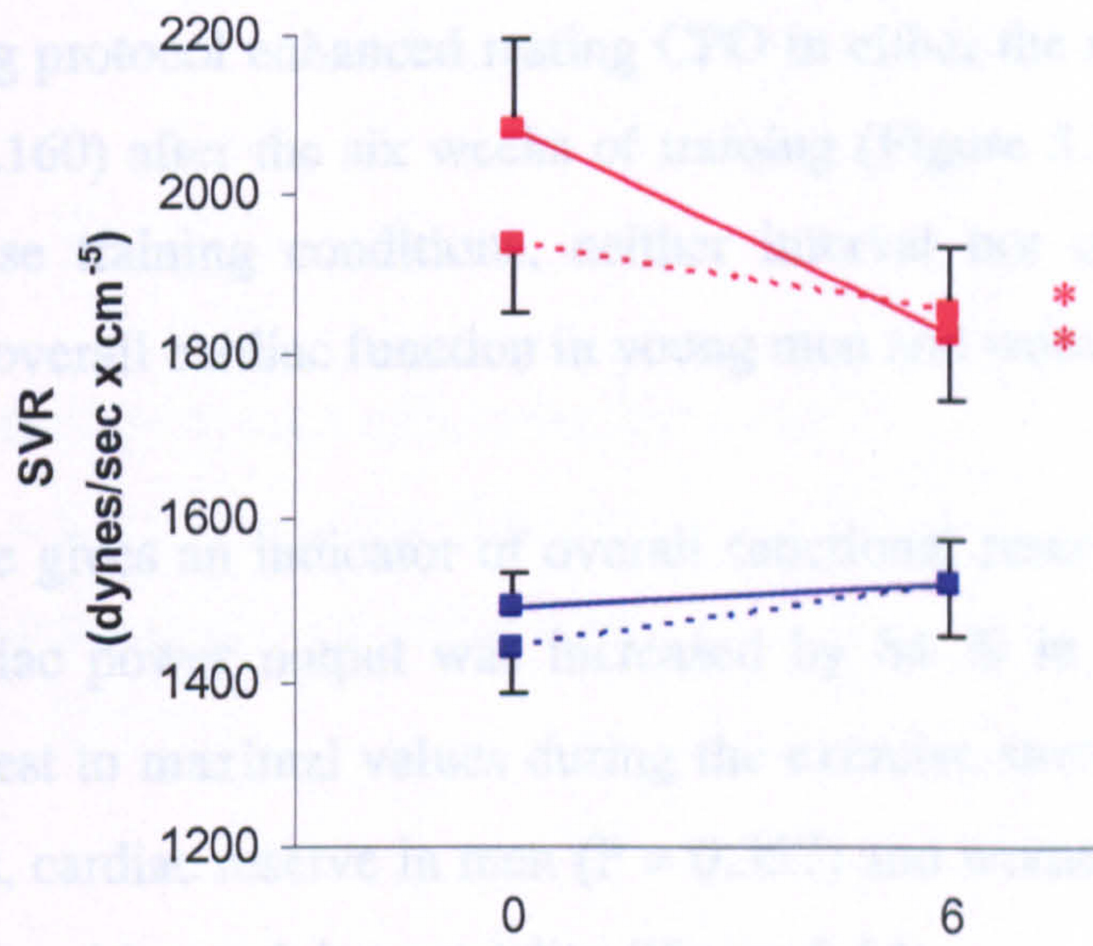


Figure 5.12: Training-induced changes in resting SVR.

Data are means ± SE in men — and women — at baseline and after 6 weeks of interval — and continuous --- exercise training.

*P < 0.05 statistically significant from pre-exercise training values (0) in both sexes.

there were no changes in resting SBP (P = 0.806), DBP (P = 0.192) or MAP (P = 0.622) after either training protocol in women (Figure 5.11). Resting SVR increased in men after both training protocols, but this was not significant (P = 0.468). There were also no changes in resting SBP (P = 0.703), resting DBP (P = 0.917) or resting MAP (P = 0.944), after either exercise protocols in the men (Figure 5.11).

5.4.4.3 Resting CPO.

Neither training protocol enhanced resting CPO in either the men (P = 0.474) or women (P = 0.160) after the six weeks of training (Figure 5.13). This suggests that under these training conditions, neither interval nor continuous training affects resting overall cardiac function in young men and women at rest.

Cardiac reserve gives an indicator of overall functional reserve during exercise. Although cardiac power output was increased by 84 % in men and 81 % in women from rest to maximal values during the exercise stress test prior to both training modes, cardiac reserve in men (P = 0.357) and women (P = 0.089) were not affected after either training modality (Figure 5.14).

5.4.5 Detraining.

Due to all participants being included in both training modalities, a de-training period was necessary to ensure baseline levels we re-attained. Each subject underwent a 6 week de-training period between exercise programmes. There were no significant differences in $\dot{V}O_{2\,max}$ (Figure 5.15) for men, at baseline between continuous (49.9 ± 3.2 ml.min.kg^{-1}) and interval 52.3 ± 5.3 ml.min.kg^{-1}; P = 0.061) exercise. This was also the case for the women (40.9±2.7 ml.min.kg^{-1} continuous; 40.2±3.7 ml.min.kg^{-1} interval; P = 0.617).

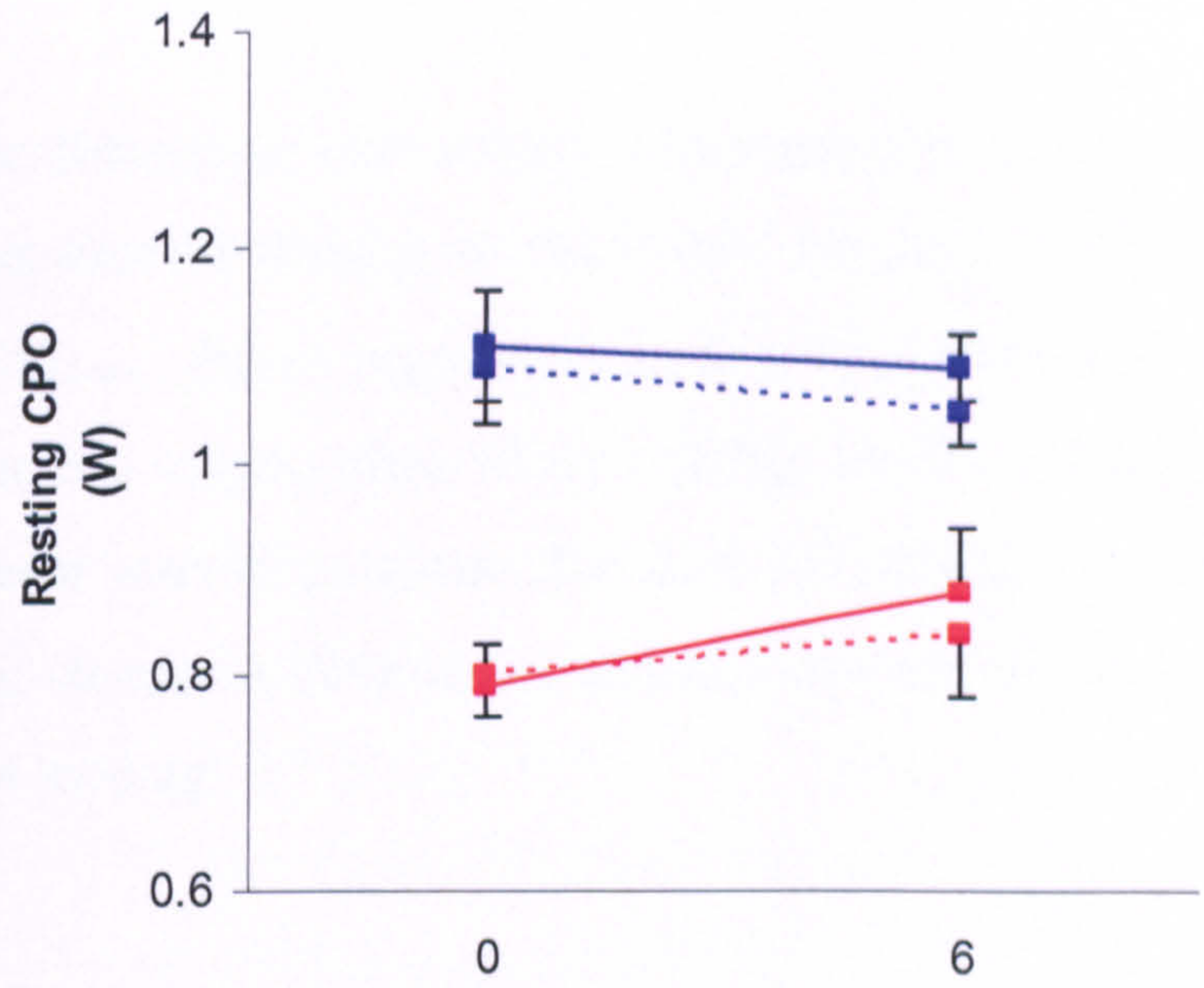


Figure 5.13: Training-induced effects on resting CPO.
Data are means ± SE in men — and women — at baseline and after 6 weeks of interval — and continuous --- exercise training.

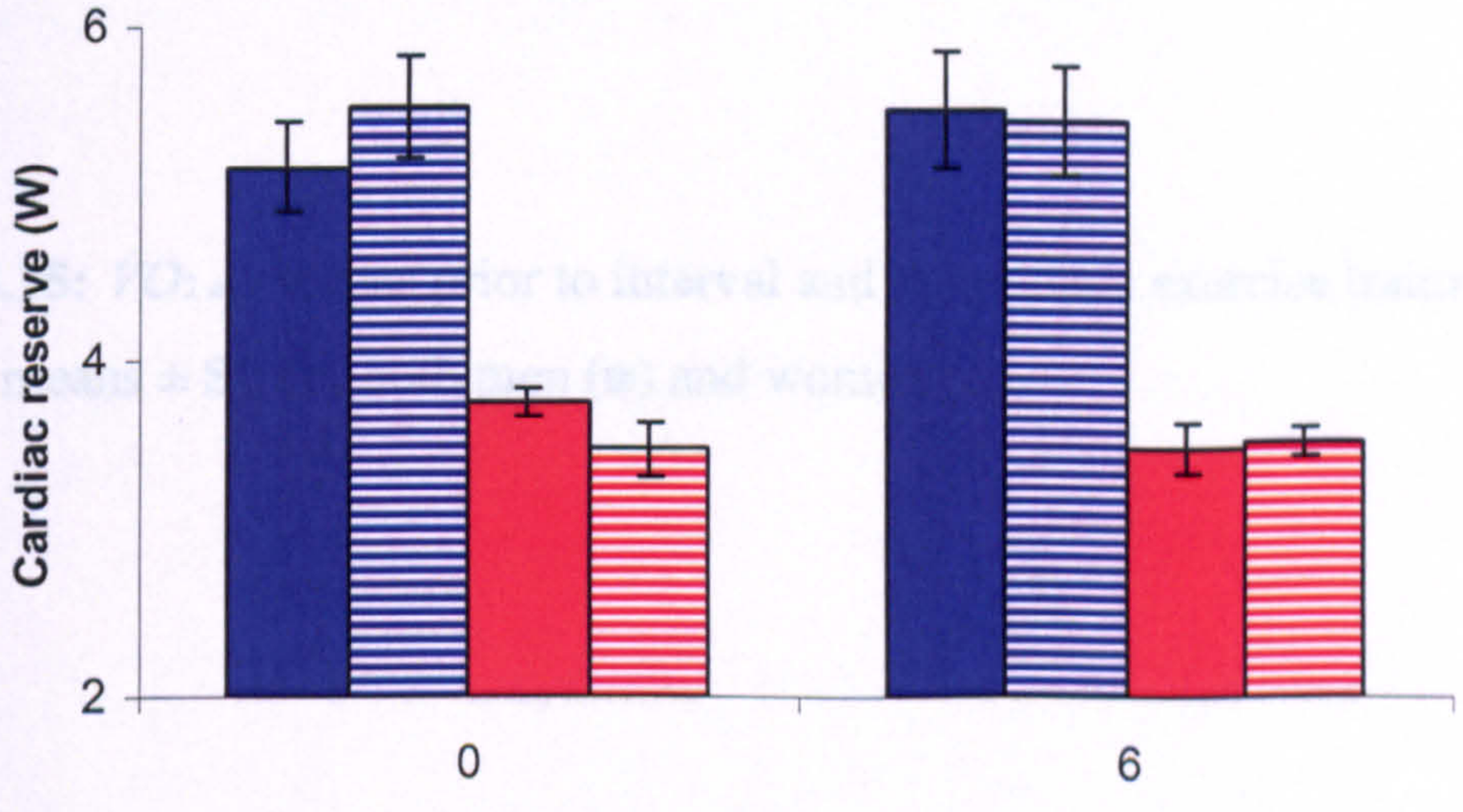


Figure 5.14: Training-induced effects on cardiac reserve capacities.
Data are means ± SE in both men (■) and women (■) with 6 weeks of interval (■) and continuous (≡) training.

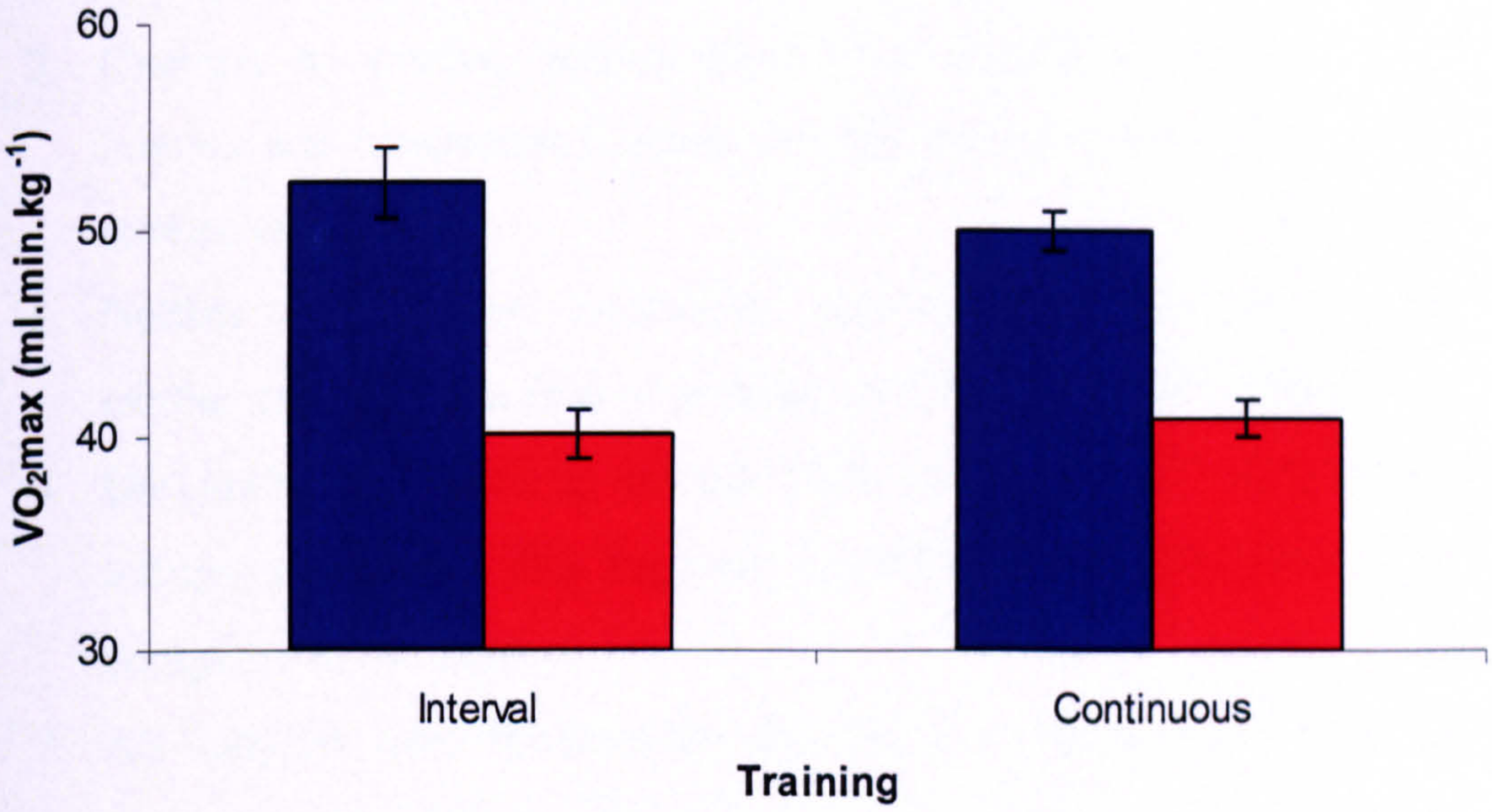


Figure 5.15: $\dot{V}O_{2\,max}$ values prior to interval and continuous exercise training. Data are means ± SE for both men (■) and women (■).

5.4.6 Summary.

No sex-specific differences were evident with regards to cardiac function after 6 weeks of either interval training or continuous training. While improvements were seen in $\dot{V}O_{2\,max}$, due to augmentations in a-vO_2 differences, there were no changes in cardiac output, after either training mode. There were also no changes in mean arterial pressure. The lack of change in $\dot{Q}$ or MAP was reflected by no change in CPO at rest and maximal exercise after both interval and continuous training.

5.5 Discussion.

5.5.1 Key findings of this study.

1. Similar improvements were seen in $\dot{V}O_{2\,max}$ after interval or continuous training for both men and women, which were attributed to increases in a-vO_2 differences. This suggests that after 6 weeks of continuous and interval training, both men and women improved their aerobic capacities through peripheral, but not central cardiac mechanisms.
2. Contrary to women, resting heart rates decreased in men after both interval and continuous training, but this had no overall affect on resting cardiac output.
3. Neither interval nor continuous exercise training affected maximal cardiac output, mean arterial pressure or CPO, regardless of sex.
4. Decreases were seen in resting SVR in women in response to both training protocols. This suggests a greater vascular response in women compared to the men.
5. All subjects were successfully detrained, as determined by measuring their $\dot{V}O_{2\,max}$ and CPO, prior to commencing the second exercise training mode.

5.5.2 Aerobic power and oxygen uptake after interval and continuous exercise training.

The majority of studies now concur that $\dot{V}O_{2\,max}$ can be enhanced, using interval and continuous exercise protocols (Cunningham *et al.*, 1979; Daussin *et al.*, 2007; Eddy *et al.*, 1977; Gorostiaga *et al.*, 1991). Our results also showed that $\dot{V}O_{2\,max}$ can be enhanced after interval and continuous training, especially when similar work-loads are employed between training protocols (Figures 5.3 and 5.4). Our study in men did show a greater increase in $\dot{V}O_{2\,max}$ after continuous training (9 %), compared to only a 5 % increase after interval training. Recent studies using the same duration of 6 weeks have found that continuous training in

men showed an improvement in $\dot{V}O_{2\,max}$ of 8 % (Jones *et al.*, 1999) and 10 % (Carter *et al.* (1999). Greater increases were seen by Convertino *et al.* (1991) who found an increase of 20 % in $\dot{V}O_{2\,max}$ after 10 weeks of cycling exercise at 75-80 % $\dot{V}O_{2\,max}$. Not only was the duration longer, but the exercise intensity undertaken was higher than that used in our continuous training protocol. This suggests that exercise intensity or training volume is an important factor in $\dot{V}O_{2\,max}$ improvement. Thomas *et al.* (1984) deduced that interval training incorporating sprint intervals of 90 % HR_{max} was more beneficial than continuous training at 75 % HR_{max}, in untrained men. This has also been seen in patients with reduced left ventricular function, with increases of 20 % in $\dot{V}O_{2\,max}$ after 8 weeks of high-intensity training (Dubach *et al.*, 1997). These studies suggest that our results should have shown greater increases in $\dot{V}O_{2\,max}$ after interval training. However, Ventura *et al.* (2003) found no change in $\dot{V}O_{2\,max}$ after 6 weeks of high-intensity training in endurance trained cyclists. Therefore, our study may not have shown large increases in $\dot{V}O_{2\,max}$ like Dubach *et al.* (1997) or Convertino *et al.* (1991) due to the participants training status. All of our participants were recreationally trained, participating in one aerobic session per week (i.e. a 5-a-side football match or an aerobics session) and thus were not sedentary before the training. However, when work-loads are identical between training modes, our results suggests that there are no differences in aerobic improvements between both interval and continuous training programmes.

Longer work periods within interval training protocols have been found to utilise more aerobic mechanisms. Khnuttgen *et al.* (1973) found that aerobic power was enhanced greater after 3 minute work periods, compared to 15 second work periods. Roskamm *et al.* (1967) also showed that 2.5 minutes of work was more effective than 1 minute in involving aerobic mechanisms. Similarly, Franch *et al.* (1998) found a 6 % increase in $\dot{V}O_{2\,max}$ after long interval training (6 x 4 min at 16.5 kmh^{-1}, 2 min recovery), compared to a 3 % improvement with short interval training (30 x 15s at 20.5 kmh^{-1}, 15s recovery). However, these improvements are still intensity dependent as 3 minute low-intensity work intervals have shown not to improve aerobic performance (Smith & Wenger, 1981). Therefore, a work period of longer than 1 minute may be needed to enhance $\dot{V}O_{2\,max}$ levels beyond

the values gained in our study. However, the women in our study found the interval training difficult to complete at the start of the programme due to the high-intensity 1 minute work periods. Therefore, increasing the duration of the work period may not have been practical with regard to participant's adherence to the programme. The four minute rest period, incorporated within our interval training protocol, provided some relief from the high-intensity exercise, but was still maintained at a moderate jogging pace at 50 % $\dot{V}O_{2\,max}$. Active rest periods were used as they have been shown to facilitate faster removal of lactic acid, compared to passive rest periods (Hermansen & Stensvolo, 1972; Belcastro & Bones, 1975) and increase the tolerance to heavier work loads, for a longer period of time (Billat, 2001; Billat *et al.*, 2000).

The women showed a similar pattern in $\dot{V}O_{2\,max}$ improvements, with increases of 7 % and 11 % after interval and continuous training respectively, slightly higher than the men. Although these results suggest a training-induced sex differences in $\dot{V}O_{2\,max}$, this 2 % difference was not statistically different. The degrees of improvements in $\dot{V}O_{2\,max}$ after both of our training protocols were consistent with previous studies on women. Cunningham *et al.* (1979) showed that improvements in $\dot{V}O_{2\,max}$ were similar between 12 weeks of continuous (20 %) and interval training (23 %). Lesmes *et al.* (1978) also showed equal increases (13 %) in $\dot{V}O_{2\,max}$ between 8 weeks of supramaximal high-intensity, short distance interval training, and high-intensity long distance interval training, in women. These studies suggest that the degree of improvement in $\dot{V}O_{2\,max}$ in women is the same regardless of training frequency, distance and intensity. Whereas, the aforementioned studies involving male participants, showed that training-induced $\dot{V}O_{2\,max}$ improvements are intensity dependant. Therefore, similar increases in $\dot{V}O_{2\,max}$ within our study were evident, as training work-loads, as determined by distance run, were equated between training protocols.

5.5.3 Cardiac function after interval and continuous exercise training.

The increases in $\dot{V}O_{2\,max}$ after both continuous and interval training in men and women can be attributed to increases in a-vO_2 differences and not a change in central function. However, unlike here, interval training has been shown to improve $\dot{V}O_{2\,max}$ through an increase in maximal cardiac output, whereas continuous training has been affirmed as an increase in maximal a-vO_2 difference (Helgerud *et al.*, 2007; Martin *et al.*, 1987). A recent study showed that 12 weeks of continuous training improved $\dot{V}O_{2\,max}$ through increased oxygen extraction at the muscle (Daussin *et al.*, 2007). Our results are therefore consistent with most studies with regard to the continuous training, but are not consistent with the studies on interval training.

Peripheral adaptations after training have been shown to occur within 3 weeks of endurance training (Hickson *et al.*, 1981). The majority of studies report increases in central cardiac adaptations after endurance training of ~ 8 weeks, therefore suggesting that the length of our training study was not long enough to enhance cardiac function. However, maximal cardiac output has been shown to increase after just 2 weeks of interval training (Adachi *et al.*, 2001) and Warburton et al. (2004) showed increases in maximal $\dot{Q}$, SV and EDV in men after 6 weeks of interval and continuous training.

Despite this, some studies suggest that a longer training period may not improve cardiac function in females. Cunningham *et al.* (1979) showed that 12 weeks of interval or continuous training improved $\dot{V}O_{2\,max}$ only through an increase in a-vO_2 differences. This peripheral change was only noted after 8 weeks of continuous or interval training. This lack of cardiac adaptation was attributed to the type of training undertaken. Kilbom (1971) also found that women undergoing interval training for 12 weeks demonstrated improvements in peripheral mechanisms only, but conversely, continuous training utilised both cardiac and peripheral mechanisms. Although not statistically significant, cardiac output did increase in our study after the continuous training in women, whereas no change was seen after interval training (Figure 5.6). The increase in maximal cardiac output in the continuous group was 5 %, which is greater than

our CV of 3.6 %. Therefore, it is plausible that females do improve their cardiac functions after continuous training, whereas interval training may enhance peripheral functions. In regards to this, studies which have used both men and women grouped together are disadvantaged, as the data could possibly be hiding sex-specific differences. This may well be why recent studies have found changes in both peripheral and central components when using a mixture of both men and women (Daussin *et al.*, 2007).

The lack of central changes after interval training may be due to exercise intensity. Helgerud *et al.* (2007) took trained men and divided them into a slow long distance group (70 % HR_{max}), a 15/15 interval running group (15s of running at 90-95 % HR_{max} followed by 15s of active rest at 70 % HR_{max}) and a 4 x 4 minutes interval running group (4 minutes of 90-95 % HR_{max} followed by 4 minutes of active rest at 70 % HR_{max}. Subjects trained 3 times per week for 8 weeks. The results showed that 15/15 interval group and 4 x 4 min group increased their $\dot{V}O_{2\,max}$ by 5.5 % and 7.2 % respectively. However, there were no changes after continuous training. The increase in $\dot{V}O_{2\,max}$ was attributed to an increase in SV_{max}, since there was no change in HR_{max}. To support these results, there were no changes in red blood cell volume or haemoglobin levels, suggesting there was no change in oxygen carrying capacity to the muscles after interval training.

However, more recently, Daussin *et al.* (2007) showed that after 8 weeks of interval training, both peripheral and central functions were improved in both men and women, with the increase in maximal cardiac output (10 %) being associated with an increased maximal stroke volume and heart rate. A similar interval training study with sedentary males aged 20-30 years, showed that after 12 weeks of training, cardiac output was increased due to equal increases in stroke volume (9 %) and heart rate (5 %; Makrides *et al.*, 1990). The increase in stroke volume after training has been associated with the utilisation of the Frank-Starling mechanism. Warburton *et al.* (2004) also found that interval training improved left ventricular diastolic function (e.g. diastolic filling), but there was little improvement in myocardial contractility. Data from M-mode and 2D echocardiography have also shown that endurance training improves left

ventricular wall thickness, end-diastolic diameter and volume, and an increase in left ventricular mass (Owen *et al.*, 2004; Giada *et al.*, 1998; Schairer *et al.*, 1993; Ehansi *et al.*, 1978). Martin *et al.* (1987) studied the effect of 12 weeks of interval training in middle-aged swimmers. The results showed that there was an increase of 7 % in peak cardiac output, which was attributed to an augmented stroke volume (10 %). There was also an increase in peak systolic blood pressure (8 %) after training, but there were no changes in ejection fraction or end-systolic volume, which could indicate improved contractile performance. However, systolic pressure/volume ratio did not change, therefore suggesting an increase in myocardial mass rather than a change in contractile state. Conversely, Doppler measurements have also shown increases in left ventricular ejection fraction after short-term high intensity interval exercise (Foster *et al.*, 1995, 1997, 1999 Upton *et al.*, 1980). This has been associated with a loss of the Frank Starling reinforcement of ventricular function, causing the maintenance of SV and $\dot{Q}$ through a decline in LV afterload.

Although our results showed no changes in maximal cardiac function, there were decreases in resting heart rates after both interval and continuous training in men (Figure 5.10A). Long-term endurance training has shown increases in parasympathetic activity and a decrease in sympathetic activity, causing a decrease in resting heart rate. Studies reporting changes in $\dot{V}O_{2\,max}$ greater than 12 ml.min.kg^{-1} have shown increases in parasympathetic control (Shi *et al.*, 1995; Smith *et al.*, 1989), whereas the studies showing smaller increases in $\dot{V}O_{2\,max}$ have shown no change (Ekblom et al, 1973; Maciel *et al.*, 1985). Katona *et al.* (1982) reported that elite male rowers, with a $\dot{V}O_{2\,max}$ of 25 ml.min.kg^{-1} greater than their sedentary counterparts, had a lower parasympathetic control over heart rate. Conversely, Shin *et al.* (1997) reported that athletes (long distance runners) had a significantly higher parasympathetic control, compared to non-athletes. These discrepancies can be attributed to the athletes' previous training histories, as Bouchard & Rankinen (2001) showed that there is considerable heterogeneity in response to regular physical activity. In our study, increases of only 5 % and 9 % in $\dot{V}O_{2\,max}$ after interval or continuous exercise training respectively in men, suggests changes in parasympathetic activity, due to

a decrease in HR_{rest} (Figure 5.10). However, studies have shown that men at rest have a greater sympathetic influence on heart rate, compared to females who in turn have greater parasympathetic control (Gregoire *et al.*, 1996; Huikuri *et al.*, 1996; Kuo *et al.*, 1999). It is possible that the men in our study could have enhanced their parasympathetic control after training, hence provoking the decrease in HR_{rest}. In contrast, the females showed no change in heart rate possibly due to greater initial parasympathetic control prior to training. Given this, we did not measure parasympathetic control, this hypothesis can only be speculative.

Arterial compliance in the central vessels determines systolic blood pressure, and this effects left ventricular afterload (O'Rouke, 1990). Our results showed no decline in maximal systolic blood pressure after either training protocols, but decreases in diastolic blood pressure were seen in the men (Figure 5.7). Such measurements of blood pressure are seldom measured at maximal exercise. Portier *et al.* (2001) showed a 3 % decrease in resting diastolic blood pressure in elite runners after 12 weeks of intense training. Sokmen *et al.* (2005) also found that resting SBP and DBP in men were reduced after 10 weeks of interval training, which were not significantly different from the group undergoing continuous training. This decrease in resting blood pressure was not apparent in our results. Endurance training, with a duration of 3 months, has been shown to decrease blood pressure through a decrease in SVR (Iwasaki *et al.*, 2003). A meta-analysis conducted by Fagard (2006) concluded that aerobic training decreased blood pressure through a decrease in SVR, in which both the sympathetic nervous system and the angio-tensin system decreased. Our results also showed decreases in maximal SVR after both interval and continuous training in men, but only after continuous training in women (Figure 5.8). Training programmes of 12 months duration have shown little enhancement of blood pressure levels from the initial increases gained in the first 12 weeks of training. In fact, Iwasaki *et al.* (2003) found that blood pressures had returned to pre-exercise values by the end of a 12 month training programme and were not affected by the higher intensity protocol initiated towards the end of this lengthy programme. Therefore, these results suggest that higher-intensity training may not be as effective as lower intensities in decreasing blood pressure. This could

explain the lack of enhancement in SVR_{max} in the females after interval training and the greater increase in SVR_{max} after continuous training.

At rest, both female training groups showed decreases in SVR_{rest}. In contrast, there were no changes in the male training groups. A recent meta-analysis by Cornelissen & Fagard (2005) showed that aerobic training decreased SVR_{rest} due to decreases in the sympathetic nervous system, and plasma nor-adrenaline (29 %). However, this meta-analysis did not disclose any sex differences in sympathetic response to exercise.

Sympathetic activity at rest has been found to be increased through the higher oestrogen and progesterone levels in women (Minson *et al.*, 2000). Oestrogen has been associated with an increase in nitric oxide and improved peripheral vascular tone (Sudhir *et al.*, 1996). All of the women in our study were taking the combined contraceptive pill. As yet, studies are still undecided on the effects of the combined contraceptive pill, but Minson *et al.* (2000) found a greater sympathetic response to these sex hormones in women taking the combined contraceptive pill, compared to normal menstruating women. The decrease in SVR at rest in this study may be due to higher oestrogen levels during the pill consumption.

Having considered the training-induced changes in $\dot{Q}$ and MAP, not surprisingly, our results showed no improvements in CPO or cardiac reserve after either training protocol in both sexes (Figures 5.9 and 5.14). Very few studies have measured CPO in response to training. Marshall *et al.* (2001) studied 9 healthy men and women participating in an endurance training protocol for 8 weeks. The results showed an improvement in overall cardiac function through an increase of 16 % in CPOpeak and 21 % in cardiac reserve. However, the training protocol was a home-based cycling protocol working at a higher intensity (75-80 % $\dot{V}O_{2\,max}$) than our continuous exercise group. Marshall *et al.* (2001) also used middle-aged sedentary participants, whereas our study used younger, recreationally active participants. Therefore, initial training status and age may have an effect in improving CPO after exercise training, with more scope for improvement from a sedentary status. Studies which depend on participants

undertaking the training programmes themselves without any supervision should be viewed with caution when producing positive results. Every training session in our study was fully supervised to ensure all the participants followed the correct protocols.

5.5.4 Detraining.

The unique aspects of our study was to investigate the same men and women after both training protocols i.e. longitudinal studies. This posed the problem of adequate detraining to ensure all cardiovascular variables had reached baseline values before the start of the second training protocol. Wang *et al.* (1997) showed a reduction in $\dot{V}O_{2\,max}$ of around 6 % within 2 weeks of detraining. Average $\dot{V}O_{2\,max}$ increases were around 7 % for men and around 9 % for women after the first training protocol of 12 weeks. Therefore, a detraining period of over at least two weeks was successful. Also the detraining time to restore blood pressure was only one-half of that needed to elevate blood pressure during the exercise training programme. The one variable which appears to take longer to return to baseline after training is a-vO_2 difference. Coyle *et al.* (1984) found that a-vO_2 difference did not to decline after 56 days of detraining. However, this study did recruit endurance trained athletes and therefore cannot be compared to newly trained participants.

From our results we conclude that all cardiovascular indices did re-gain baseline values after 6 weeks of detraining. Therefore, it was plausible to use the same participants to compare different training protocols, but more research needs to be undertaken to ascertain the minimal de-training period required to restore pre-training cardiovascular levels. Our measurements in this study focussed mainly on cardiac indices, hence the effects of the detraining on muscle metabolism is also unknown.

5.5.5 Limitations.

5.5.5.1 Training period.

It is not clear from the literature what the shortest effective time is to induce changes in cardiac function through exercise training. Although 6 weeks, 3 times a week, was effective in increasing $VO_{2\,max}$ it did not affect cardiac function. A longer time of training may have been more fruitful. Similarly, the duration and intensity used during the sprint periods in the interval training. Such considerations needed to be more extensively studied to design more effective protocols, which distinguish differences (if any) between continuous (fixed intensity) and interval training, whilst crucially ensuring that the participants undertake the same amount of work.

5.5.5.2 Menstrual cycle phase.

All females were on the combined oral contraceptive pill and were tested within the non-menstrual phase. Due to the length of each training modality, each woman would not have been tested in the same phase of their cycle. Menstrual cycle phase has been shown to affect cardiovascular functions (Stoney *et al.*, 1986) and therefore may have impacted the results. However, the use of the contraceptive pill should have balanced oestrogen and progesterone levels throughout the 21 non-menstruating days.

5.5.5.3 Equating exercise training workloads.

Few studies have equated workloads to directly compare two different training modalities. We equated our workload by using distance covered and $\dot{V}O_{2\,max}$ equated work loads during the training sessions. We believe this to be crucial, however other parameters such as calorific usage, oxygen uptake kinetics

and heart rate could have been selected. However, oxygen uptake and heart rate are both widely variable during exercise training, especially during interval training. Therefore, distance covered was easy to calculate, did not require extra equipment, and did not fluctuate with training. Heart rates were recorded with exercise training and were not significantly different between training modalities in either men or women. While crucially controlled for the timing of food and caffeine (Study 1) intake prior to making measurements, overall dietary intake was not controlled even though the same diet was recommended over the study periods.

5.5.6 Summary.

This study was the first to directly compare interval and continuous training, at the same maintained work loads, in both men and women. It is unclear why many other studies have not used the same participants in each training mode, but our results suggest that after 6 weeks of either interval or continuous training, a detraining period of 6 weeks is sufficient to restore baseline cardiorespiratory values. Therefore, to clarify the widespread discrepancies within the literature, regarding the effects of interval and continuous training on central function, the recruitment of the same participants for each training study would be advisable and the work loads matched accordingly.

Aerobic fitness is improved in men and women after both interval and continuous training protocols. In agreement with previous studies, training modality does not appear to affect the extent of the improvement in $\dot{V}O_{2\,max}$. The equated training work loads between our interval and continuous training protocols have shown similar increases in $\dot{V}O_{2\,max}$. Therefore, setting work loads in relation to $\dot{V}O_{2\,max}$ and distance covered is acceptable in being able to directly compare different training modalities. Although aerobic power is improved after 6 weeks of training, this time period is too short to induce central adaptations. However, the decreases in resting heart rates after both the continuous and interval training protocols in the men, suggests an increase in parasympathetic control, and the decreases in maximal and resting SVR, indicating an

improvement in vascular function. These changes could be viewed as early adaptations, subsequently leading to changes in overall cardiac function. However, a longer training period may be required to start to induce central adaptations in response to both interval and continuous training.

5.5.7 Future directions.

This study was initiated to determine whether interval training is more effective in improving cardiac function. The results from study 2 suggest a lack of central adaptation in response to 30 weeks of continuous endurance training. Although the present study was undertaken with younger subjects, Makrides *et al.* (1990) has stated that high-intensity interval training elicits similar cardiovascular changes in both young and old men. This therefore suggests that if a similar protocol was instigated using older subjects and interval training, no changes in cardiac function would occur after 6 weeks. Therefore, to ascertain whether older or younger subjects do adapt centrally to a high-intensity interval programme, a longer training period is probably required. However, it is important to highlight that the present study and study 2 have both shown improvements in peripheral function, as defined by differences in a-vO_2 differences.

The periphery can be divided into two areas. Firstly, vascular blood flow and secondly muscle metabolism. Vascular blood flow has been investigated with regard to sex-specific responses to exercise training, but no studies have yet reported sex-specific differences in muscle metabolism with regard to interval and continuous exercise training. In the last decade, studies have shown sex differences in cardiac muscle, especially in relation to differences in cardiomyocyte number (Olivetti *et al.*, 1995), but little research has been undertaken concerning sex-specific protein expression in skeletal muscle after different modes of exercise training. Since a-vO_2 differences relate to oxygen extraction by muscle, it would seem important to examine changes in muscle protein profiles before and after exercise. This has been undertaken in study 4.

Chapter 6

Study 4 - The Effects of Exercise Training on Muscle Protein Expression in Young Men and Women.

6.1 Background.

Gorostiaga *et al.* (1991) were the first to compare interval and continuous training protocols at the same overall work loads. The results showed similar improvements in oxygen uptake, but these increases were associated with different metabolic processes. The improvement in O_2 uptake after interval training was attributed to higher utilisation of ATP from anaerobic respiration during work periods (Essen *et al.*, 1977), while lactate removal was accelerated through oxidation and/or glycogen synthesis within the muscle during the rest phase (McLane and Holloszy, 1979; Essen & Kaijser, 1978). After continuous training, ATP production was synthesised through aerobic lipid metabolism (Newsholme. 1984), with very little contribution from anaerobic glycolysis (Essen *et al.*, 1977). Nonetheless, interval training has become popularised due to the 'active phase' being considerably shorter than the 'active phase' during endurance training, resulting in less work being required to gain the same cardiovascular advantages.

6.1.1 Metabolic energy systems during exercise.

Interval training combines both near maximal work and low-intensity activity. For immediate energy requirements, ATP (adenosine triphosphate) reserves are used. This involves the hydrolysis of ATP, via ATPase, which produces ADP (adenosine diphosphate) and Pi (inorganic phosphate). Peak rates of ATP turnover are around 15 mmol/kg dm/sec, which can fuel approximately 1-2 seconds of maximal work (Gaitanos *et al.*, 1993; Parolin *et al.*, 1999). Once ATP stores become depleted, ATP is regenerated from other metabolic processes, including the phosphocreatine system, which is required for a high rate of energy release. Phosphocreatine (PCr) and ADP are catalysed by creatine kinase to form ATP and creatine. There are approximately 80 mmol/kg dm of stored intramuscular PCr, with maximal turnover rates of 9 mmol ATP/kg dm/sec during maximal work, causing a large depletion of stores within 10 seconds (Bangsbo *et al.*, 2001). Creatine kinase is a key catalyst which encodes 5 known subunits. Creatine kinase-B (B for 'brain') and creatine kinase-M (M

enzyme chain exist, and hence the regulation of cellular metabolism. The TCA cycle consists of a series of enzyme-catalysed reactions which occur in the matrix of the mitochondrion. The energy made available by the TCA cycle is transferred as energy-rich electrons to the respiratory co-enzyme chain. The respiratory co-enzyme chain, otherwise known as the electron transport chain, removes electrons from an electron donor (NADH or FADH2) and passes them to a terminal electron acceptor via a series of redox reactions, made up of four complexes (Figure 6.1).

Fast oxidative fibres (IIa) have similar oxidative capacity to type I fibres, but possess a faster rate of contraction and are relatively fatigue resistant. Slow and type IIa fibres have higher capillary densities than IIx fibres. However, the fibre volume occupied by mitochondria is around 6 % in type I, 4.5 % in IIa and 2.3 % in IIx (Howald *et al.*, 1985). Although IIx fibres contain a lower mitochondrial content, they have the fastest rate of contraction and a poor resistance to fatigue (Spangenburg & Booth, 2003). During exercise, glycogen decreases first in the slow fibres, followed by the fast fibres. This order has been suggested to reflect the order of motor unit recruitment.

6.1.3 Skeletal muscle contraction.

Skeletal muscles are largely heterogeneous in nature, possessing a range of mechanical and energetic properties, which are essential to manage different demands. However, individual fibres vary in force development. This is due to a number of determinants, including the rate of calcium uptake and release from the SR, calcium sensitivity of the contractile apparatus and the tension developed when actin and myosin heads interact to form cross-bridges (Bottinelli & Regiani, 2000).

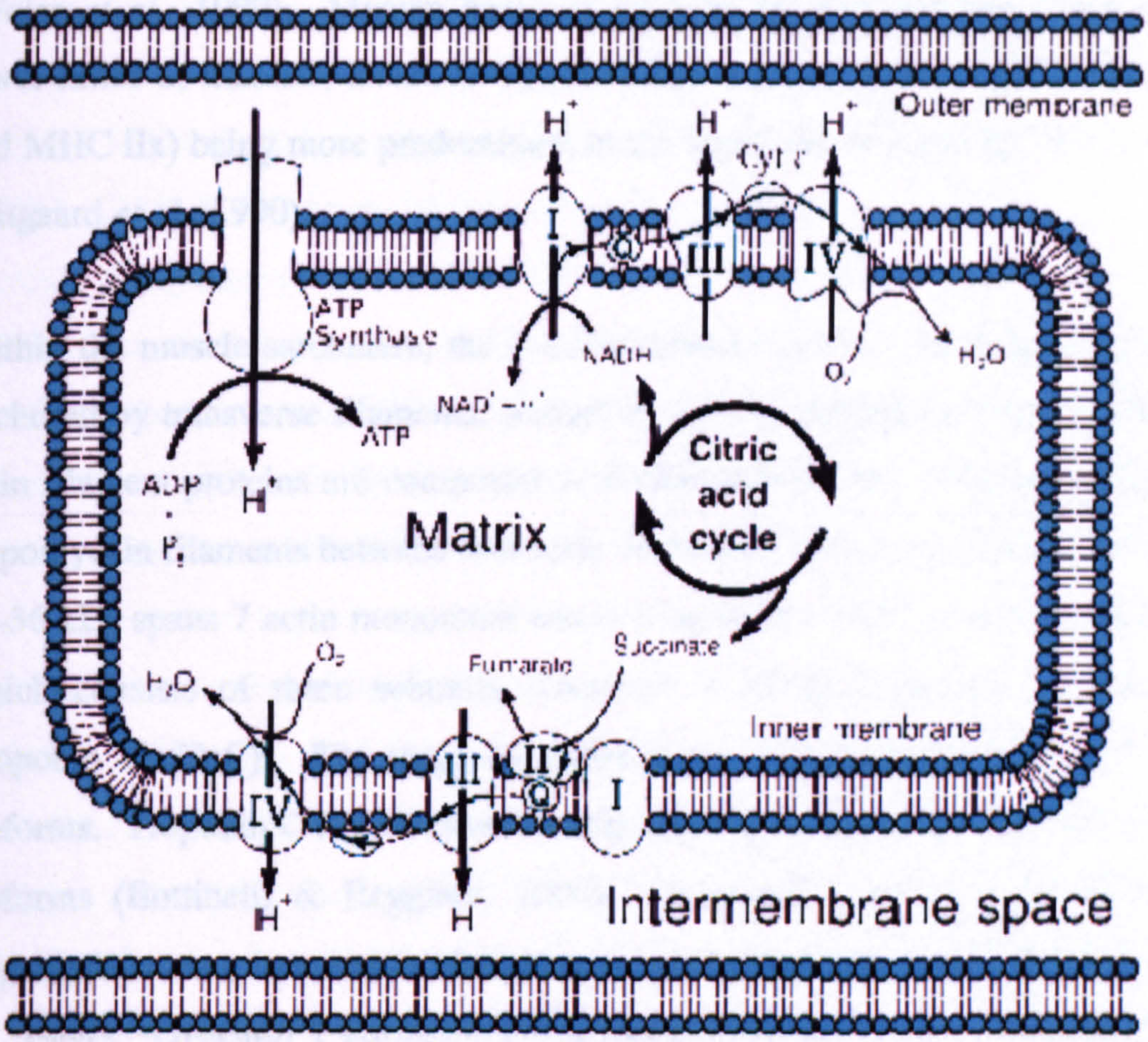


Figure 6.1: The TCA cycle and the electron transport chain in the mitochondria.

Myofibrillar proteins and contraction play an important role in converting chemical energy in to mechanical energy. Myofibrils are made up of sarcomeres, in ordered alignment for the interaction of myosin and actin filaments. The myosin molecule is composed of two myosin heavy chains (MHCs; mol. Wt 200-220 kD) and four myosin light chains (MLCs; mol. Wt 20-16 kD). Eight genes have been identified as sarcomeric class II MHCs in the human genome (Weiss *et al.*, 1999). Myosin heavy chain I is the most abundant isoform in lower limbs of humans, followed by MHC IIa, with the fast isoforms (MHC IIa and MHC IIx) being more predominant in the upper limbs (Harridge *et al.*, 1996; Klitgaard *et al.*, 1990).

Within the muscle sarcomere, the Z band consists of two sets of thin filaments anchored by transverse filaments, formed by actinin (Briggs & Schachat, 1996). Thin filament proteins are composed of double helical actin filaments, with two tropomyosin filaments between two actin filaments. Each tropomyosin (mol. Wt 34-36 kD) spans 7 actin monomers where it combines with a troponin complex, which consists of three subunits, Troponin T (TnT), Troponin I (TnI) and Troponin C (TnC). The troponin subunits are also present in two or more isoforms. Troponin C is a calcium binding protein, and expressed as fast or slow isoforms (Bottinelli & Reggiani, 2000). Troponin I stabilises the troponin-tropomyosin complex, and is also present as fast and slow isoforms (Salviati *et al.*, 1984). Troponin T consists of 3 isogenes, fast TnT, slow TnT and cardiac TnT. The fast TnT gene can produce up to 64 variants by alternative splicing. However, only a limited amount of spliced variants occur in human muscles (Briggs & Schachat, 1996). The slow TnT gene is expressed in slow twitch muscles also consisting of two further isoforms (TnT1s and TnT2s; Schmitt & Pette, 1990). Integrated actions of metabolic and myofibrillar constituents determine muscle contractility. Therefore, changes in muscle protein expression should be viewed as the overall proteomic modifications, rather than individual changes (Isfort *et al.*, 2002; Yan *et al.*, 2001).

6.1.4 Heat shock proteins (HSPs).

The first response to heat shock was identified by Ritossa (1962). It is now well established that heat shock proteins respond to a variety of stresses, especially exercise intensity. The production of heat shock proteins have been found in nearly all eukaryotic cells and have been identified with a variety of molecular weights, due to differences in amino acid residues. HSPs range from 8 to 110 kDa in molecular mass and are categorised by both size and function. The most common HSPs in skeletal muscle are small HSPs (20, 27), HSP60, HSP70 and HSP90. HSP 20 has been related to muscle contraction, mainly in slow-twitch muscles (Inaguma *et al.*, 1996) and HSP 27 has been associated with the stabilisation of microfilaments and cytokine signal transduction (Moseley, 1997). Another important small HSP is alpha B-crystallin, which serves as a molecular chaperone to either facilitate protein folding or preventing the build up of denatured proteins (Horowitz, 1992).

In response to stress, heat shock protein 70 is the most abundant, and has 4 isoforms. When new proteins emerge from the ribosomes, HSP70 interacts with them by its substrate-binding domain recognising the hydrophobic amino acid residues, and by stimulating ATPase activity and increasing ATP hydrolysis. HSP70 then acts as a chaperone by passing new, unfolded proteins to members of the HSP60 family. These aid proteins folding and their translocation into cellular compartments. HSP70 is also involved in cell homeostasis, proliferation, differentiation and cell death (Matranga *et al.*, 2002). Therefore, HSP expression can be indicative of a wide range of muscle metabolic responses and can be useful to measure muscle responses to exercise training.

6.1.5 Changes in skeletal muscle after exercise.

Skeletal muscle can change the amount and type of proteins expressed when exposed to stress. Exercise-induced protein expression involves the transcription of DNA of genes to create new proteins. Changes in protein expressions are determined by training frequency, duration and intensity. Depending on the

protein in question, messenger RNA (mRNA) peaks 3-12 hours post exercise, with values returning to baseline within 24 hours. If training is long-term, then the increased mRNA expression of a particular protein leads to a new and higher baseline level (Mahoney *et al.*, 2005).

Seo *et al.* (2006) studied the effects of unloading of skeletal muscle in regards to altered contractility in rats. The results showed that muscle tension decreased during unloading. This was attributed to decreases in MLC2, α-actin, tropomyosin β-chain and troponin T1 and T2, therefore suggesting decreases in slow contractile proteins. The increase in shortening velocity was due to an increase in fast-type MLC2, glycolytic enzymes and creatine kinase. This confirms a conversion to a faster contractile state, elevating the capacities of glycolytic and phosphogen supplies. In contrast, chronic low-frequency stimulation of muscle fibres has been extensively used to understand the physiological properties of muscle fibres (Pette, 2001; Pette & Staron, 1997). Chronic stimulation of muscle fibres enhances aerobic-oxidative capacity (Reichmann *et al.*, 1991), half relaxation times (Ohlendieck *et al.*, 1999), increases time to peak twitch tension (Pette & Staron, 2000), and decreases fibre cross sectional area, thus improving fatigue resistance and a fast to slow fibre conversion (Donoghue et al., 2005; Hicks *et al.*, 1997) as shown in Figure 6.2 in animals.

6.1.6 Changes in skeletal muscle after training.

Training-induced adaptations, in relation to cardiovascular and aerobic fitness, have been extensively studied using endurance training, but the modality of the exercise training has been shown to affect the degree of alteration to muscle metabolism.

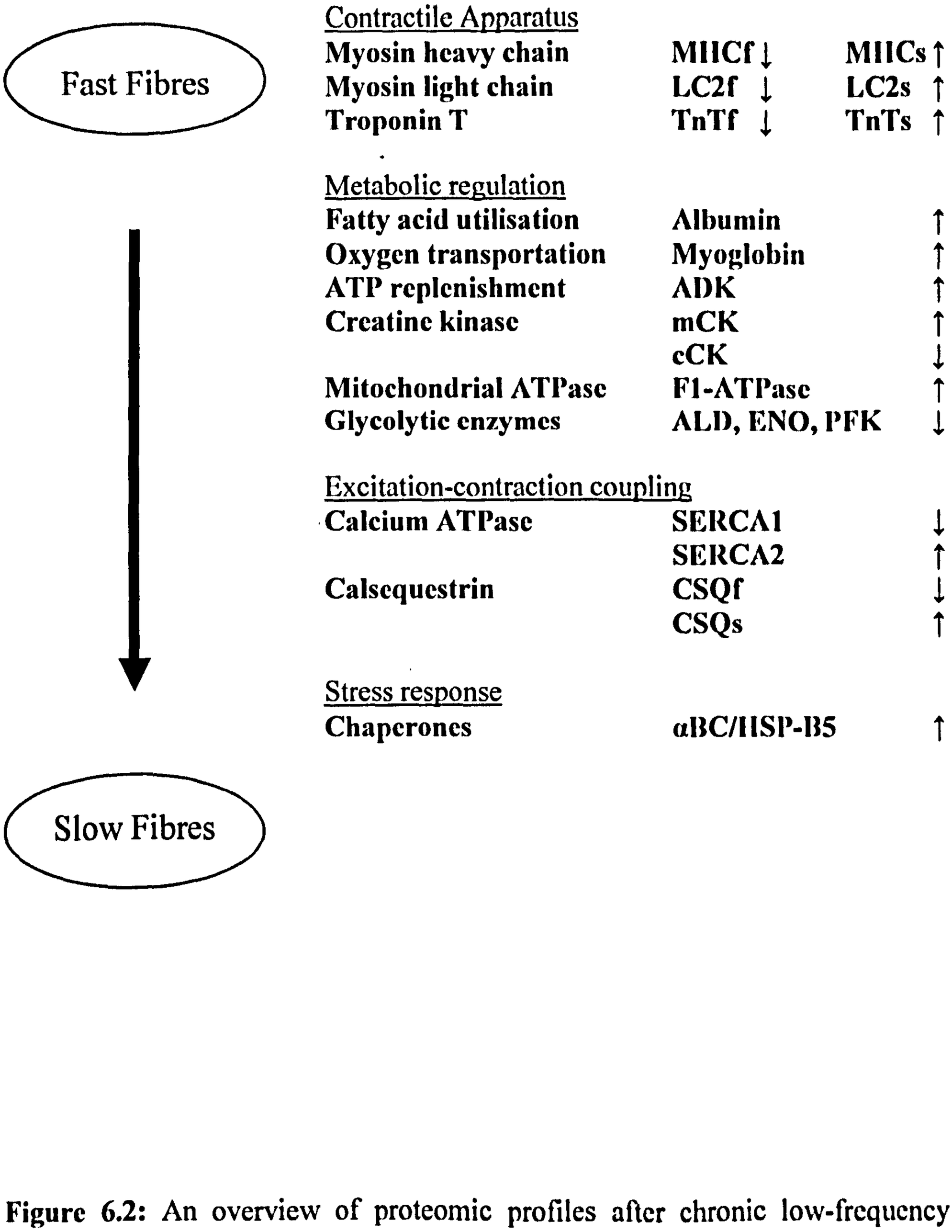


Figure 6.2: An overview of proteomic profiles after chronic low-frequency stimulation of a fast muscle in a rabbit (Donoghue *et al.*, 2005).

It is of general consensus that sedentary ($\dot{V}O_{2\,max}$ <45 ml.kg.min^{-1}) and recreational individuals ($\dot{V}O_{2\,max}$ 45-55 ml.kg.min^{-1}) need several years of training to gain the $\dot{V}O_{2\,max}$ of a highly trained athlete ($\dot{V}O_{2\,max}$ >60 ml.kg.min^{-1}). However, Hickson *et al.* (1977) showed that 10 weeks of high-intensity interval training could improve sedentary/recreationally active individuals $\dot{V}O_{2\,max}$ by 44 %. Long-term and short-term training studies using sedentary individuals have both shown improvements in $\dot{V}O_{2\,max}$ (Green *et al.*, 1987) which are attributed to an increase in oxygen delivery to the muscles (Green *et al.*, 1990, 1991) and muscle oxygen utilisation (Green *et al.*, 1989, 1991, 1992).

Muscular adaptations after exercise training are correlated to the duration, intensity, amount and distribution of muscle loads (Dudley *et al.*, 1982). Regular endurance training has been known to facilitate numerous physiological adaptations (Hawley, 2002). It has long been recognised that a change in skeletal muscle substrate metabolism is a prominent adaptation to training (Holloszy & Coyle, 1984). Even short endurance training programmes have been shown to increase glycogen availability and decrease glycogen catabolism, causing an increase in endurance capacity (Chesley *et al.*, 1996; Green *et al.*, 1992, 1995). Endurance exercise causes changes in neural recruitment, muscle bioergenetrics, metabolic substrates and acid-base status in skeletal muscle (Hawley, 2002). The resulting adaptations cause increased muscle glycogen stores and glycogen sparing through the increase of fat oxidation. (Gollnick *et al.*, 1973; Hickson *et al.*, 1977; Holloszy, 1967; Kiens, 1997).

Interval training has shown improvements in enzymatic activities relating to glycolysis (phosphofructokinase, PFK; lactate dehydrogenase, LDH; glucose 3-phopshate, GP; Cadefau *et al.*, 1990), metabolite concentrations of phosphocreatine (PCr) and glycogen (Linossier *et al.*, 1993; 1997), lactate removal/ transport (Juel *et al.*, 2004), ionic regulation (Harmer *et al.*, 2000), sarcoplasmic reticulum function (Ortenblad *et al.*, 2000) and skeletal muscle blood flow (Krustrup *et al.*, 2004). These therefore suggest that interval training enhances the performance which utilises aerobic metabolism. MacDougall *et al.* (1998) found that interval training involving sprinting significantly increased

$\dot{V}O_{2\,max}$ by 7 % after 7 weeks. This was correlated with an increase in muscle oxidative enzyme activities (PFK ~49 %, citrate synthase (CS) ~36 %, malate dehydrogenase ~29 % and succinate dehydrogenase ~65 %). Recently, Gibala *et al.* (2006) found an increase in maximal cyclooxygenase (COX) activity (COX IV, nuclear and COX II, mitochondrial) after interval training, suggesting an enhancement of the electron transport chain. Consequently intense, yet brief, exercise up-regulates both glycolytic and oxidative enzyme activities in untrained individuals, improving the energy state of the muscle and the preservation of high-energy phosphates. Therefore, endurance training may induce aerobic changes, but with both active and passive phases with the interval training, it is conceivable that both aerobic and anaerobic metabolism can be enhanced.

The recovery time of the interval training is also important as intense and brief muscle loads with long recovery periods are suggested to induce PCr metabolism (Thorstensson *et al.*, 1975). Parra *et al.* (2000) studied 10 healthy men and designed two high-intensity programmes with the same work load, but different rest periods (SP trained everyday for 2 weeks, LP trained for 6 weeks with a 2 day rest following each session). The results showed that glycogen consumption was greater (25 %) in the LP group, compared to the SP group (15 %). Therefore glycolysis rates were higher in LP group, whereas the SP group generated more ATP anaerobically with less lactate production. This suggests that a shorter rest period between training bouts enhances PFK and CK activities, causing a greater improvement in short-term performance.

To directly compare interval and continuous training responses, Gibala *et al.* (2007) studied 16 healthy active men who performed 6 training sessions over 2 weeks. One group undertook sprint interval training and another group undertook endurance training. The exercise mode, frequency and duration were the same between the interval training and the endurance trained groups, but the total training volume was ~90 % lower for the interval training. The results showed that both groups had similar improvements in muscle buffering and oxidative capacities after training. Similar studies have found increases in

mitochondrial enzyme activity with both interval and continuous training (Henriksson & Reiman, 1976; Saltin *et al.*, 1976). However others have reported changes only with continuous training (Fournier *et al.*, 1982; Gorostiaga *et al.*, 1991). These discrepancies between studies are due to different exercise intensities and different training volumes between interval and continuous trained groups. However, it is interesting that similar changes are found between interval and continuous training after different training volumes.

Mitochondrial protein content can be enhanced by 50-100 % with ~6 weeks of endurance training (Zierath & Hawley, 2004). Using histochemical techniques, this increase in mitochondrial density after long-term endurance training has been shown due to a decrease in type IIb fibres (Anderson & Henriksson, 1977; Baumann *et al.*, 1987) and an increase slow type I fibres by around 50-56 % (Howald *et al.*, 1985) and 41-47 % (Simoneau *et al.*, 1985). Conversely, interval training has shown to augment oxidative capacity in type II fibres (Poole & Gaesser, 1985) and increase type II fibre number by 10 % (Dawson *et al.*, 1998). Ogura *et al.* (2006) recently showed that 8 weeks of sprint interval training improved both anaerobic and aerobic enzymes and caused hypertrophy of type IIa fibres. However, some studies have also found an increased expression of type I fibres after sprint interval training (Linossier *et al.*, 1993), as type I fibres may play an important role during the resting phase of interval training.

Different types of exercise training can induce changes in the pattern and concentration of key contractile enzymes and structural proteins (Booth & Thomason, 1991). High-intensity training has shown a bidirectional transformation of myosin heavy chain I and IIb to myosin heavy chain IIa (Anderson *et al.*, 1994) due to an increase in type IIa fibre expression and a decrease in type IIb fibre expression (Allemeier *et al.*, 1994). Sprint training have also shown to up-regulate muscle Na^+-K^+-ATPase content (Harmer *et al.*, 2000) and sarcoplasmic Ca_2^+-ATPase (Laursen & Jenkins, 2002), which is critical for muscle contractility. With muscle contractility being improved after high intensity training, increases in HSP70 and HSP90 have been noted, which are known chaperones for proteolysis pathways. Also due to the intense nature of the exercise, heat shock proteins have been seen to respond to the metabolic,

mechanical and oxidative stress in muscle cells after interval training (Liu & Steinacker, 2001; Locke, 1997). Two smaller heat shock proteins (HSP20 and HSP27) have shown to affect actin dynamics through modifying smooth muscle relaxation (Tessier *et al.*, 2003; Lavoie *et al.*, 1993). High-force eccentric contractions have shown to induce HSP27 and HSP70 expression in untrained muscle, therefore suggesting that the work phase of interval training may improve muscle contractility. It has been well acknowledged that endurance training induces HSP70 in skeletal muscles (Desplanches *et al.*, 2004; Liu *et al.*, 2004; Liu *et al.*, 1999; Milne & Noble, 2002; Naito *et al.*, 2001; Skidmore *et al.*, 1995), but little attention has been given to interval training.

6.1.7 Sex-specific changes in skeletal muscle after exercise training.

Although interval training has been studied extensively in regards to muscle metabolism, the majority of these studies have only included men. When women have been included, they have been in mixed sex cohorts, thus concealing any sex-specific differences. From studies using endurance training, the results suggest growing evidence of sex-specific differences in relation to substrate selection during exercise (Horton *et al.*, 1998; Tarnopolsy *et al.*, 1990, 1995, 1997). It has been noted that females have a larger proportion of type I fibres in the vastus lateralis muscle (Simoneau and Bouchard, 1989). This suggests that in the vastus lateralis muscle, females have a greater aerobic potential than males and therefore may respond differently to interval training. If so, this may be due to female sex hormones. They may alter VO_2 kinetics since oestrogen and progesterone administrations in rats have shown an increase in pyruvate dehydrogenase kinase, which inactivates the PDH (pyruvate dehydrogenase) complex (Campbell *et al.*, 2003), resulting in a decrease in carbohydrate-derived substrate for the TCA cycle. Consequently this slows the activation of muscle O_2 consumption at the start of exercise, especially during the luteal phase of the menstrual cycle (Timmons *et al.*, 1998). Previous studies have also found that oestrogen and progesterone supplementation alters the control of blood flow (Ettinger *et al.*, 1998; Kirwan *et al.*, 2004; Moreau *et al.*, 2003; Sudhir *et al.*, 1997). Gurd *et al.* (2007) studied the effects of the whole menstrual cycle on

VO_2 response to moderate-intensity exercise. The results showed that the menstrual cycle phases did not affect pulmonary VO_2 or muscle ΔHHb levels. Therefore, Gurd *et al.* (2007) stated that it may not be necessary to control for menstrual cycle phase when examining VO_2 kinetics in young, healthy women.

6.1.8 Proteomics.

No single analytical protocol has so far been developed which can characterise all the complex protein mixtures within a muscle sample. This fact has stimulated the evolution of varying methods of proteomics. The majority of studies that have researched muscle substrate utilisation after exercise training have measured markers in blood serum or used Western blot analysis using muscle biopsy samples. Western blot analysis identifies individual proteins, but cannot account for any spliced variants of proteins or post-translational modifications, unless antibodies are specifically raised against such epitopes. Previously, observations suggested that one gene is equivalent to one protein end product, whereas now it is envisaged that there are more like 6-8 proteins derived from one gene (Strohman, 1994). There are roughly between 20,000 and 25,000 genes in humans (Aparicio *et al.*, 2000; Liang *et al.*, 2000; Roest Crollius *et al.*, 2000), which could amount to a million proteins when splice variants and post-translational modifications are included (Binz *et al.*, 1999; Hoogland *et al.*, 1999; Wilkins *et al.*, 1996, 1999). Proteomics has been designed to analyse and characterise complex mixtures of proteins. Proteomics for separating and qualitatively and quantitatively analysing complex protein mixtures within a tissue sample (Hunter *et al.*, 2002) is currently the most powerful method (Gevaert & Vanderkerckhove, 2000), when combined with high-throughput mass spectrometry (MS) techniques, as it allows simultaneous analysis of numerous proteins.

Two-dimensional electrophoresis first separates proteins by isoelectric focussing (a technique for separating different molecules by their electric charge). Then in the second dimension, resolves by SDS-PAGE, based on protein size. Numerous staining methods have been developed to allow visualisation of the array of

proteins (Gorg *et al.*, 1988; Klose & Kobalz, 1995). Presently, there are no stains which cover the dynamic range of all cellular proteins (Wirth & Romano, 1995). Studies have compared numerous stains including colloidal Coomassie blue, Daiichi silver, Sypro orange, Sypro red, Sypro ruby and Sypro tangerine, and have shown that all these stains are compatible with MALDI methods and have a greater dynamic range compared to more traditional methods (Lauber *et al.*, 2001; Patton, 2002; Steinberg *et al.*, 1996a, 1996b). However, there are two main staining methods which are predominantly used in 2D gels. Coomassie blue staining has the advantage of being highly sensitive but with a shorter staining time. This method also requires destaining, giving a clearer background (Neuhoff *et al.*, 2005). Silver staining results in a poorer MS sequence coverage than Coomassie blue staining due to the stain modifying proteins (Scheler *et al.*, 1998).

The most common method of protein identification involves the excision of protein spots, proteolytically digesting them, and then extracting the peptide products. Lim *et al.* (2003) found a 95 % success rate of protein identification using ZipTip sample clean up for 162 Coomassie-stained 2D gel spots. These peptides are then analysed using mass spectrometry and the peptide peaks correlated with protein sequence databases (Eng *et al.*, 1994). The preferred mass spectrometry technique for 2D gels is peptide mass mapping, using matrix-assisted laser ionisation time of flight (MALDI-TOF) mass spectrometry. This technique allows databases to recognise fragmented ion spectra, which relates to various peptide chains. Peptide mass fingerprint analysis takes the protein of interest, after it has been enzymatically or chemically cleaved, and analyses the peptide mixture. The peptide mass fingerprint is comparable to other fingerprints which have been found through the theoretical cleavage of protein sequences. Unlike techniques such as electrospray ionisation (ESI), MALDI tolerates mild salt and buffer solutions to produce singly charged ions (Gevaert & Vanderkerckhove, 2000). MADI-TOF mass spectrometry has enabled a greater increase in the databases of genetic sequences for a greater variety of organisms (Hunter *et al.*, 2002).

Two-dimensional electrophoresis (2-DE) is technically complex and time consuming, with large gels requiring a few days to complete. 2-DE is not capable of resolving the entire muscle proteome. The abundance of proteins spans more than 6 orders of magnitude whereas contemporary 2-DE techniques and proteins stains achieve a dynamic range of 4 orders of magnitude. In addition, it is widely observed that hydrophobic proteins and proteins at the extremes of the molecular weight range (i.e. < 10 kDa and >150 kDa) are poorly resolved by 2-DE. Nonetheless, 2-DE and colloidal Coomassie staining can be used to resolve several hundred abundant proteins. Whilst the genome is static in nature, the proteome is dynamic and represents the product of the continuous fluctuations in the expression of gene transcripts and the translation, covalent modification and degradation of proteins. 2-DE is associated with inherent technical variation and sophisticated image analysis is required to distinguish biologically important differences between experimental groups from the background variation due to technical and biological noise. Despite these disadvantages, 2DE currently remains the best method for separating and displaying individual proteins and their subunits.

A recent study in rats used quantitative proteomics to assess the acute effects of high-intensity exercise training on skeletal muscle and found the technique to be sensitive enough to detect changes in muscle protein levels (Guelfi et al., 2006). Proteomic analysis of the chronic effects of interval training has not yet been performed.

6.2 Rationale.

There is still much debate within the literature about muscle metabolic adaptation to exercise training. Sex-specific differences in muscle metabolism, and the response to endurance exercise, have been reported. However, there are no known studies that have examined the effects of interval and continuous exercise training (with the same maintained exercise work loads between groups) in both men and women. Interval training has been recently popularised due to its short high-intensity nature, but the potential sex-specific effects of interval training

have not been investigated. Previous proteomic work has shown many muscle proteins on 2D gels appear as multiple spots, suggesting different isoforms, splice variants and post-translational modifications of the gene products, and 2-D electrophoresis is unrivalled in its ability to resolve these different protein species. To date, the response of human muscle to exercise training has not been investigated using proteomics techniques. Proteomics techniques can provide a broad assessment of changes in the expression of large numbers of proteins and enable us to discover novel information relating to changes in muscle structure and function.

Therefore the aims of this study were:

- To establish how interval and continuous exercise training effects the protein expression in the vastus lateralis muscle.

- To determine any sex-specific differences with regards to muscle protein expressions, after either or both modes of exercise training.

6.3 Methods.

6.3.1 Subjects.

Recreationally active females (n = 5) and males (n = 5), between the ages of 18-24 years, were recruited from the cohort of study 3 (chapter 5). This recruitment was initiated through a presentation and information sheet given to all participants involved in study 3. A meeting was held to familiarise prospective volunteers with the equipment and laboratories.

Female subjects were on the combined, monophasic contraceptive pill. All muscle biopsies were performed during the non-menstrual phase (i.e. during late follicular or luteal phases) of the cycle.

6.3.2 Health Screening.

Each subject underwent a health screening session to ascertain their suitability for the project (see chapter 5 for full details).

6.3.3 $\dot{V}O_{2\,max}$ test.

Each subject performed a $\dot{V}O_{2\,max}$ test to volitional exhaustion before and after 6 weeks of either continuous or interval training. For the full $\dot{V}O_{2\,max}$ protocol, refer to chapter 5.

6.3.4 Exercise Training.

Subjects were randomly assigned to either interval or continuous training using a cross-over design. Subjects participated in both interval and continuous training

programmes, interspersed by a 6 week detraining period. Each exercise programme was performed for 6 weeks, training 3-times a week, with at least one rest day between training sessions. Each individual's average heart rate and the distance covered on the treadmill were recorded during each session (appendix 9.2). Subjects were fully supervised throughout both training protocols.

6.3.4.1 Interval training.

The interval training consisted of a one minute bout of 90-100 % VO_{2max}, then 4 minutes at 50 % VO_{2max} on a treadmill (Cosmos, Nussdorf-Traustein, Germany). This interval set was repeated 6 times over 30 minutes.

6.3.4.2 Continuous training.

Continuous training was performed at a work rate of 70 $\%\dot{V}O_{2\,max}$ on a treadmill (Cosmos, Nussdorf-Traustein, Germany), covering exactly the same distance as when interval training. The exercise time varied between 28-34 minutes, depending on the individual and their initial fitness level.

6.3.5 Muscle biopsies.

Muscle biopsies were taken before, and after, 6 weeks of both the interval and continuous exercise training programmes. Subjects fasted overnight and did not exercise during the 5 days prior to the muscle biopsy being taken.

Muscle biopsy samples were obtained from the vastus lateralis (~10-20 mg of tissue) under local anaesthesia (0.5 % marcaine) using a Pro-Mag 2.2 biopsy gun (MD-TECH, Manan Medical Products, Northbrook, IL, USA) and immediately frozen in liquid nitrogen and stored at –80 °C. Before the training interventions

commenced, $\dot{V}O_{2\,max}$ tests were performed 48 hours after the biopsy had been taken, without any discomfort. After completing each training programme, biopsies were taken again, this time 72 hours after the last training session. The final $\dot{V}O_{2\,max}$ test was again performed 48 hours after the biopsy.

The following sections deal, in detail, with the separation, quantification and identification of the individual proteins within each muscle biopsy. This enabled comparison of the effect of the 2 exercise training programmes on the male and female participants.

6.3.6 2-Dimensional Electrophoresis.

Electrophoretic separation techniques are based on the movement of electrically charged molecules (Lorentz force) within an electrical field. Molecules in a sample do not migrate through the gel at similar rates when a sample is subjected to a negative to positive electro-magnetic field. Therefore, SDS (sodium dodecyl sulfate) is used to denature the proteins and coat them with a negative charge. 2-D electrophoresis separates molecules according to differences in both their isoelectric points (stage 1; section 6.3.9) and their masses (stage 2; section 6.3.10). So, proteins are moved to their isoelectric point by applying a pH gradient and an electric potential across the gel, causing one end of the gel to be more positive than the other. Proteins will be charged at all pHs across this gradient, except at their isoelectric points (stage 1). Therefore, positively charged molecules are pulled towards the cathode end of the gel, and negatively charged molecules towards the anode end, until they reach their isoelectric points.

When the protein samples are treated with SDS, they attract a number of SDS molecules relative to their proteins mass. A second electrical current can now be applied at 90 degrees to the first field (pH gradient). The proteins now move to the positive side of the gel with their movement through the gel slowed by frictional forces. The gel acts like a sieve, causing larger proteins to be retained

higher in the gel and smaller proteins passing through the sieve to settle in lower regions of the gel (Hanash, 1998). The individual proteins appear as discrete spots on the gel (stage 2).

6.3.7 Preparation of muscle samples.

Muscle samples were ground up using a pestle and mortar, under liquid nitrogen. The powder was accurately weighed (20 mg) and homogenised in 10 volumes of lysis buffer, using a Polytron homogeniser (Polytron, Switzerland). The Lysis and solubilising buffer consisted of 8 M urea, 4 % CHAPS (3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate,) 40 mM Tris and Complete protease inhibitor cocktail (Roche, Indianapolis, USA), which solubilises proteins. Homogenates were centrifuged at 12,500 rpm, 4 °C for 45 minutes, the supernatant decanted and stored in 200 µl aliquots at -80 °C.

6.3.8 The Bradford Assay.

A Bradford Assay (Bradford 1976) was performed to ascertain the protein concentration in each sample. Bovine serum albumin (BSA) standards (from 0.2 mg ml^{-1} to 1.0 $mg.ml^{-1}$ in 0.2 $mg.ml^{-1}$ increments) were prepared from a commercial stock solution (1.0 $mg.ml^{-1}$; Sigma-Aldrich, Poole, Dorset) and pipetted in triplicate (5 µl per well) onto a microtitre plate. Muscle supernatants were diluted 1:40 to bring them within this Standard range and pipetted (5 $\mu g.\mu l^{-1}$) in duplicate. Bradford reagent (Sigma) was added (250 µl per well) and the mixtures incubated at room temperature for 5 minutes, before reading at a wavelength of 595 nm (MultiskanSpectrum, Thermo Labsystems, Vantaa, Finland). The BSA standard curve was used to determine the protein concentration in each muscle supernatant sample, for subsequent loading onto the gels.

Prior to loading the sample onto the gel, a lysis buffer was used containing chaotropes and detergents to solubilise almost all proteins (muscle processing). The amount of protein loaded onto the gel was standardised after a Bradford assay, and run in batches so as not to introduce technical bias.

The proteins in these supernatant fractions were then separated into discrete spots by 2-Dimensional-SDS-polyacrylamide gel electrophoresis. As mentioned, the separation procedure was based on differences in the isoelectric points (Stage 1; section 6.3.9) and masses (stage 2; section 6.3.10) of the various proteins. Subsequently, the protein spots were visualised (section 6.3.11), quantified (section 6.3.11), xcised (section 6.3.12), digested into peptide fragments (section 6.3.12), identified using mass spectrometry and reference databases (section 6.3.13).

6.3.9 Stage 1: 1st Dimensional separation.

6.3.9.1 Isoelectric focusing.

Supernatant samples (60 µl) were diluted in 5 volumes of acetone, vortexed (mixed) and stored at -20 °C for 1 hour. After centrifugation (5,000 rpm) at room temperature, the supernatants were decanted and the pellets allowed to dry in air for 10 minutes at room temperature. Each pellet was resuspended in 260 µl of rehydration solution (8 M urea, 2 % CHAPS, 2.8 mg/ml DTT and 5 µl/ml IPG 3-10 NL buffer) and centrifuged at 8,000 rpm for 2 minutes to remove any particulate debris. The supernatant (250 µl) was pipetted into a strip holder (Amersham Biosciences, Germany Now GE healthcare) and an immobilised pH-gradient (IPG) strip (pH 3-10 non-linear strip GE Healthcare, Sweden) laid over the sample solution. Strips were covered in Dry Strip Cover Fluid and the strip holder placed onto an Ettan IPGphor machine (Amersham Biosciences, Germany) with a plastic sheet with foam inserts to ensure firm contact between the strip holder and electrodes (Figure 6.3). During isoelectric focussing, the electrical current decreases while the voltage increases, as proteins and other

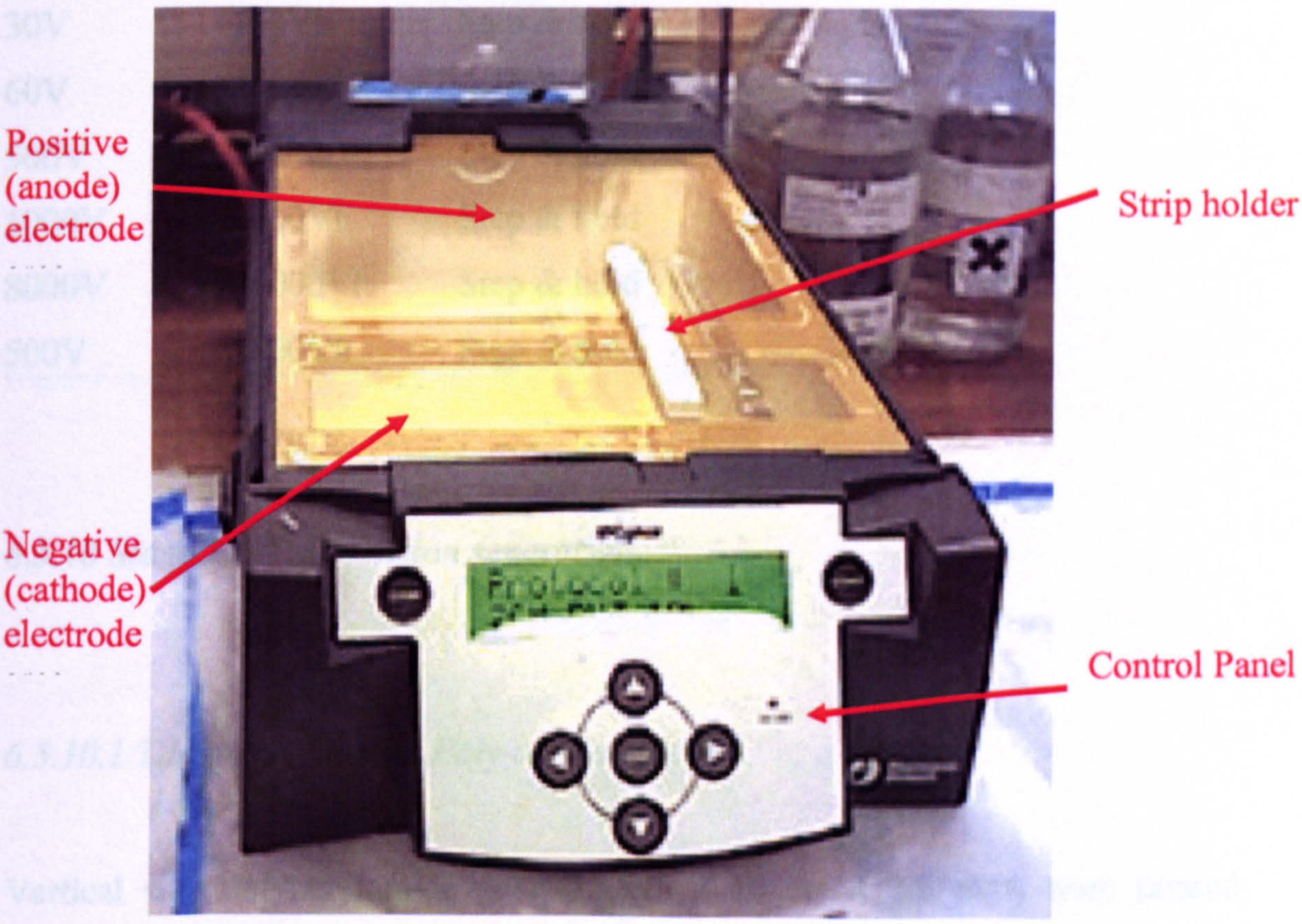


Figure 6.3: Ettan IPGphor machine (Amersham Biosciences, Germany) used for the first-dimension separation based on isoelectric focussing.

charged components migrate to their equilibrium positions. The protocol proceeds through a series of voltage steps that begin at a relatively low value (see below). The voltage was gradually increased to the final desired focusing voltage, which was held for up to several volt hours (Vh). The isoelectric focussing protocol, which took around 24 hours to complete, consisted of the following phases:

Voltage	**Time**	**Gradient**
30V	150Vh	Step & hold
60V	300Vh	Step & hold
500V	500Vh	Step & hold
1000V	1000Vh	Step & hold
8000V	48000Vh	Step & hold
500V	2000Vh	Step & hold

6.3.10 Stage 2: 2nd dimension separation.

6.3.10.1 The preparation of Poly-acrylamide gel.

Vertical slab polyacrylamide gels (13 cm x 16 cm x 1.5 mm) were poured manually. Glass plates were cleaned with 70 % ethanol to ensure all traces of protein were removed. Cassettes were formed (130 × 160 mm) by two glass plates separated by spacers of the required thickness (1.5 mm) placed at the sides (Figure 6.4a). The glass plates were then assembled as shown in Figure 6.4b. A 12.5 % Acrylamide mix, consisting of 30 % acrlyamide, 0.8 % N, N-methylenebisacrylamide (ratio of acrylamide:bis-acrylamide), resolving buffer (1.5 M Tris base, pH 8.8 HCl, ddH_2O), 10 % SDS and ddH_2O, was poured in between the glass plates up to 1 cm from the top. Iso-butanol was then pipetted over the top of the gel to ensure a flat surface and left at room temperature for 1 hour. The iso-butanol was subsequently removed with ddH_2O, and the gels overlayed with storage solution (resolving buffer, 10 % SDS and ddH_2O) and stored overnight at 4 °C.

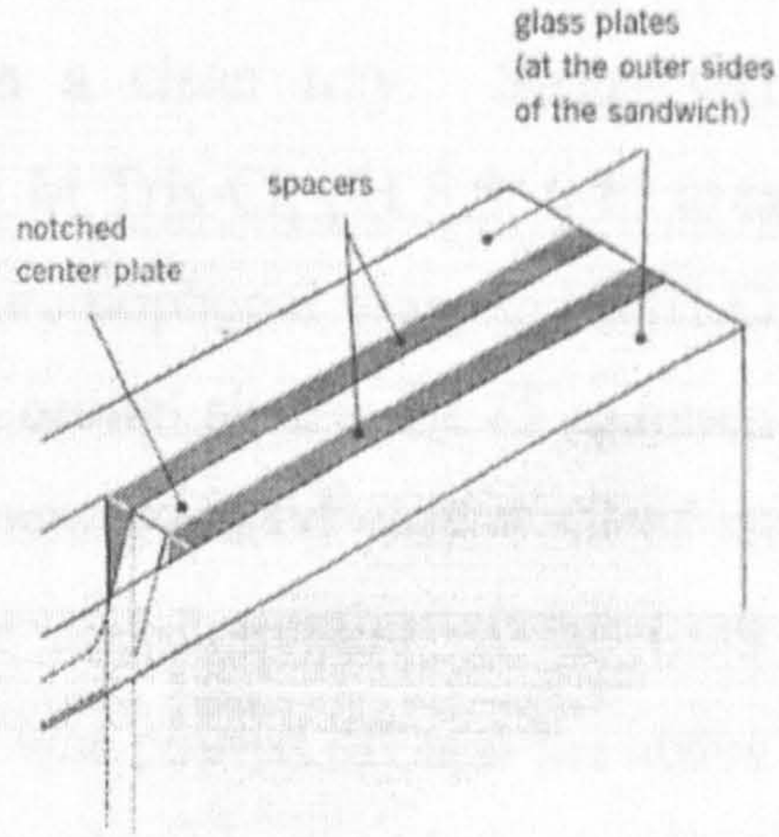


Figure 6.4a: Cassette assembly for acrylamide gels

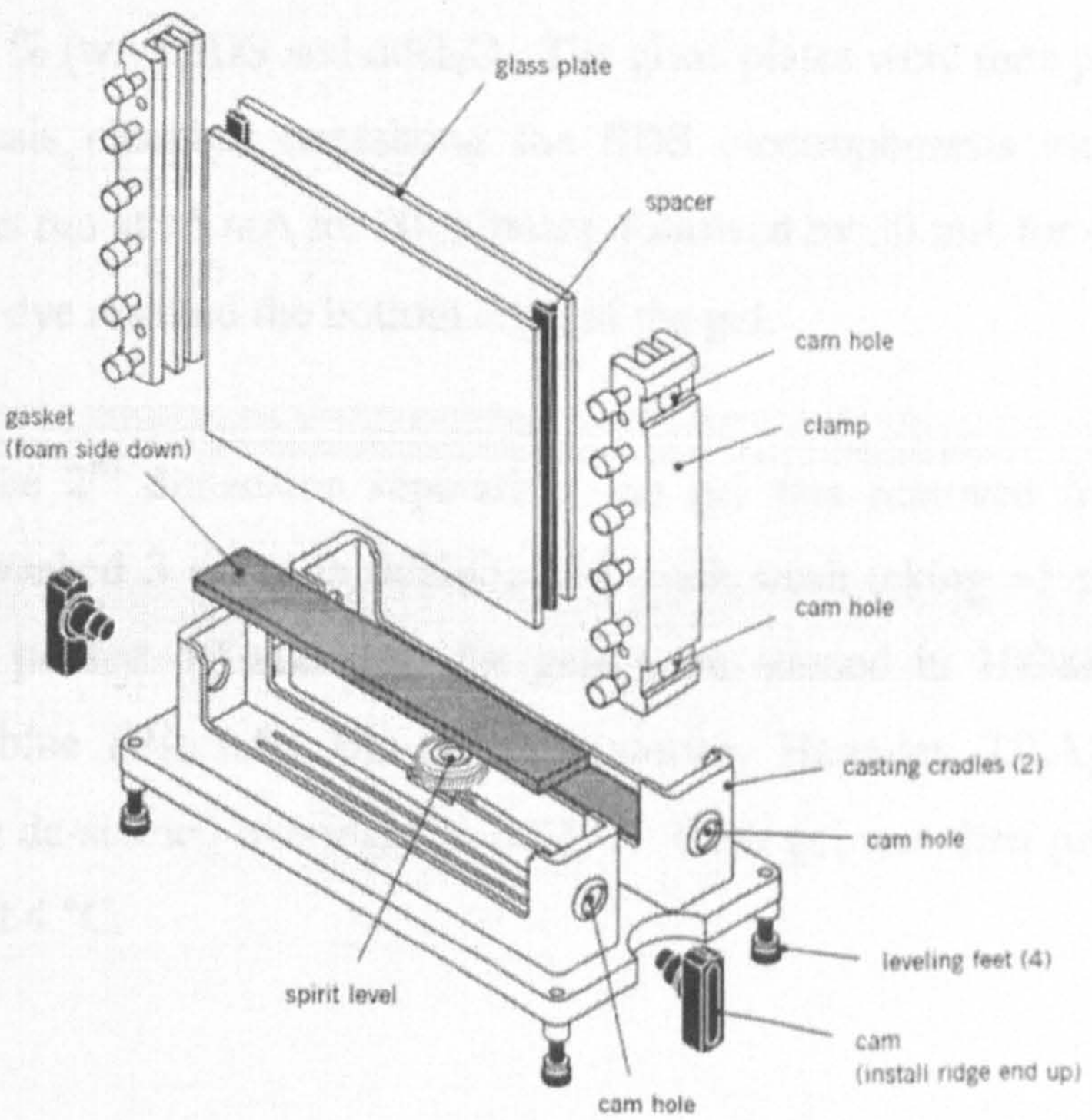


Figure 6.4b: Glass plate set-up for making the acrylamide gels.

6.3.10.2 2nd dimension separation.

Following the isoelectric focusing, IPG strips were removed from the strip holders and placed in a clean tray. Strips were equilibrated with 4 ml equilibrium buffer (1.5 M Tris-Cl; pH 8.8; 6 M urea; 30 % (v/v) glycerol; 2 % (w/v) SDS; a trace of bromophenol blue, and ddH_2O), with 40 mg dithiothreitol added, and put onto an orbital shaker for 15 minutes at room temperature. This solution was decanted and replaced with 4 ml of strip equilibrium buffer, with 100 mg iodoacetamide added instead of the SDS and shaken again for 15 minutes. This solution was poured off and the strips were then placed on top of the gel within the glass plates assembled previously. Broad range molecular weight markers were blotted onto a paper strip and placed at the positive end of the IPG strip and warm 0.5 % agarose-NA solution (0.5 % agarose, trace bromophenol blue) poured over the top of the gel to form a seal. The electrophoresis tank (SE600 ruby; Figure 6.5) was filled with a sodium dodecyl sulfate (SDS) electrophoresis buffer containing 25 mM Tris base, 192 mM glycine, 0.1 % (w/v) SDS and ddH_2O. The glass plates were then placed into the electrophoresis chamber containing the SDS electrophoresis buffer solution. Each gel was run at 15 mA for 30 minutes, followed by 30 mA for ~4 hours until the tracking dye reached the bottom edge of the gel.

Following the 2nd dimension separation, the gel was removed from the glass plates and washed 3 times in ddH_2O, with each wash taking ~5 minutes. The ddH_2O was poured off and then the gels were stained in 100ml of colloidal Coomassie blue (Bio-safe, Bio-rad laboratories, Hercules, USA) for 1 hour before being de-stained overnight in ddH_2O. Each gel was then put into ddH_2O for storage at 4 °C.

6.3.11 Image Analysis using Progenesis PG220 Same Spots Analysis.

Not all gels appear identical after electrophoresis due to small differences in the migration of some protein spots. Therefore, before biological differences can be

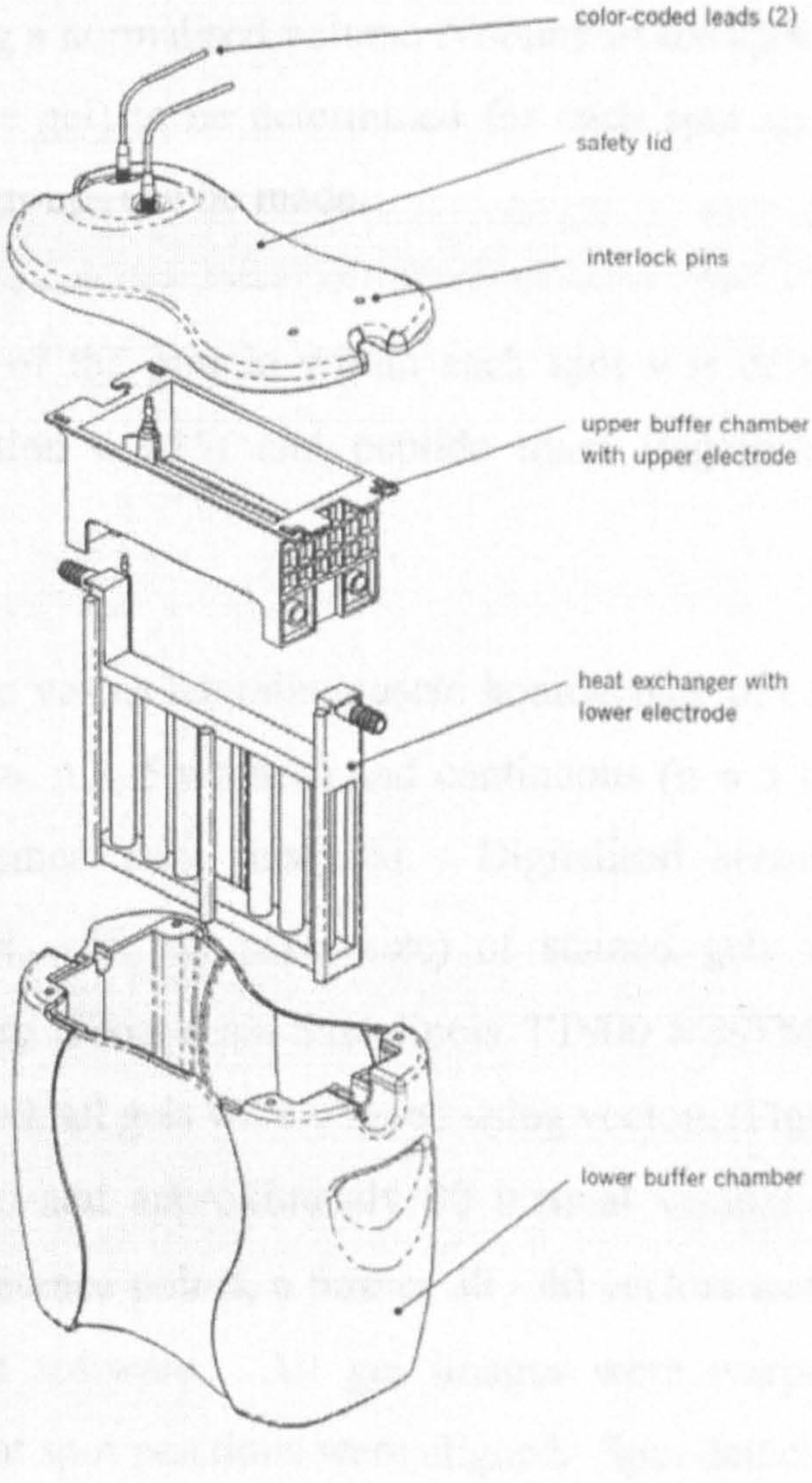


Figure 6.5: 2nd-dimension buffer chamber, containing SDS electrophoresis buffer solution.

compared it is necessary to align the relevant spots across each gel in each experiment. Progenesis SameSpots software (Non-Linear Dynamics, Newcastle, UK) addresses this issue and reduces spot volume variance between gels, by aligning/warping the gels and applying the same spot outline consistently to each gel within an experiment (Karp *et al.*, 2008). The density of each spot is also measured, enabling a normalised volume (volume of the spot divided by the total spot volume in the gel) to be determined for each spot so direct comparisons between exercise groups can be made.

The identification of the protein within each spot was determined using mass spectrometry (section 6.3.13) and peptide mass fingerprinting, as described below.

A 2-D gel from the vastus lateralis muscle homogenate of each participant after interval (n = 5 men, n = 5 women) and continuous (n = 5 men, n = 5 women) exercised programmes were analysed. Digitalised scanned images (16-bit greyscale, 300 dpi, ~85 μm pixel size) of stained gels were aligned using SameSpots software (Progenesis SameSpots TT900 S2STM). A reference gel was chosen, to which all gels were aligned using vectors (Figure 6.6). Prominent spots were used to add approximately 50 manual vectors to each gel image. Based on these reference points, a further 30 - 40 vectors were added to each gel by the SameSpots software. All gel images were warped to the common reference gel so that spot positions were aligned. Spot detection were performed on the reference gel and edited manually to remove gel artifacts. The resulting spot outlines were then applied consistently to each gel within the set and manually verified, producing a data set with no missing values. Spot volumes, expressed relative to total spot density, were used to identify changes in normalised spot volumes. Gel images were arranged into interval (20 gel images pre and post) and continuous training (20 gel images) groups, both before and after 6 weeks training, to allow the direct comparison of sex-differences with exercise training. Data were exported to SPSS (v.14) and statistical analyses were undertaken using two-way repeated measures ANOVA and paired t-tests (Figure 6.7).

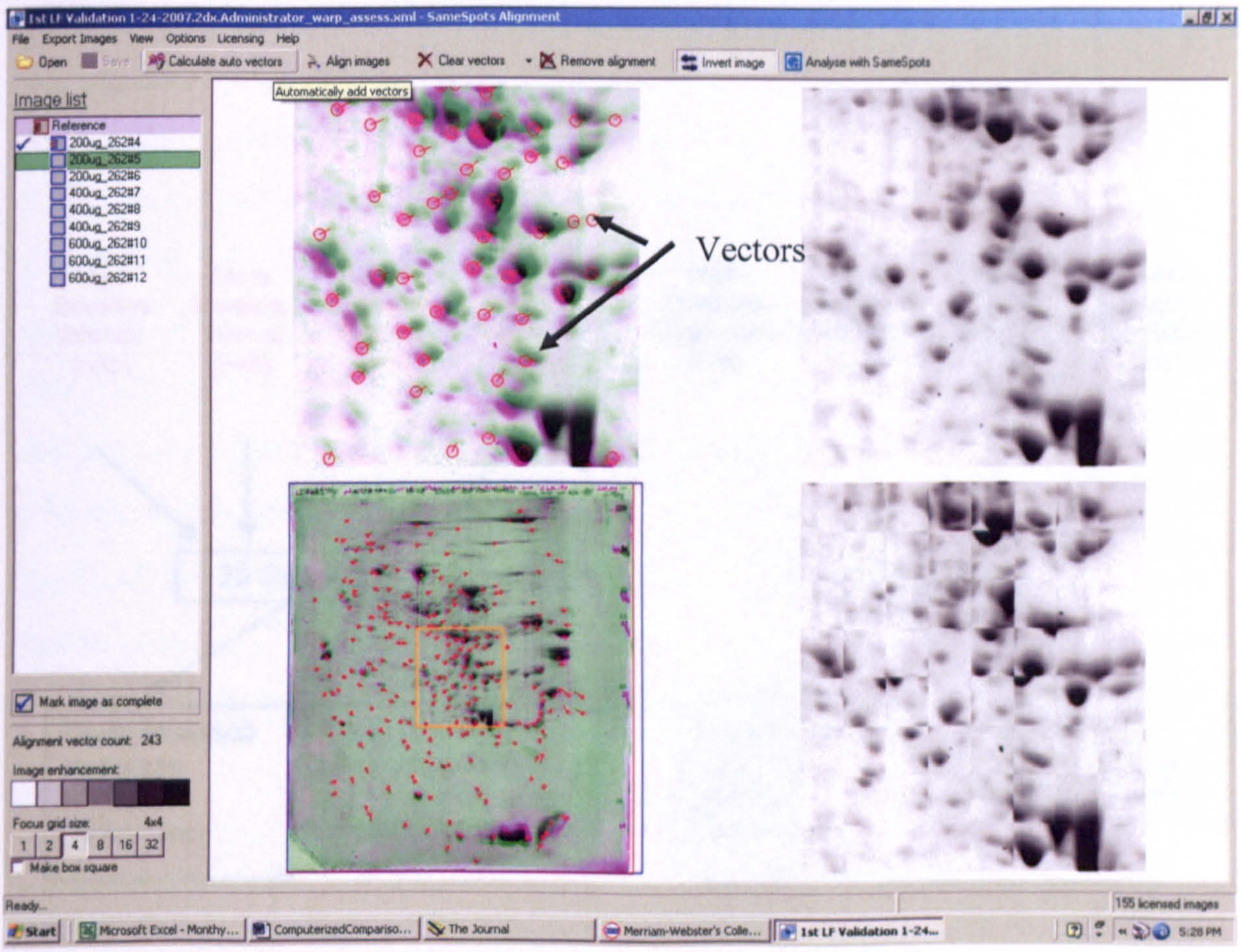


Figure 6.6: Gel image alignment using manual and automatic vectors in progenesis software.

The pink spots are the reference gel spots and the green spots are a gel being aligned.

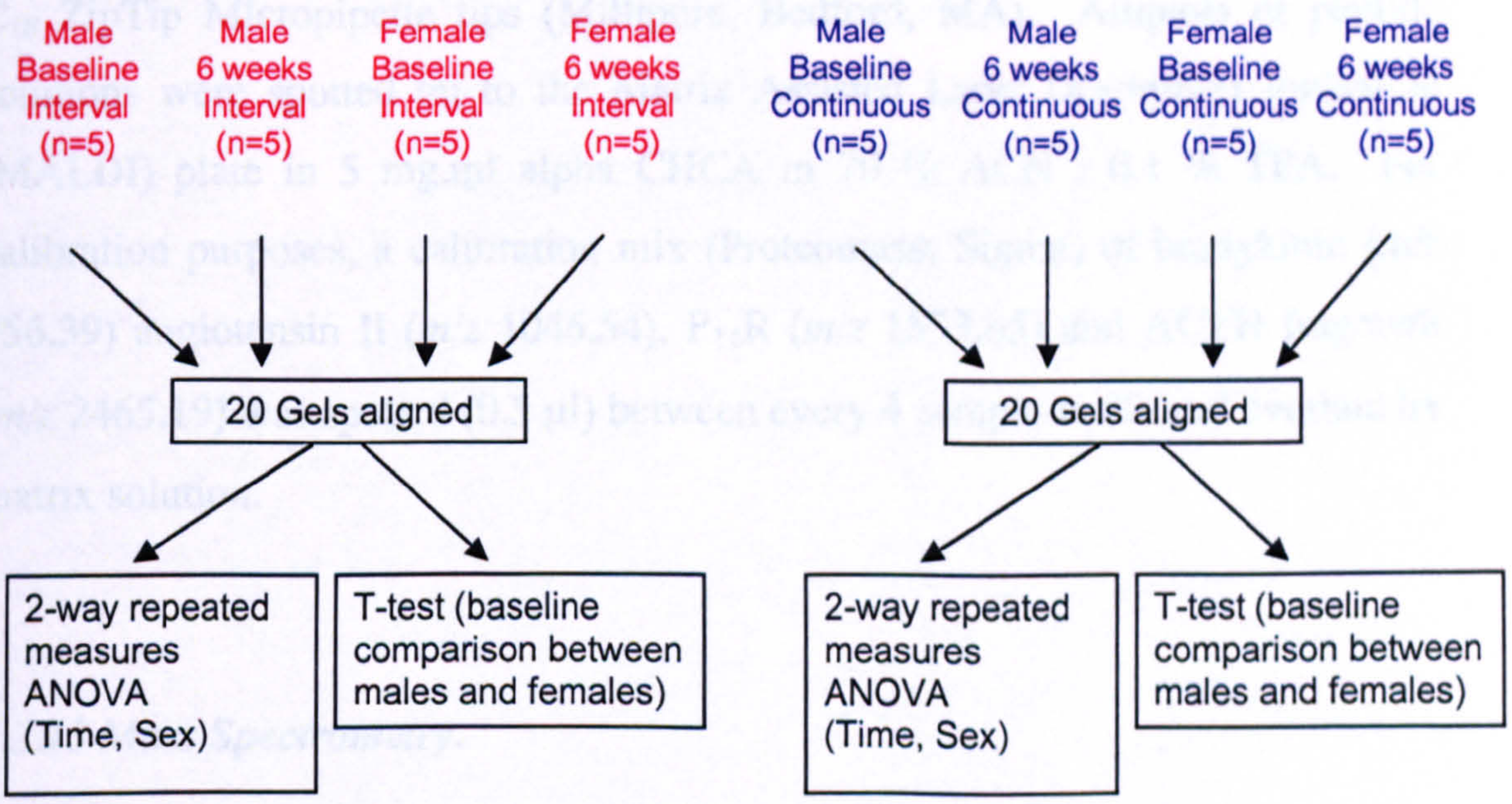


Figure 6.7: Gel alignment and statistical analyses.

6.3.12 Spot cutting and digestion.

In-gel digestion of each spot is part of the preparation of each sample for mass spectrometry, as whole proteins are difficult to analyse. The protein in each spot is enzymatically digested into peptides by the serine protease, Trypsin (Promega). The permeation of the gel to trypsin is facilitated by the dehydrating of the gel by treating it with acetronitrile. Gel spots of interest were cut (Xcise, Shimadzu Biotech Corporation, Tokyo, Japan; Figure 6.8), producing gel plugs. These were de-stained in 3 changes of 25 mM ammonium bicarbonate in 50 % Acetonitrile by the Xcise. Dehydrated gel plugs were incubated with 35 µl of 1.25 µg/ml porcine trypsin (Promega, Madison, WI) in 50 mM ammonium bicarbonate overnight at 37 °C. Peptides were concentrated and desalted using C_{18} ZipTip Micropipette tips (Millipore, Bedford, MA). Aliquots of peptide solutions were spotted on to the Matrix Assisted Laser Desorption Ionisation (MALDI) plate in 5 mg.ml alpha CHCA in 70 % ACN : 0.1 % TFA. For calibration purposes, a calibration mix (Proteomass; Sigma) of bradykinin (*m/z* 756.39) angiotensin II (*m/z* 1046.54), $P_{14}R$ (*m/z* 1533.85) and ACTH fragment (*m/z* 2465.19) was spotted (0.5 µl) between every 4 sample-wells and overlaid by matrix solution.

6.3.13 Mass Spectrometry.

Mass spectrometry measures mass-to-charge ratio of charged particles. The most widely used mass spectrometer is MALDI-TOF (Matrix-assisted laser desorption ionisation-time of flight mass spectrometer) due to its large range of masses. The peptide sample, overlaid with a co-precipitate of an UV-light absorbing matrix, is irradiated by a nanosecond laser pulse. The matrix solution absorbs most of the laser energy to prevent fragmentation of the sample. The ionised protein sample is accelerated within an electric filed and enters the flight tube. During the flight in this tube, different molecules are separated according to their mass to charge ratios and reach the detector at different times, yielding a distinct signal. This therefore creates a spectrum where each peak represents the mass of a peptide.

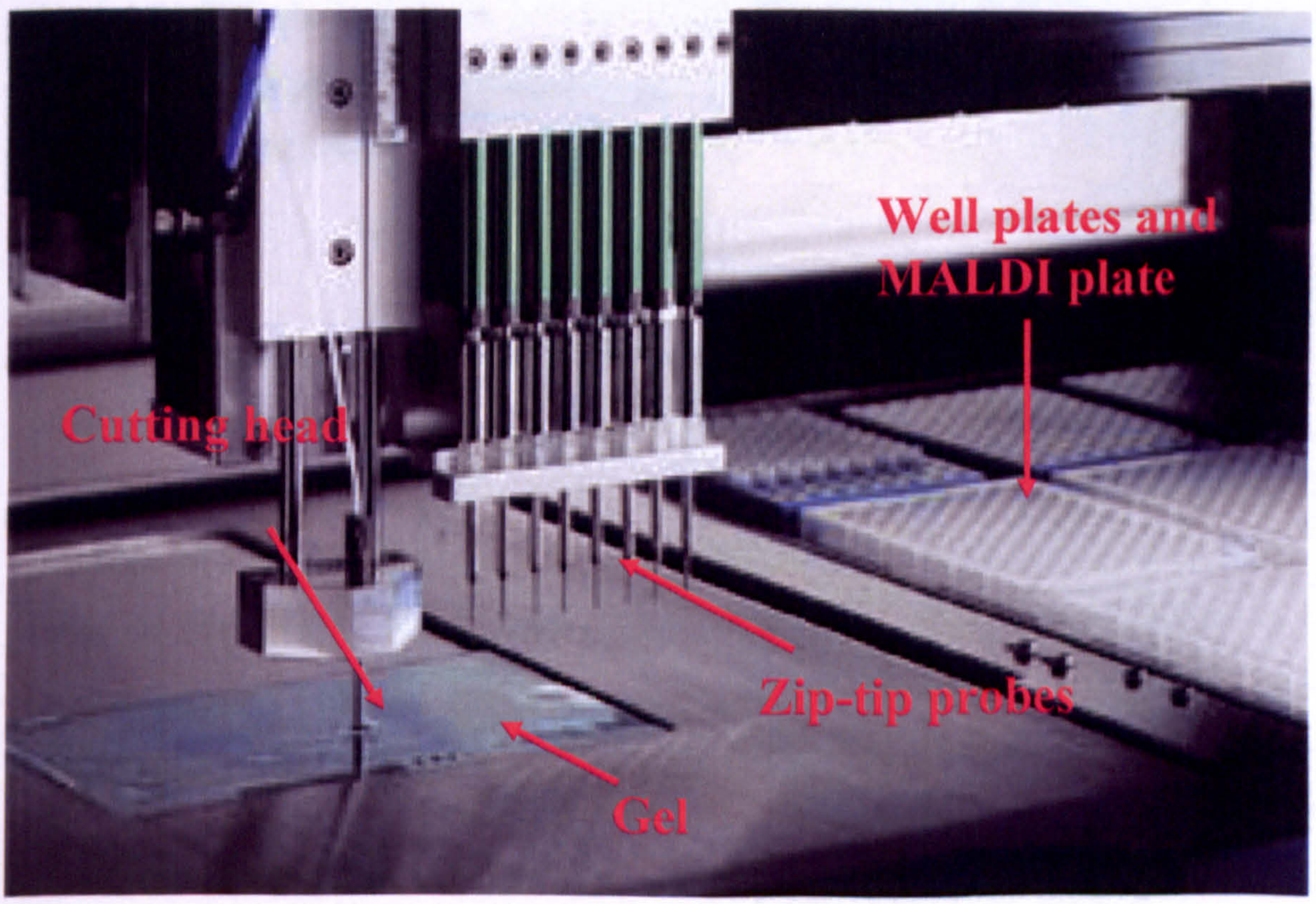


Figure 6.8: The Xcise machine, with a specifically designed cutting head for precise excision of the gel spots.

The MALDI-TOF allows the detection of low (10^{-15} to 10^{-18} moles) quantities of sample with an accuracy of 0.1 - 0.01 %. The addition of trypsin (proteolytic enzyme; section 6.3.12) to the protein sample creates peptides of 6-20 residues which are fairly unique to their parent protein.

Trypsin also introduces fixed information, i.e. cleaves C-term of lysine (K) and arginine (R) resides unless followed by Proline (P), therefore all tryptic peptides should end in K or R unless they are from the C-terminal of the protein. The peptides are then matched by their constituent fragment masses (peptide masses) to the theoretical peptide masses generated from a protein or DNA database. This is known as peptide mass fingerprinting (PMF). A peptide sequence tag (a piece of information about a peptide obtained by tandem mass spectrometry, MS/MS) can also be used to identify a peptide in a protein database.

Mass spectrometric (MS) and tandem mass spectrometric (MS/MS) analyses were performed using a matrix-assisted laser desorption ionisation tandem time of flight (MALDI-ToF/ToF) mass spectrometer (Axima TOF^2; Shimadzu Biotech, Manchester, UK) in positive reflectron mode over a mass/charge *m/z* range of 700-3500. Monoisotopic peptide masses were searched against the known peptide masses stored in the Swiss-Prot database. Samples not confidently identified by their peptide mass fingerprint were investigated further using MS/MS analysis.

Peptide ions were isolated using an ion gate, and high-energy fragmentation was induced with a collision-induced dissociation (CID) with helium as the collision gas. Fragment ions were resolved and collected using a curved field reflectron and a maximum of 42 ions over 6 segments, encompassing 5 - 95 % of the precursor ion m/z.

All searches were conducted on a locally implemented MASCOT (www.matrixscience.com) server. Taxonomy was restricted to 'Humans', the enzyme specificity was set as trypsin (allowing 1 missed cleavage), carbamidomethyl modification of cysteine (fixed), oxidation of methionine

(variable) and an *m/z* error of ± 0.3 Da (tolerance ± 0.5 Da parent, ± 0.8 Da fragments) using the database constraints described for peptide mass fingerprinting. If no significant identification was returned, the search was repeated against the NCBInr database. The National Center for Biotechnology Information (NCBI) is part of the United States National Library of Medicine (NLM) and houses genome sequencing data in GenBank as well as other information relevant to biotechnology. For both peptide mass fingerprinting and MS/MS analysis, protein identification was accepted based on a significant Mowse probability score.

6.4 Results.

6.4.1 Subjects.

Five health-screened recreationally active men (age 21 ± 1 years, body mass 70.6 ± 2.9 kg, and body fat 15 ± 1 %) and 5 women (age 20 ± 1 years, body mass 54.8 ± 1.6 kg, and body fat 23 ± 1 %) completed the training programme after giving their informed consent to the ethically approved procedures. Each male and female participant completed the entire study with an adherence rate of 100 % out of 36 training sessions, of both training modalities. A control group as such was not used in this study, since subjects acted as their own internal controls between training modalities and the programme was only 6 weeks in duration. During each training session, participants recorded the distance covered on the treadmill and their average heart rates (appendix 9.2). Participants CV between interval and continuous training showed an average CV for distance covered of 1 % in men and 2 % in women, and an average CV for heart rate of 6 % in men and 8 % in women throughout the six weeks of training. These results suggest that both the interval and continuous training protocols for work loads were well matched.

6.4.2 Maximal aerobic power ($\dot{V}O_{2\,max}$) after 6 weeks of exercise training.

In men after interval exercise training average $\dot{V}O_{2\,max}$ increased by 4 % (P = 0.001) from 51.2 ± 1.6 ml kg^{-1} min^{-1} to 53.49 ± 1.7 ml kg^{-1} min^{-1}. After continuous training, $\dot{V}O_{2\,max}$ increased by 9 % (P = 0.025; from 49.5 ± 0.3 ml kg^{-1} min^{-1} to 54.2 ± 0.8 ml kg^{-1} min^{-1}: Figure 6.9). In women, interval exercise training increased average $\dot{V}O_{2\,max}$ by 7 % (P = 0.001; from 40.4 ± 1.7 ml kg^{-1} min^{-1} to 43.6 ± 1.5 ml kg^{-1} min^{-1}), and by 8 % (P = 0.025; from 41.3 ± 1.4 ml kg^{-1} min^{-1} to 45.0 ± 2.2 ml kg^{-1} min^{-1}) after continuous training (Figure 6.9).

These increases in aerobic capacity in men were associated with significant 6 % (average of 42 seconds) increases in $\dot{V}O_{2\,max}$ test durations after interval (P = 0.017) and continuous training (P=0.036; Figure 6.10). Likewise, there were

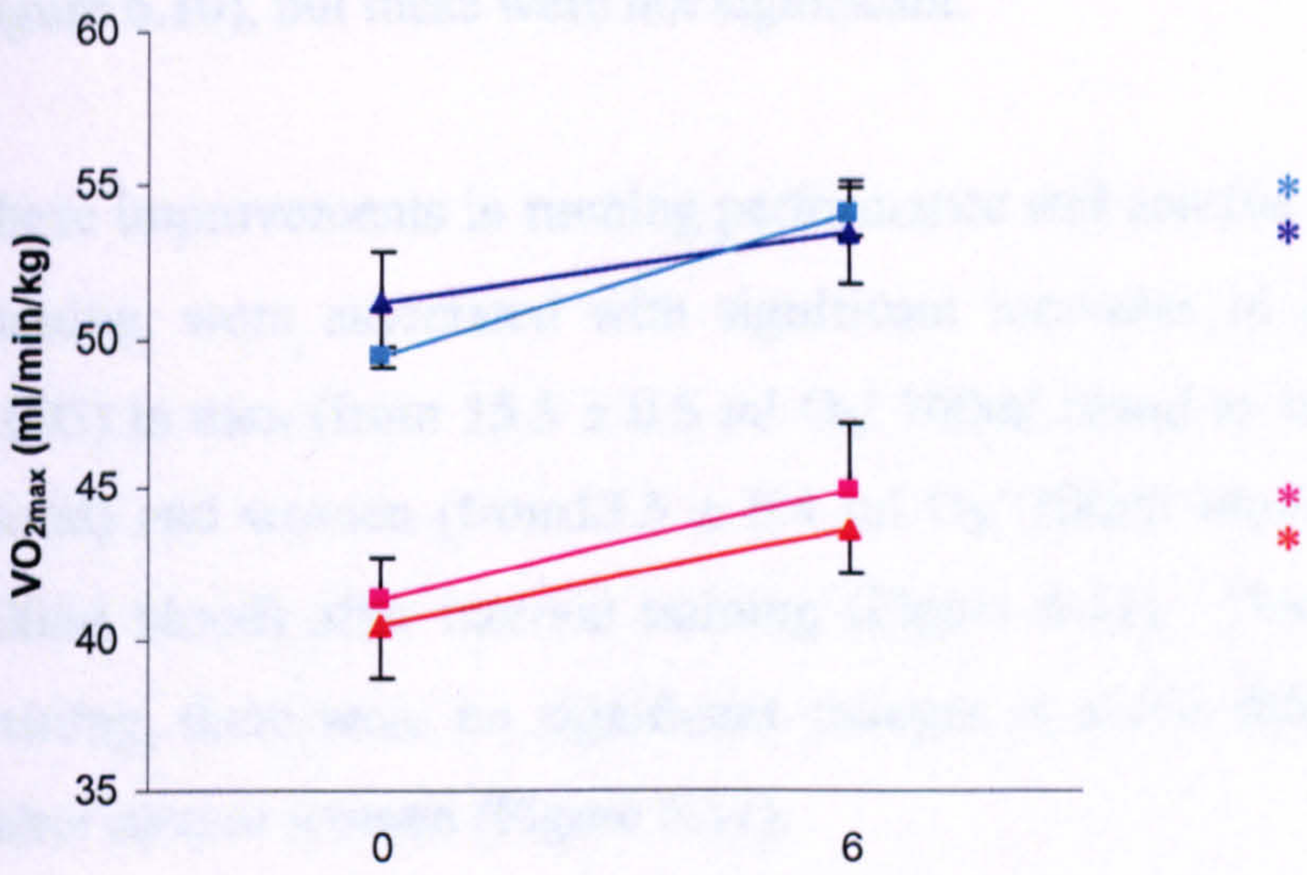


Figure 6.9: Training-induced changes in $\dot{V}O_{2\,max}$.

Data are means ± SE after 6 weeks of interval training for men (—) and women (—), and continuous training for men (—) and women (—).

*P < 0.05 statistically significant from pre-exercise training (0) values.

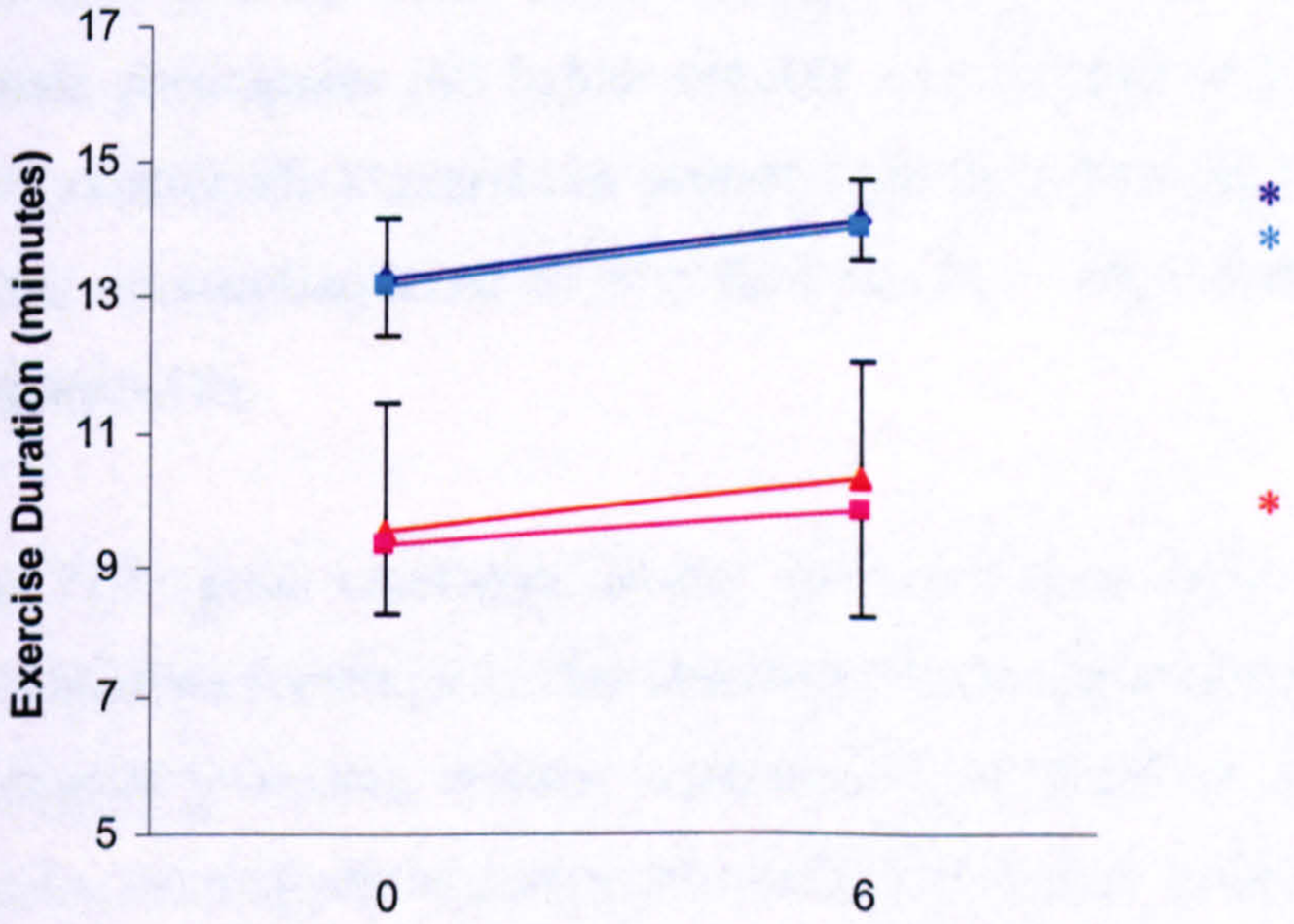


Figure 6.10: Training-induced changes in exercise duration during the $\dot{V}O_{2\,max}$ test.

Data are means ± SE after 6 weeks of interval training for men (—) and women (—), and continuous training for men (—) and women (—).

*P < 0.05 statistically significant from pre-exercise training (0) values.

increases (7 %) in $\dot{V}O_{2\,max}$ test exercise durations after interval training (P = 0.279) in the women, and a 5 % increase after continuous training (P=0.312; Figure 6.10), but these were not significant.

These improvements in running performance and aerobic capacities after interval training, were associated with significant increases in a-vO_2 differences (P = 0.005) in men (from 15.5 ± 0.5 ml O_2/ 100ml blood to 16.2 ± 0.8 ml O_2/ 100ml blood) and women (from13.5 ± 0.4 ml O_2/ 100ml blood to 15.0 ± 0.5 ml O_2/ 100ml blood) after interval training (Figure 6.11). However, after continuous training, there were no significant changes in a-vO_2 differences (P = 0.966) in either men or women (Figure 6.11).

6.4.3 Proteome Profiling.

Differential analysis was conduction on 237 protein spots for the interval training and 256 spots for continuous training, matched across 20 gels from male and female participants (10 before training and 10 after training). Gene products were confidently identified by peptide mass fingerprinting in 117 of these protein spots, representing about 50 % of the total spots visible during the image analysis (Figure 6.12).

The GO (gene ontology) project has developed three structured controlled vocabularies (ontology's) that describe gene products in terms of their associated biological processes, cellular components and molecular functions. Within our results, the majority of gene products identified were cytoplasmic (35%), but also included myofibrillar (26 %), cell surface/ extracellular (20 %) and mitochondrial (9 %) gene products (Figure 6.13A). The 4 most common (representing 67 % of all identifications; Figure 6.13B) Biological Process GO phrases associated with the identified gene products were related to regulation of muscle contractility, glycolysis, anti-apoptosis, and cellular transport. The 4 most common (representing 69 % of all identifications; Figure 6.13C) Molecular

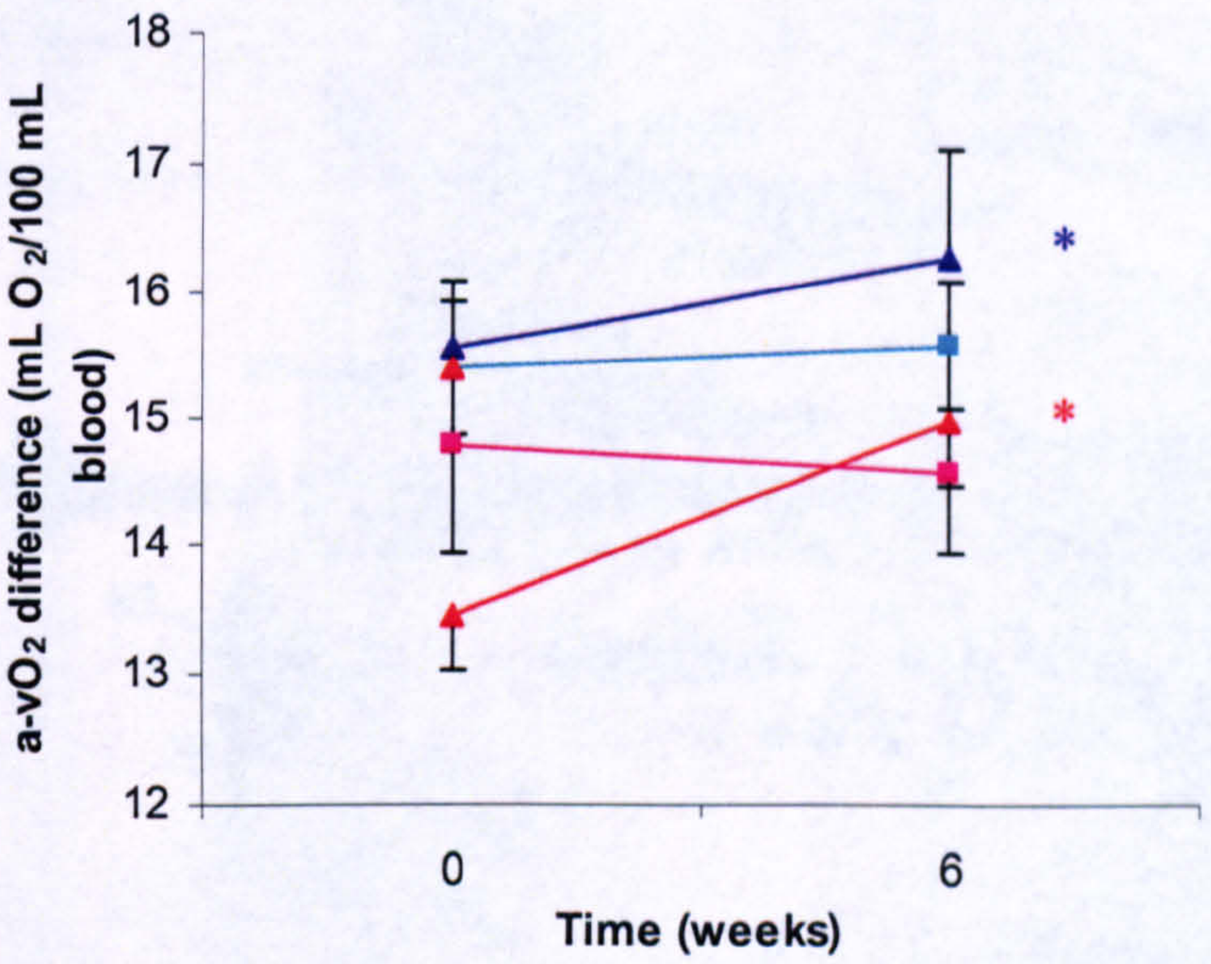


Figure 6.11: Training-induced changes in a-vO_2 differences.

Data are means ± SE after 6 weeks of interval training for men (—) and women (—), and continuous training for men (—) and women (—).

*P < 0.05 statistically significant from pre-exercise training (0) values.

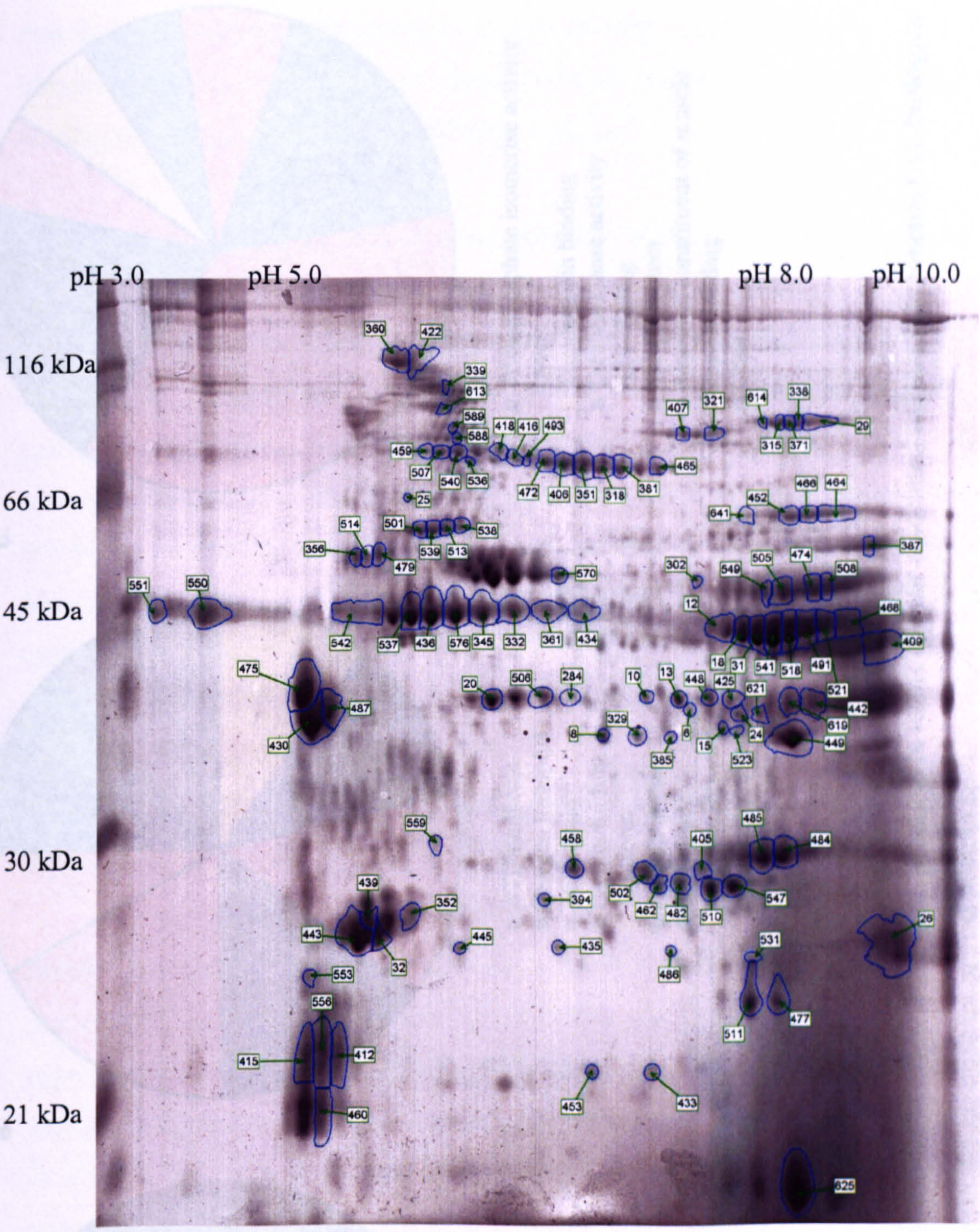


Figure 6.12: 2-D gel stained with colloidal Coomassie blue to show the proteins identified by peptide mass fingerprinting.

The numbers identified on this map correspond to the numbers reported (Table 9.3).

Proteins are separated according to isoelectric point (PI; pH 3-10NL) from left to right, and resolved molecular weight (200 kDa – 20 kDa) from top to bottom.

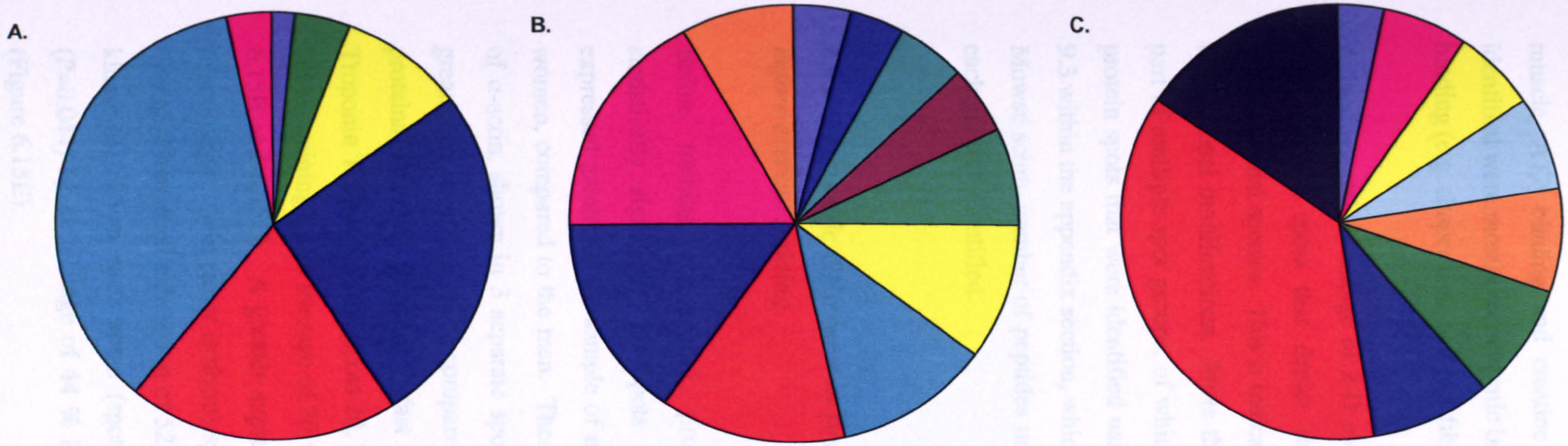


Figure 6.13: The Gene Ontology (GO) vocabularies describing gene products in terms of their associated cellular components (A), biological components (B), and molecular components (C) for each identified protein spot.

Function GO phrases were defined as protein binding, structural constituent of muscle, ATP binding and creatine kinase activity. Therefore, the proteins identified were mostly cytoplasmic being involved in energy metabolism, protein binding (e.g. chaperones) and myofibrillar proteins.

A fundamental advantage of 2-D electrophoresis is that it can resolve proteins into multiple spots that depict their different post-translational and post-transcriptional species. This is indicative of spliced variants of proteins and post-translational modifications. More than one-half of the proteins identified were part of multiple spot groups, of which an example is shown in Figure 6.14. All protein spots that were identified using mass spectrometry, are present in Table 9.3 within the appendix section, which details the Mascot outputs (database entry, Mowse score, number of peptides matched etc.) and the expected M_r and p*I* for each gel spot identified.

6.4.4 Sex-specific differences in protein expressions between men and women before exercise training.

Before training commenced, protein expression profiling identified 13 statistically significant gel spots (5 different gene products) which were expressed greater in the sample of muscle taken from the vastus lateralis in the women, compared to the men. These included a greater expression ($P = 0.015$) of α-actin, shown in 3 separate spots (spots 332, 345, 576), with an averaged greater expression of 46 % compared to men (Figure 6.15A). Other contractile proteins including troponin T fast isoform (spots 6, 10, 15; $P = 0.041$) and Troponin T slow isoform (spots 20, 284, 621; $P = 0.030$) were expressed greater before training by an average of 30 % and 38 % respectively in women (Figures 6.15B and 6.15C). A greater expression before training was also seen in two myosin light chain (slow isoform) spots (spots 32 and 352; $P = 0.013$), with an averaged increased expression of 52 % before training (Figure 6.15D). Creatine kinase-M, shown in 2 spots (spots 18 and 31), were also expressed more ($P=0.044$) by an average of 44 % in women compared to men before training (Figure 6.15E).

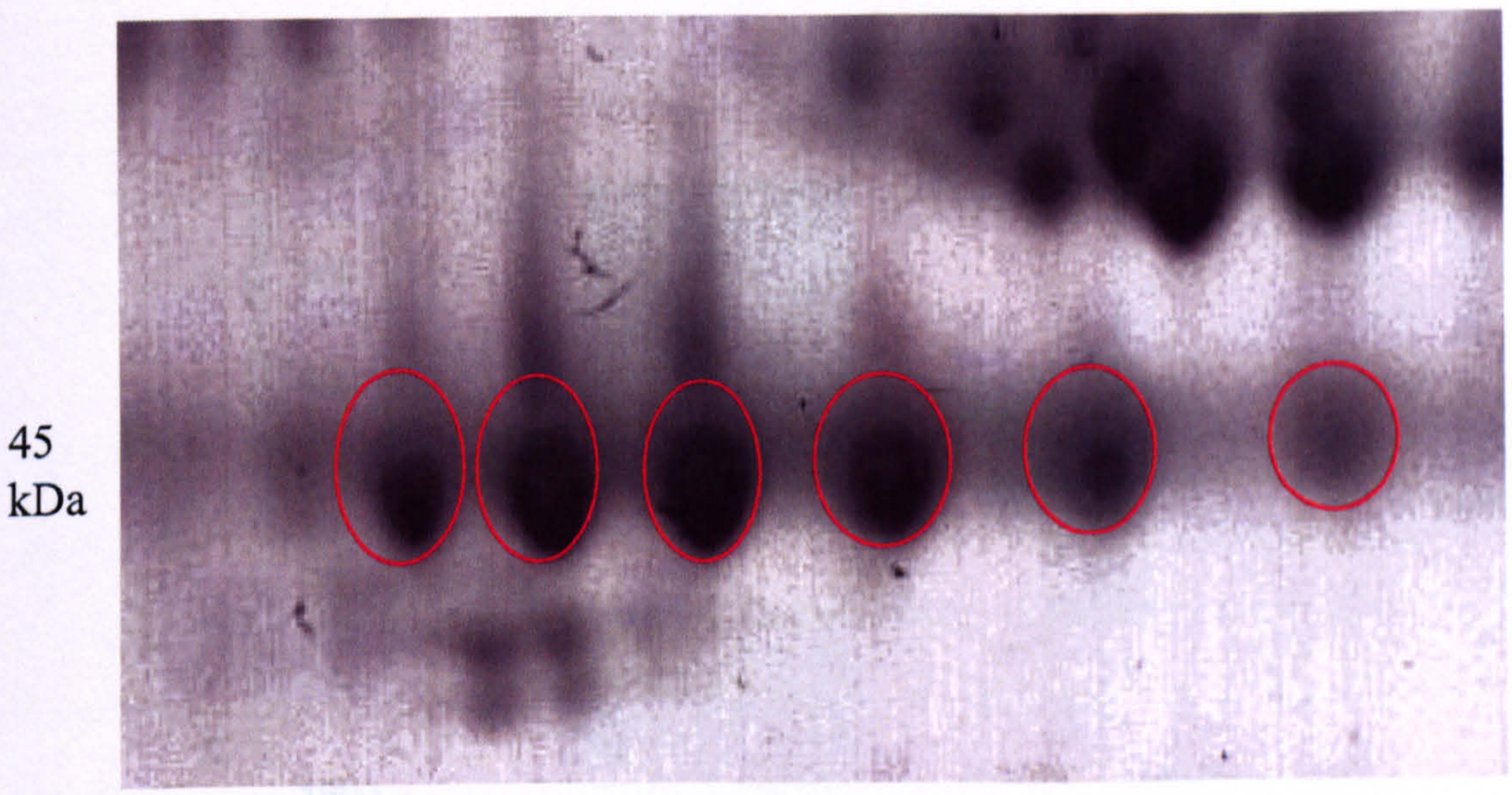


Figure 6.14: Multiple alpha actin spots on a gel.

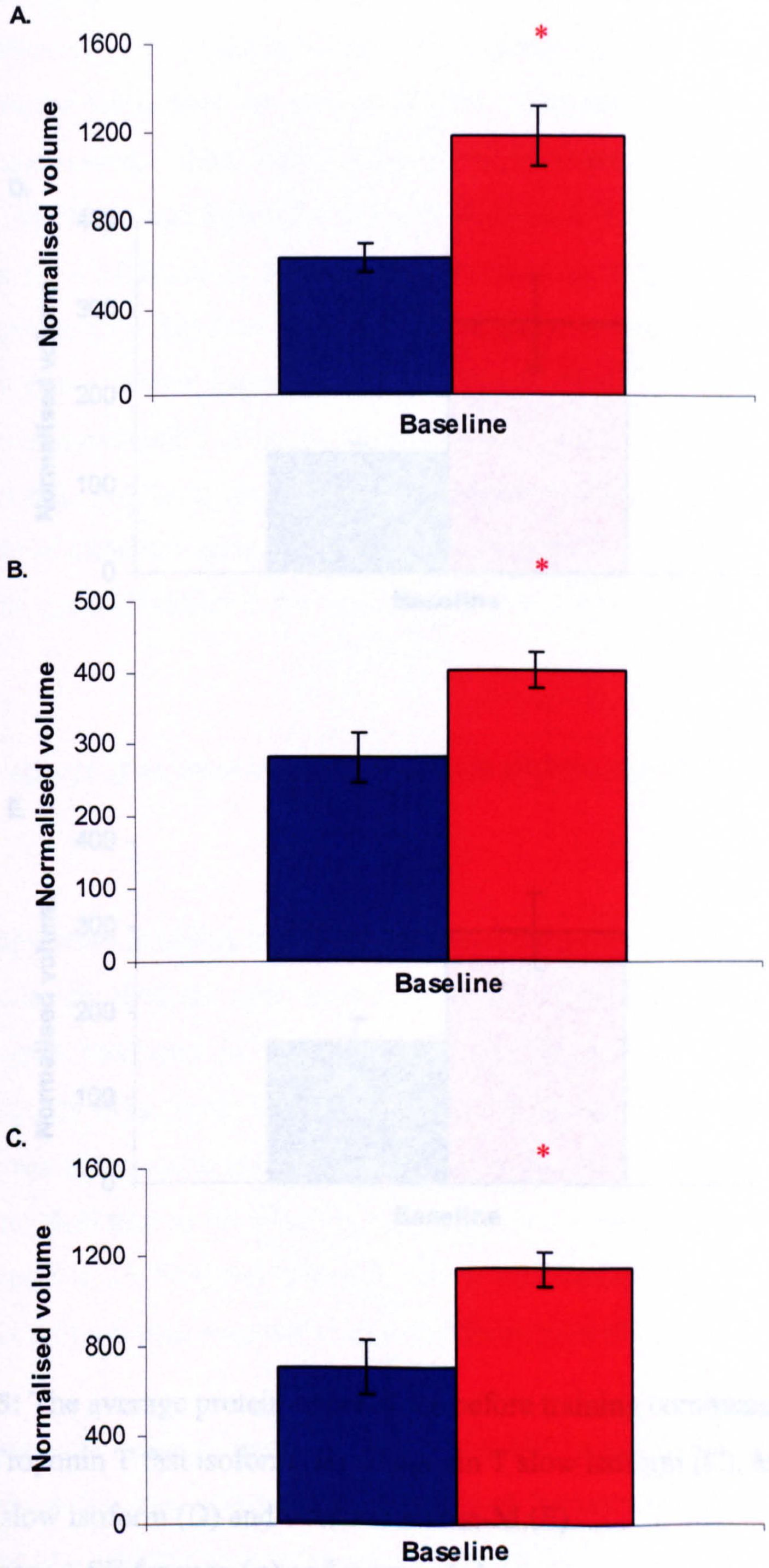


Figure 6.15: The average protein expressions before training commenced of α-actin (A), Troponin T fast isoform (B), Troponin T slow isoform (C), Myosin light chain slow isoform (D) and Creatine kinase-M (E).

Data are means ± SE for men (■) and women (■).

*P < 0.05 statistically significant between pre-exercise values.

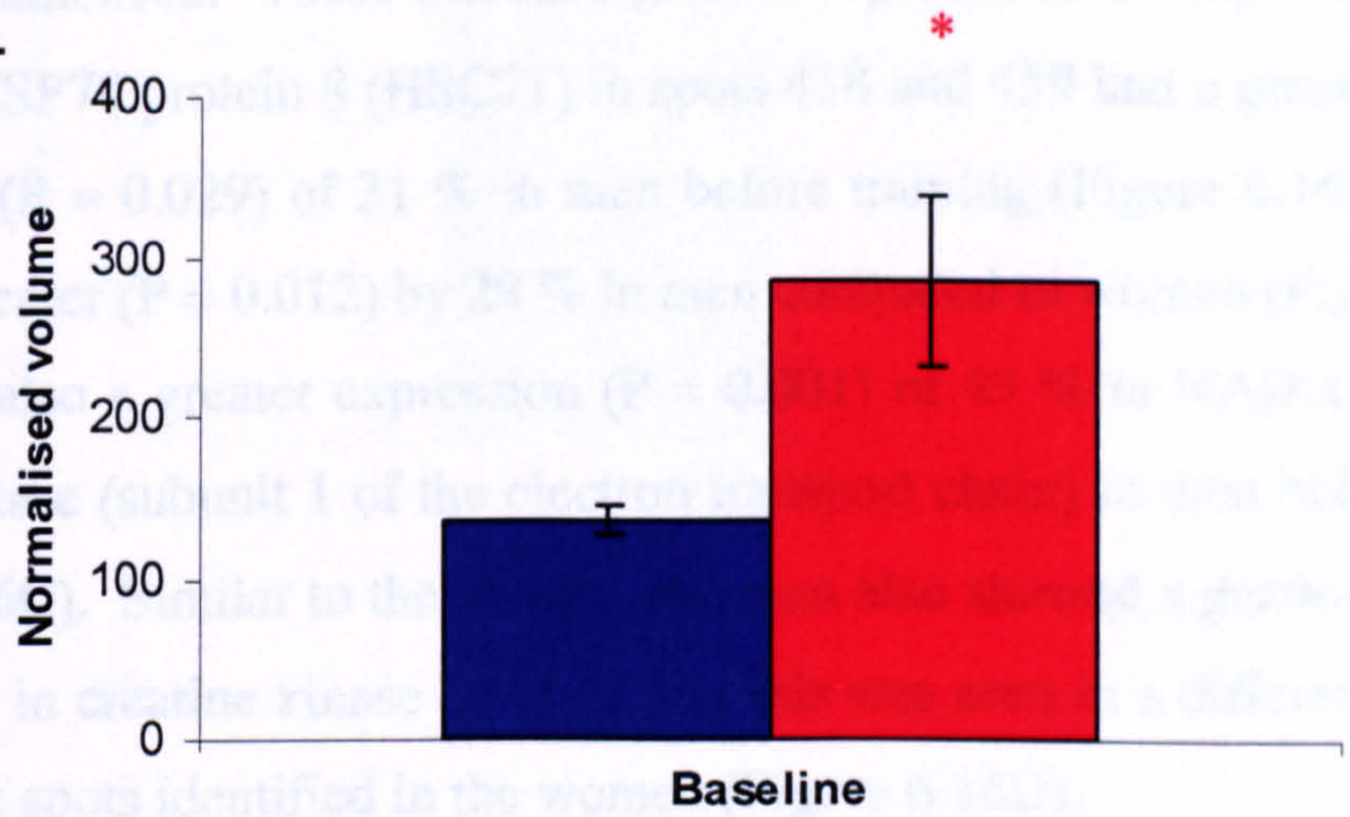


Figure 6.15: The average protein expressions before training commenced of α-actin (A), Troponin T fast isoform (B), Troponin T slow isoform (C), Myosin light chain slow isoform (D) and Creatine kinase-M (E).

Data are means ± SE for men (■) and women (■).

*P < 0.05 statistically significant between pre-exercise values.

In the men, protein expression profiling identified 5 statistically significant gel spots (4 different gene products) which were expressed to a greater extent in the biopsy samples taken from the vastus lateralis, compared to the women before training commenced. These included greater expressions in heat shock proteins (HSPs). HSP70 protein 8 (HSC71) in spots 418 and 459 had a greater averaged expression (P = 0.029) of 31 % in men before training (Figure 6.16A). HSP60 was also greater (P = 0.012) by 28 % in men compared to women (Figure 6.16B). There was also a greater expression (P = 0.001) of 43 % in NADH ubiquinone oxidoreductase (subunit 1 of the electron transport chain) in men before training (Figure 6.16C). Similar to the women, the men also showed a greater expression (P = 0.032) in creatine kinase of 41 %, but this was seen in a different spot (spot 12) than the spots identified in the women (Figure 6.16D).

6.4.5 The effects of interval training on muscle protein expressions in men and women.

Protein expression profiling identified statistically significant changes in 14 gel protein spots (9 different gene products) in men and 2 gel protein spots (2 different gene products) in women in response to interval exercise training (Table 6.1). Of these spots in men, 3 of the 9 expressed gene products were present as multiple protein spots. As such, the expression of these multiple-spots series were identified as creatine kinase (spots 12, 18, and 31), troponin T fast isoform (spots 6, 13, 553) and troponin T slow isoform (spots 284, 506). Figure 6.17 shows a full protein map where the significant spot changes are highlighted.

In men, interval training significantly (P < 0.04) changed the expression of creatine kinase M-type, which were identified as 3 separate spots (spots 12, 18 and 31) out of a multiple-spot series (spots 468, 491, 518, 521, 541; Figure 6.18). Spot 12 increased in expression by 51 % (Figure 6.19A), spot 18 by 56 % (Figure 6.19B) and spot 31 by 42 % (Figure 6.19C). In contrast the female group did not increase creatine kinase expression with interval training, conversely showing a non-significant averaged decrease (P = 0.713) of 14 % (Figure 6.19).

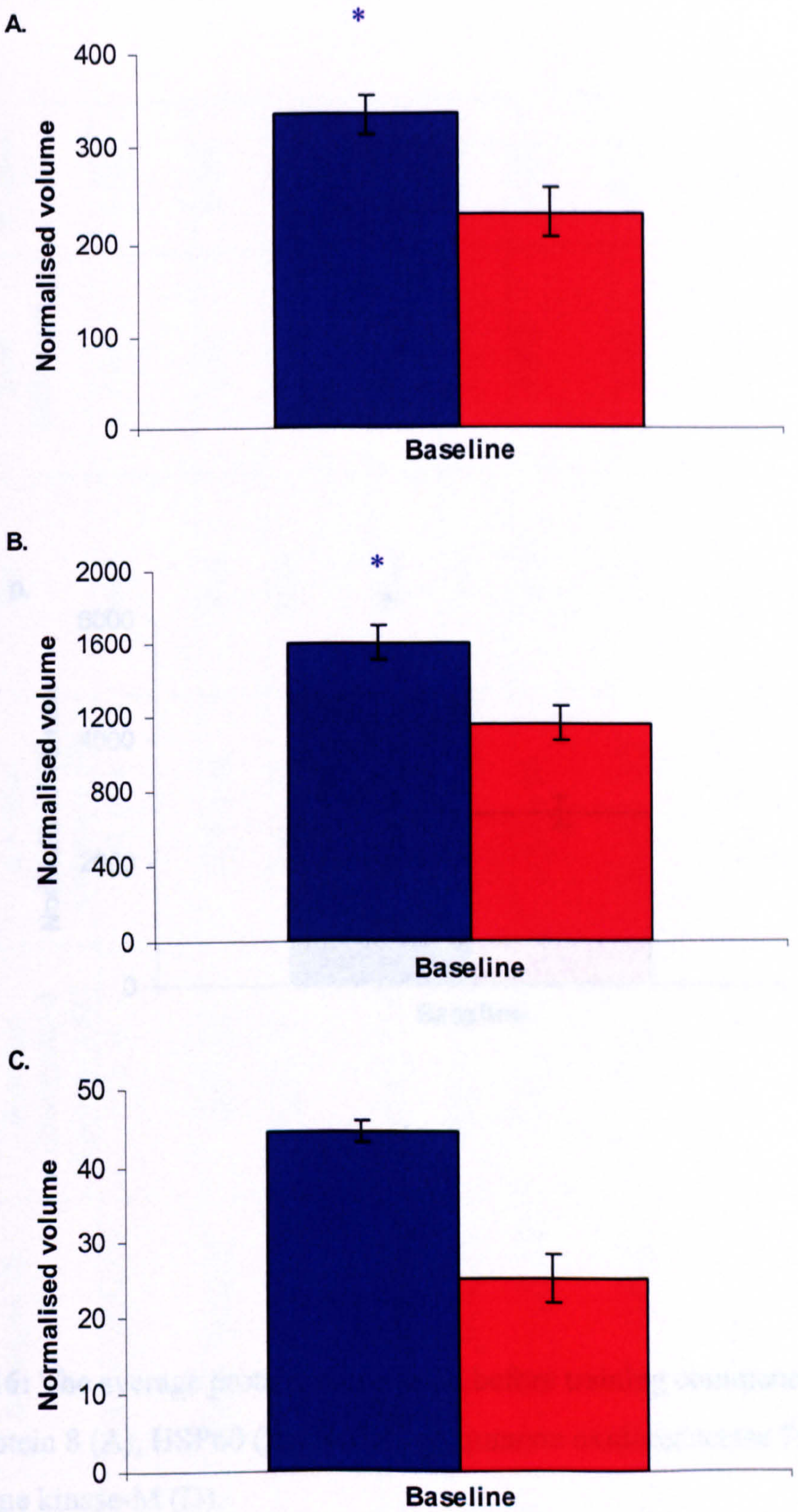


Figure 6.16: The average protein expressions before training commenced of HSP70 protein 8 (A), HSP60 (B), NADH ubiquinone oxidoreductase 75 kDa (C) and Creatine kinase-M (D).

Data are means ± SE for men (■) and women (■)

*P < 0.05 statistically significant between pre-exercise values.

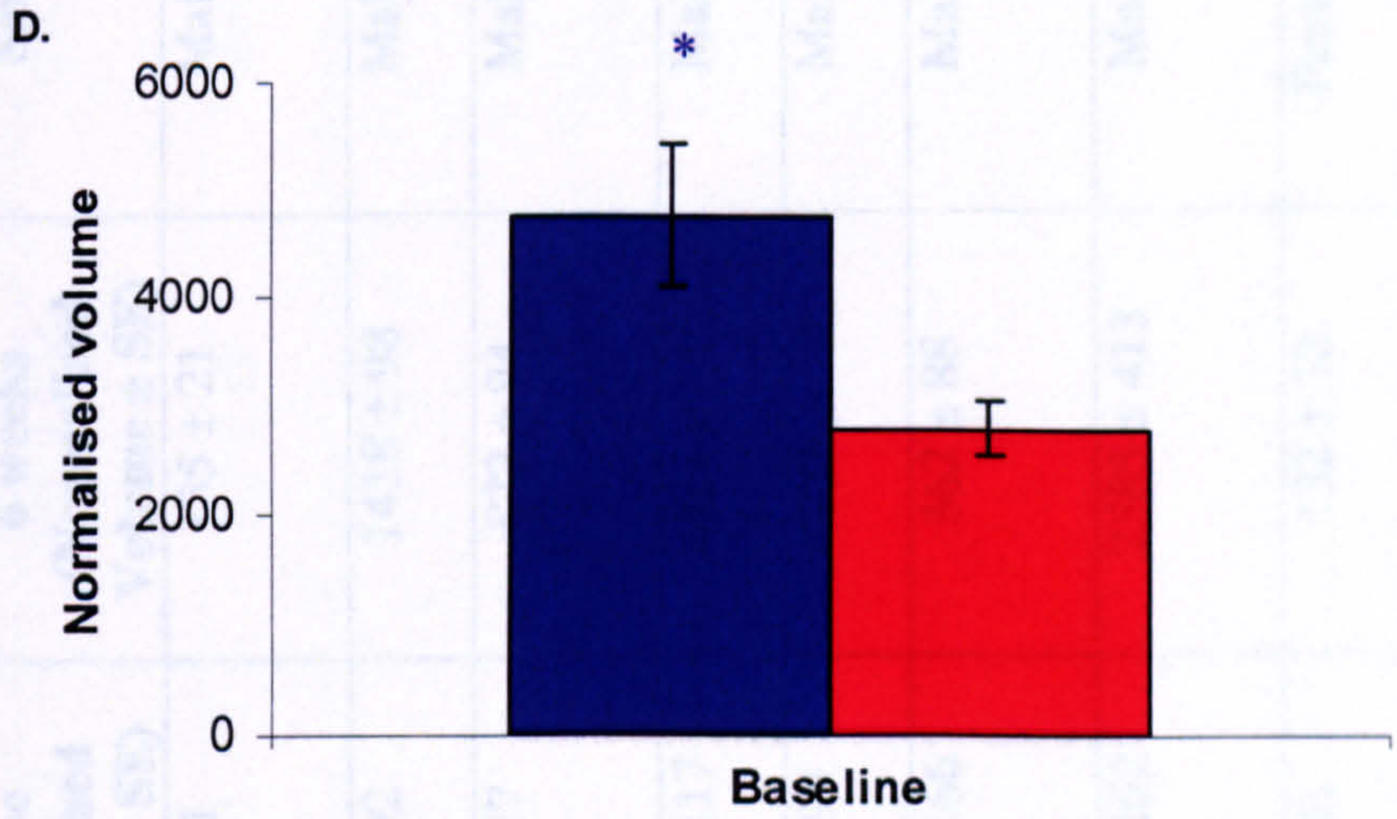


Figure 6.16: The average protein expressions before training commenced of HSP70 protein 8 (A), HSP60 (B), NADH ubiquinone oxidoreductase 75 kDa (C) and Creatine kinase-M (D).

Data are means ± SE for men (■) and women (■)

*P < 0.05 statistically significant between pre-exercise values.

Table 6.1: Mascot search results (database entry and protein ID) and the normalised volume before (baseline) and after (6 weeks), fold difference and the significance level, after interval training.

Spot number	Description	Swissprot ID	Baseline (Normalised Volume ± SE)	6 weeks (Normalised Volume ± SE)	Sex	Fold difference	P value
6	Troponin T, fast skeletal muscle (TnTf)	TNNT3_HUMAN	67 ± 11	135 ± 21	Male	2.034	0.024
12	Creatine kinase M-type	KCRM_HUMAN	698 ± 92	1418 ± 98	Male	2.031	0.018
13	Troponin T3, skeletal, fast isoform 2	TNNT3_HUMAN	264 ± 27	822 ± 94	Male	3.116	0.04
18	Creatine kinase M-type	KCRM_HUMAN	1028 ± 117	2321 ± 115	Male	2.259	0.04
31	Creatine kinase M-type	KCRM_HUMAN	2723 ± 99	4716 ± 79	Male	1.732	0.015
284	Troponin T, slow skeletal muscle (TnTs)	TNNT1_HUMAN	1018 ± 166	362 ± 88	Male	-2.813	0.015
412	Myosin regulatory light chain 2,	MLRV_HUMAN	1977 ± 262	2585 ± 413	Male	1.307	0.015
553	Troponin T, fast skeletal muscle	TNNT3_HUMAN	249 ± 31	132 ± 32	Female	-1.899	0.027
506	Troponin T, slow skeletal muscle (TnTs)	TNNT1_HUMAN	1755 ± 296	1178 ± 181	Male	-1.49	0.001

Spot number	Description	Swissprot ID	Baseline (Normalised Volume ± SE)	6 weeks (Normalised Volume ± SE)		Fold difference	P value
433	Heat-shock protein beta-6 (HspB6)	HSPB6_HUMAN	155 ± 14	227 ± 25	Male	1.459	0.009
439	Myosin light polypeptide 3	MYL3_HUMAN	2179 ± 158	1556 ± 138	Male	-1.4	0.049
465	Serum albumin	ALBU_HUMAN	418 ± 40	322 ± 55	Male	-1.3	0.007
493	Heat shock 70 kDa protein 1 (HSP70.1)	HSP71_HUMAN	133 ± 30	144 ± 25	Male	1.087	0.012
559	Heat-shock protein beta-1 (HspB1)	HSPB1_HUMAN	59 ± 9	108 ± 24	Female	1.532	0.032

Values are means ± SE.

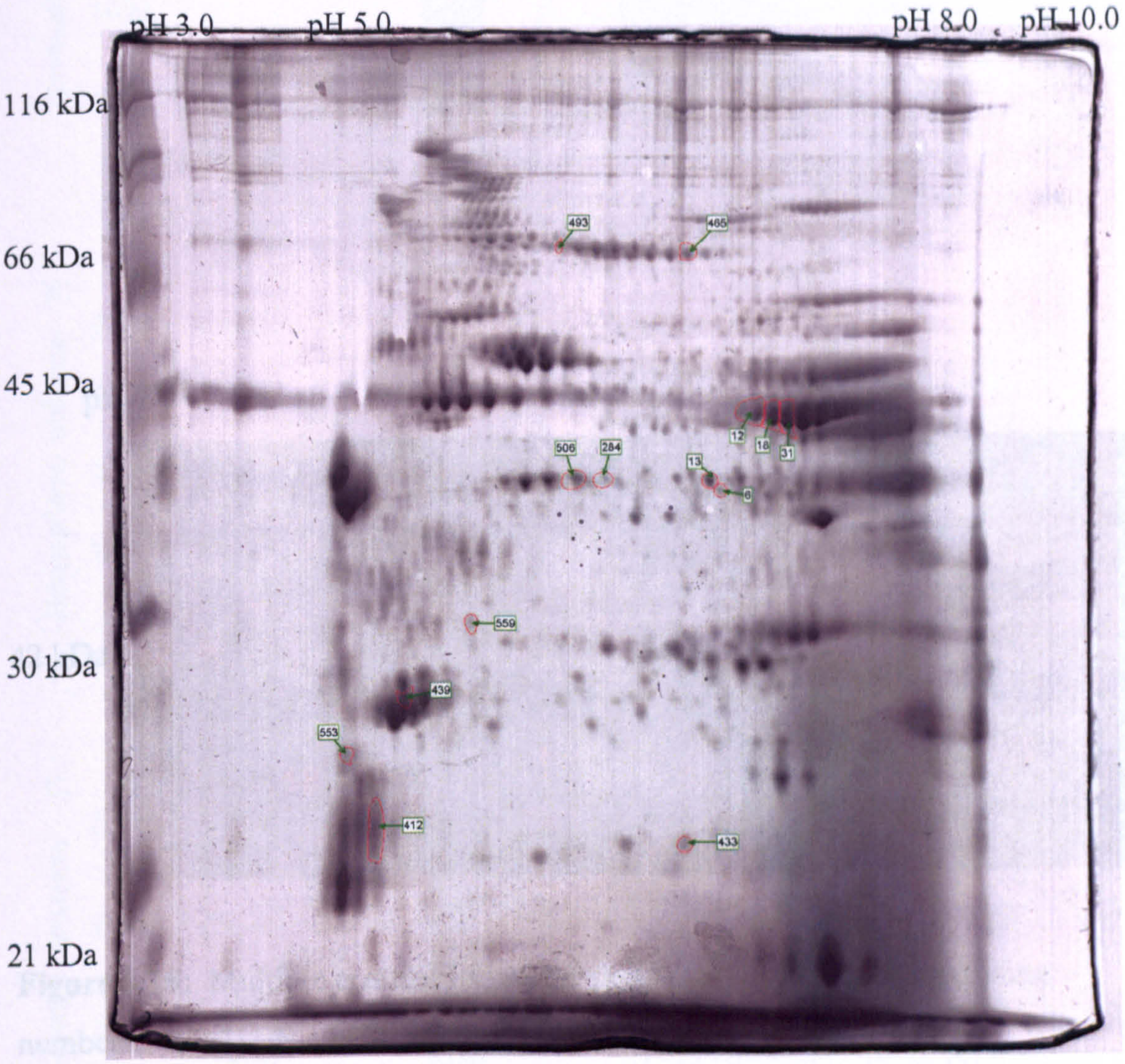


Figure 6.17: The identification of changed expression in proteins after interval training.

The numbers identified on this map correspond to the numbers reported (Table 6.1).

Proteins are separated according to isoelectric point (PI; pH 3-10NL) from left to right, and resolved molecular weight (200 kDa – 20 kDa) from top to bottom.

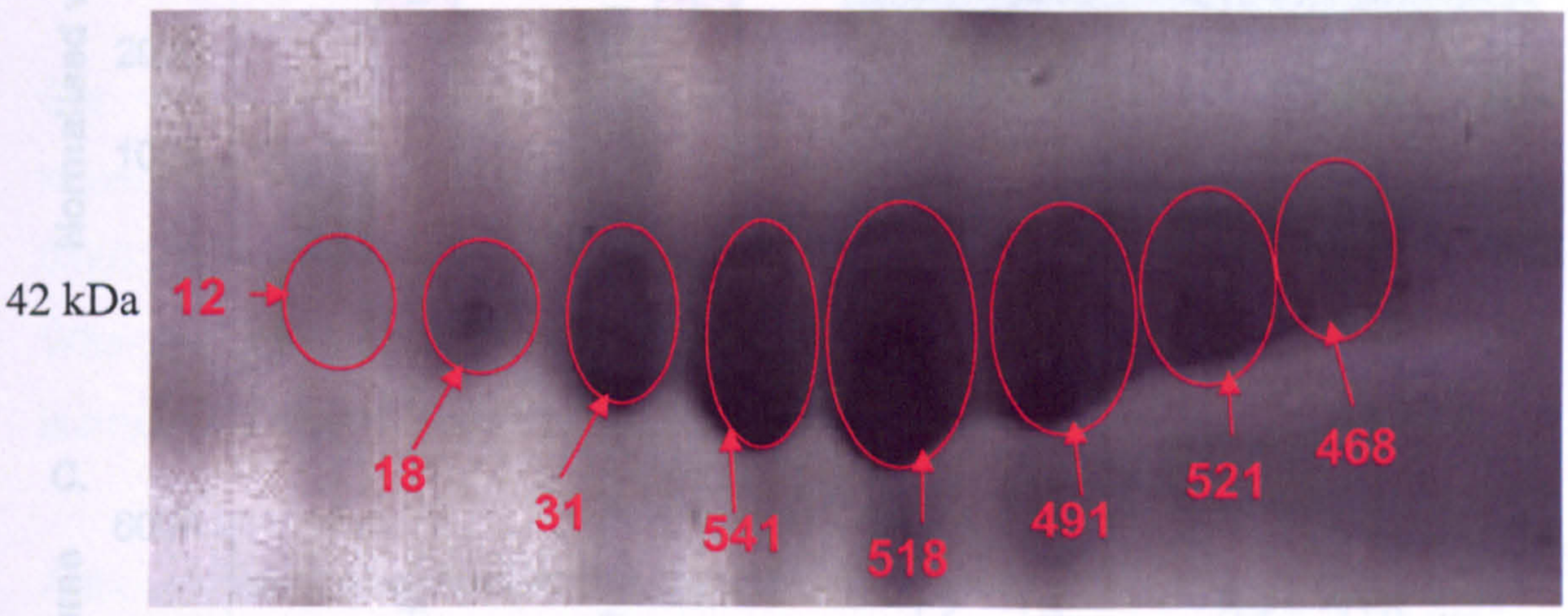


Figure 6.18: Multiple creatine kinase-M spots in series with corresponding numbers.

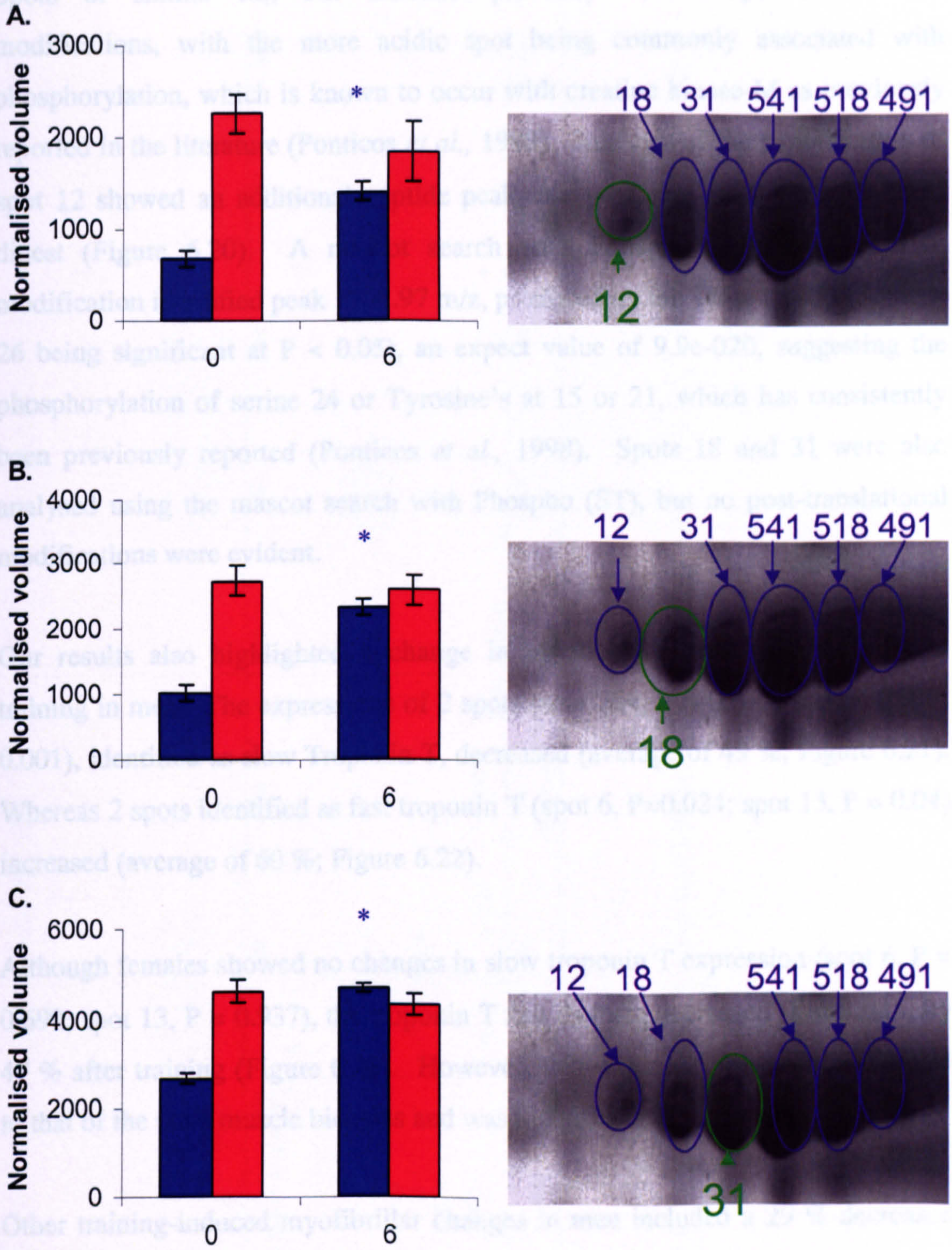


Figure 6.19: Training-induced changes in creatine kinase M-type for spot 12 (A), spot 18 (B) and spot 31 (C) after interval training.

Data are shown as means ± SE for men (■) and women (■) at 0 and 6 weeks of training.

*$P < 0.05$ statistically significant from pre-exercise training (0) values.

Spots of similar M_r, but different p*I*, may indicate post-translational modifications, with the more acidic spot being commonly associated with phosphorylation, which is known to occur with creatine kinase-M as previously reported in the literature (Ponticos *et al.*, 1998). Analysis of the peptide mass of spot 12 showed an additional peptide peak, not predicted from the theoretical digest (Figure 6.20). A mascot search with Phospho (ST) as a variable modification identified peak 1768.97 m/z, produced an ion score of 232 (Score > 26 being significant at $P < 0.05$), an expect value of 9.9e-020, suggesting the phosphorylation of serine 24 or Tyrosine's at 15 or 21, which has consistently been previously reported (Ponticos *et al.*, 1998). Spots 18 and 31 were also analysed using the mascot search with Phospho (ST), but no post-translational modifications were evident.

Our results also highlighted a change in myofibrillar proteins after interval training in men. The expressions of 2 spots (spot 284, P = 0.015; spot 506, P = 0.001), identified as slow Troponin T, decreased (average of 49 %; Figure 6.21). Whereas 2 spots identified as fast troponin T (spot 6, P=0.024; spot 13, P = 0.04), increased (average of 60 %; Figure 6.22).

Although females showed no changes in slow troponin T expression (spot 6, P = 0.692; spot 13, P = 0.937), the troponin T fast isoform decreased (P = 0.021) by 47 % after training (Figure 6.23). However, this was a different spot compared to that of the male muscle biopsies and was identified at a lower p*I* (spot 553).

Other training-induced myofibrillar changes in men included a 29 % decreased expression in myosin light polypeptide 3 (spot 439, P = 0.049, Figure 6.24), but an increased expression of 24 % in myosin regulatory light chain (spot 412, P = 0.015, Figure 6.25). However, these patterns were not found in the female muscles, with no changes in either myosin light polypeptide 3 (P = 0.826, Figure 6.24) or myosin regulatory light chain (P = 0.451, Figure 6.25) expressions after interval training.

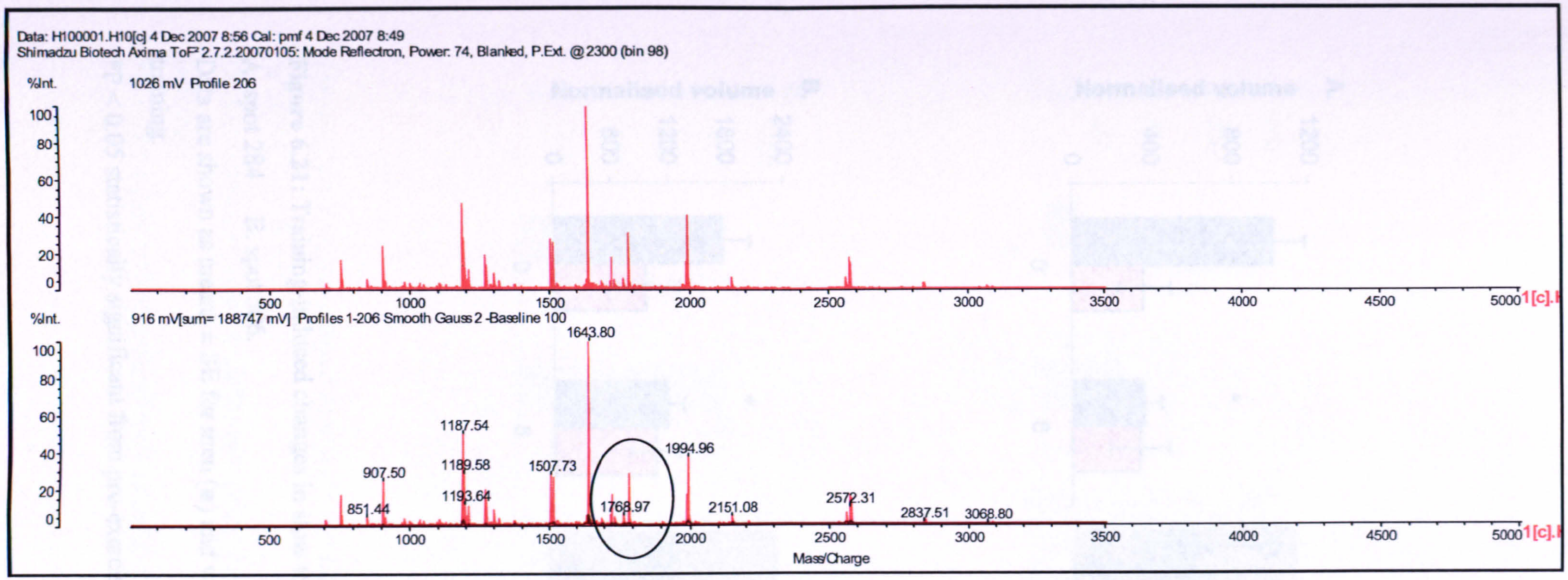


Figure 6.20: Peptide Spectra for Spot 12 (creatine kinase) showing an additional peptide peak (residue 1768.97), not within the theoretical range.

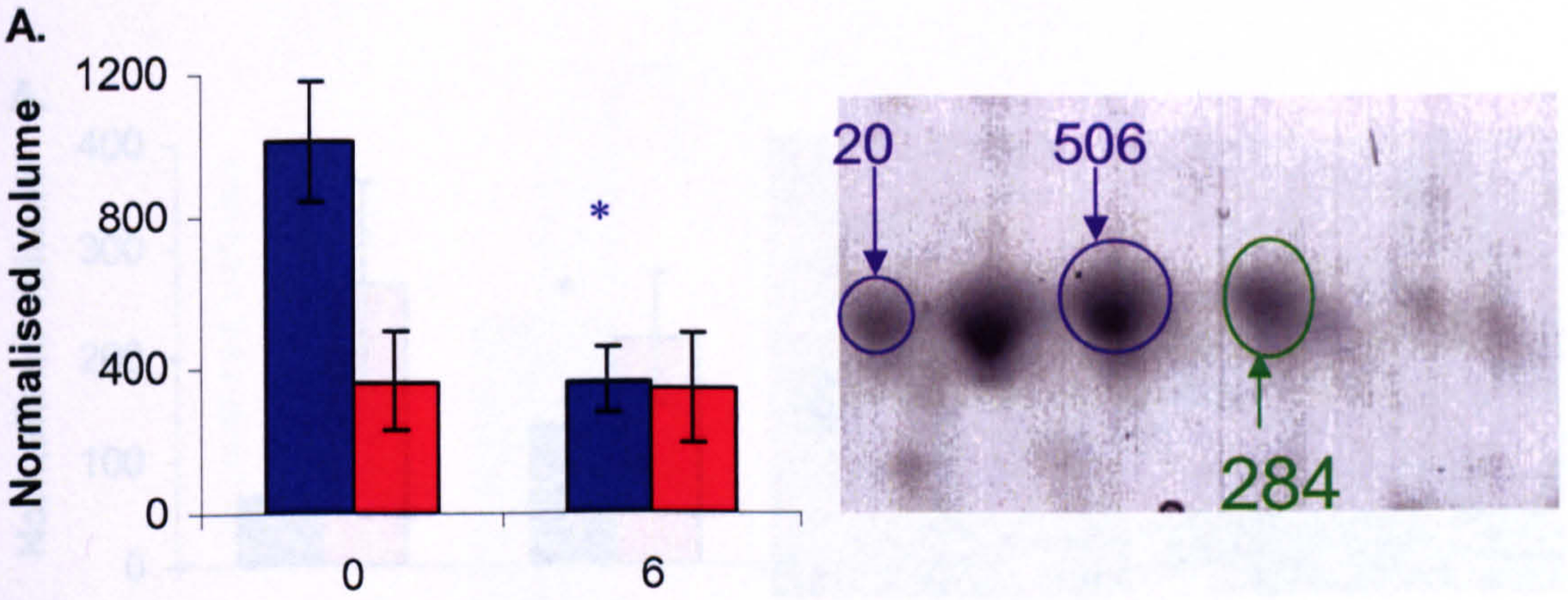


Figure 6.21: Training-induced changes in slow troponin T after interval training.

A. spot 284 B. spot 506.

Data are shown as means ± SE for men (■) and women (■) at 0 and 6 weeks of training.

*P < 0.05 statistically significant from pre-exercise training (0) values.

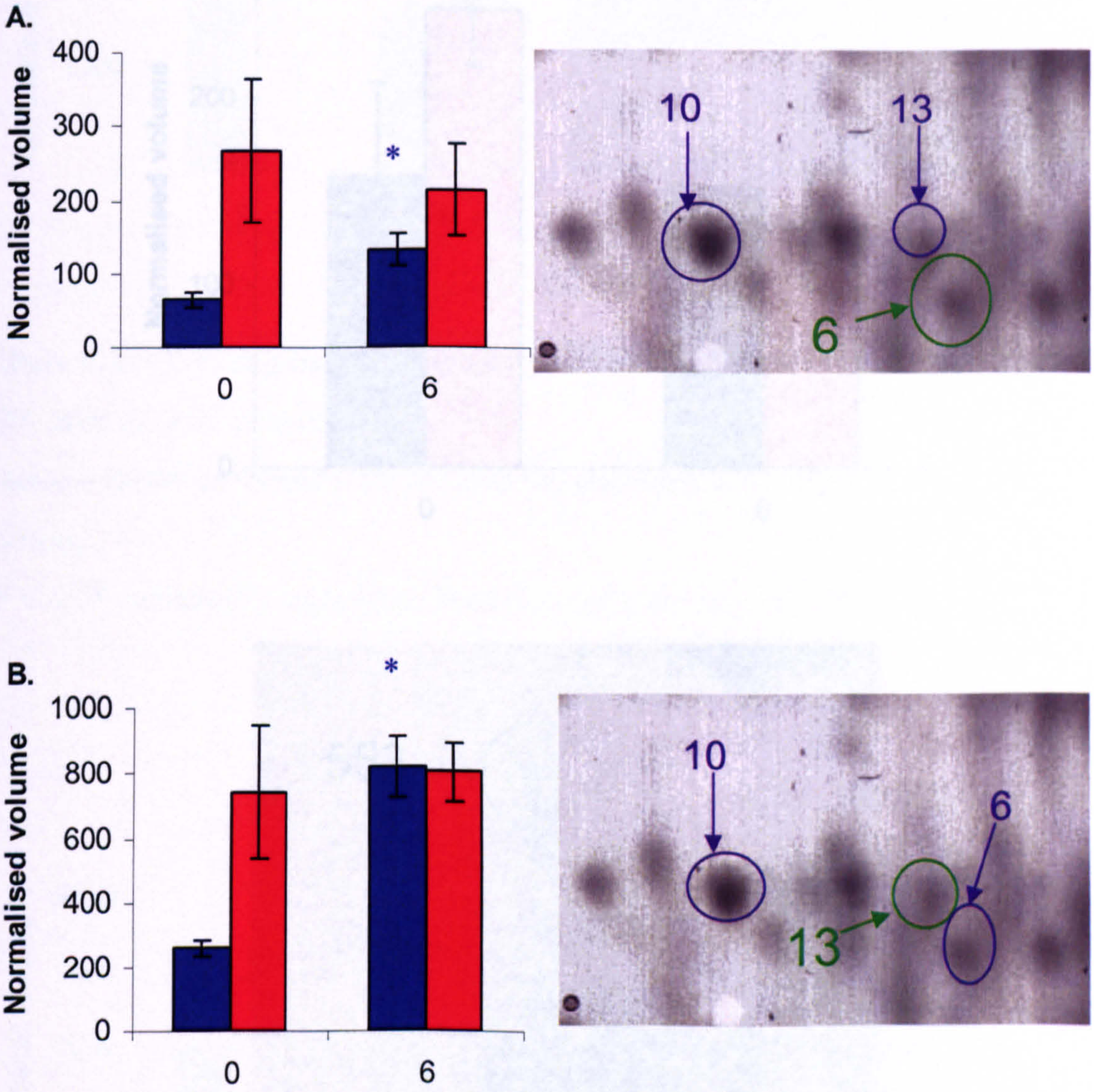
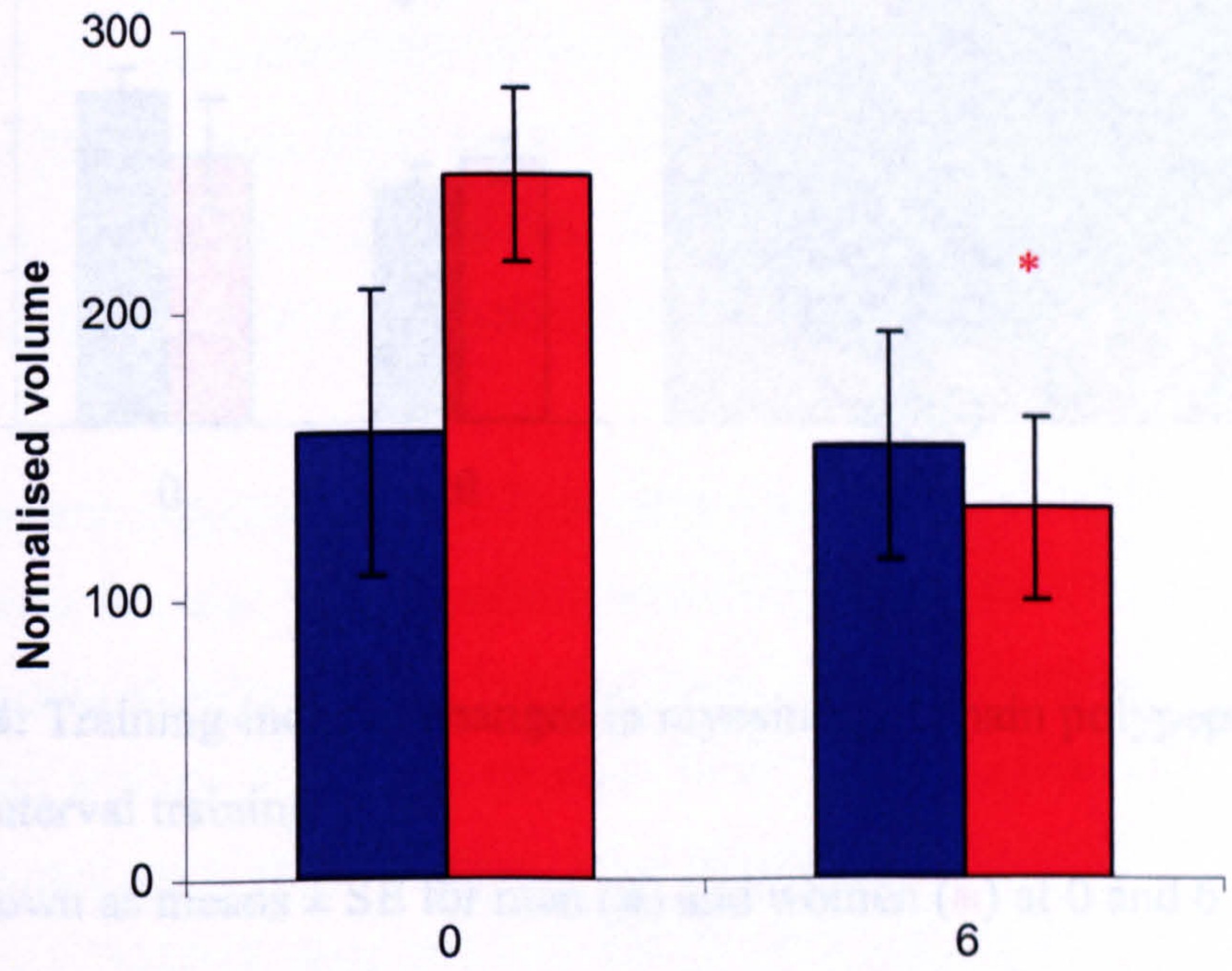


Figure 6.22: Training-induced changes in fast troponin T after interval training.

A. spot 6 B. spot 13.

Data are shown as means ± SE for men (■) and women (■) at 0 and 6 weeks of training.

*P < 0.05 statistically significant from pre-exercise training (0) values.



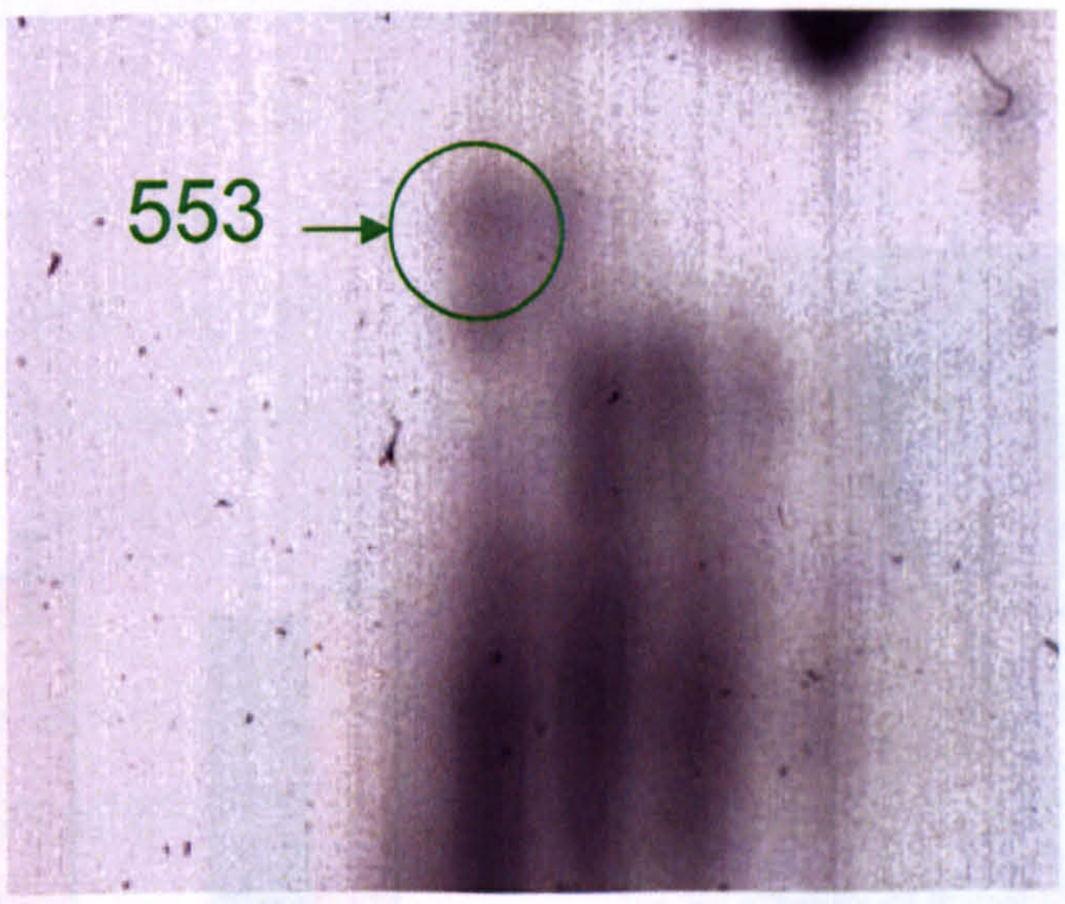


Figure 6.23: Training-induced changes in fast troponin T (spot 553) after interval training.

Data are shown as means ± SE for men (■) and women (■) at 0 and 6 weeks of training.

*P < 0.05 statistically significant from pre-exercise training (0) values.

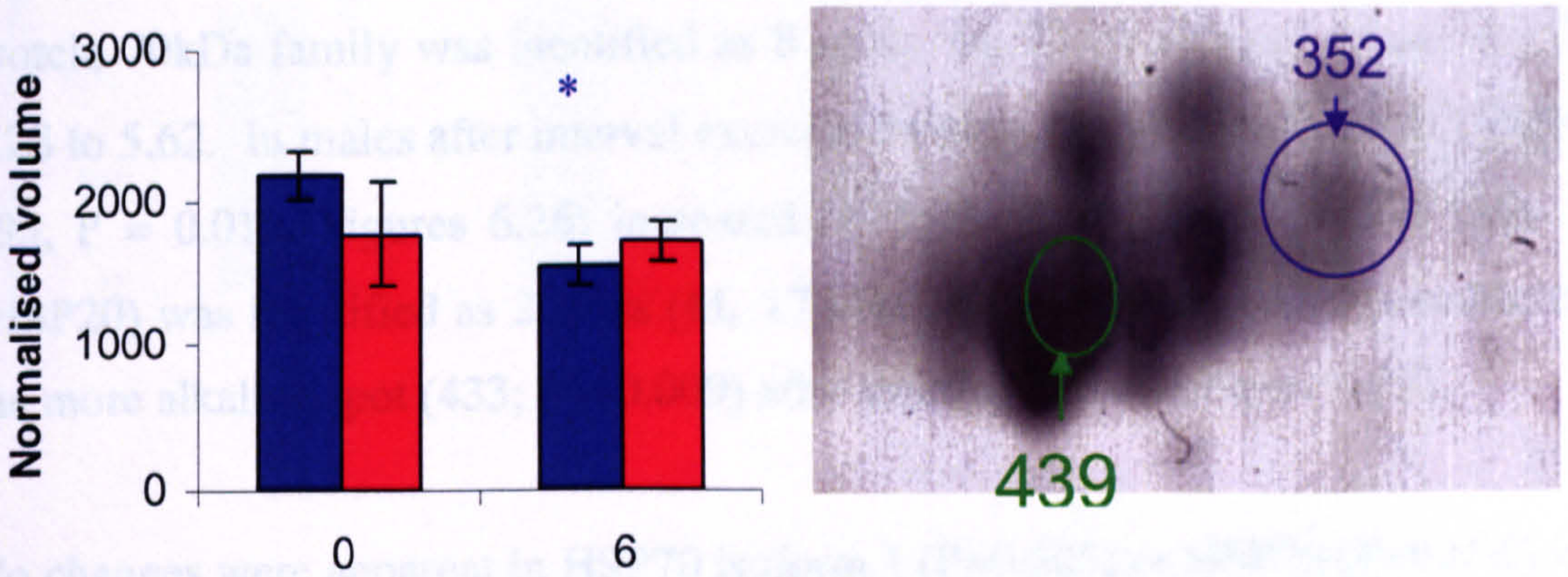


Figure 6.24: Training-induced changes in myosin light chain polypeptide 3 (spot 439) after interval training.

Data are shown as means ± SE for men (■) and women (■) at 0 and 6 weeks of training.

*P < 0.05 statistically significant from pre-exercise training (0) values.

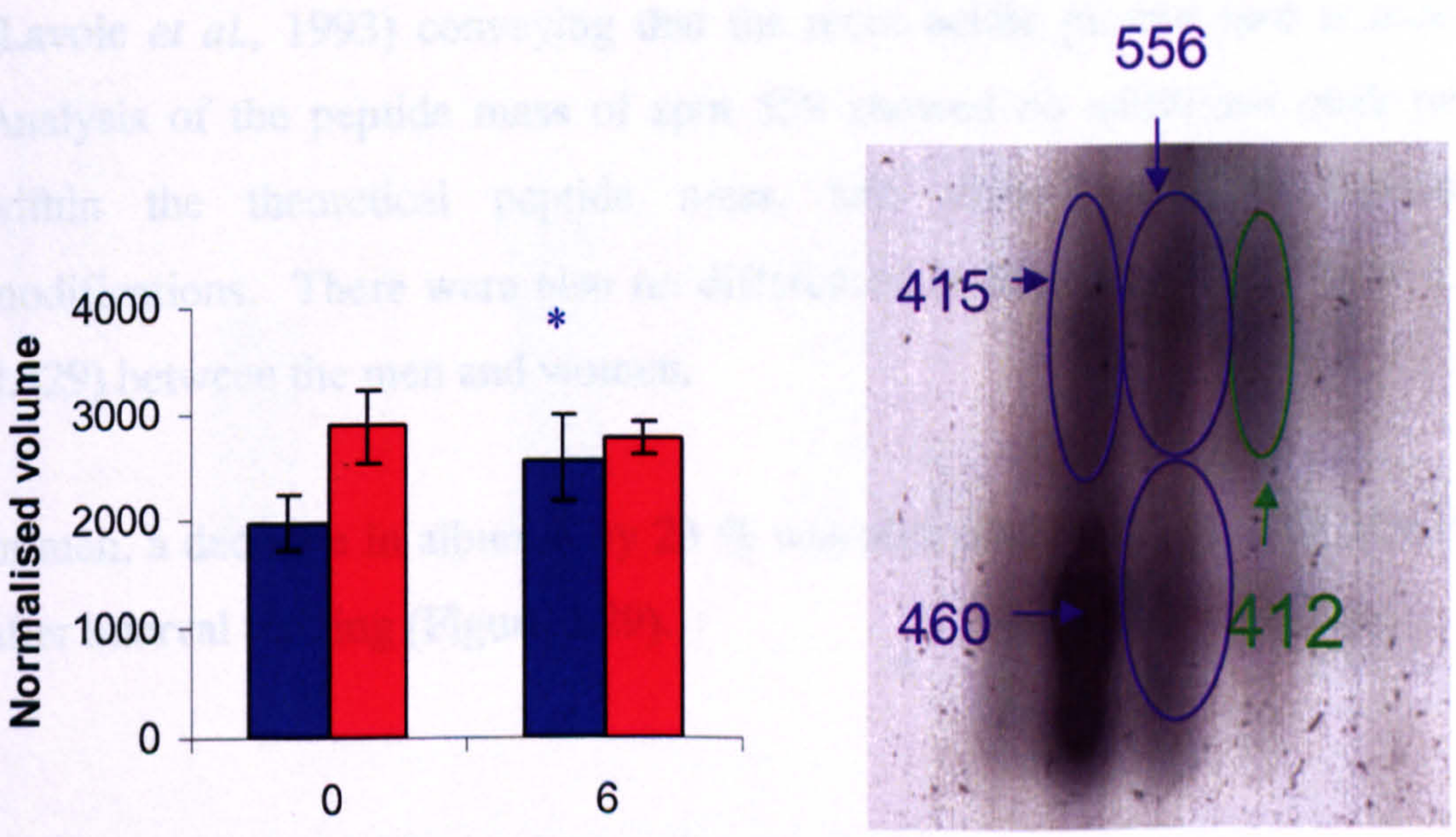


Figure 6.25: Training-induced changes in myosin regulatory light chain (spot 412) after interval training.

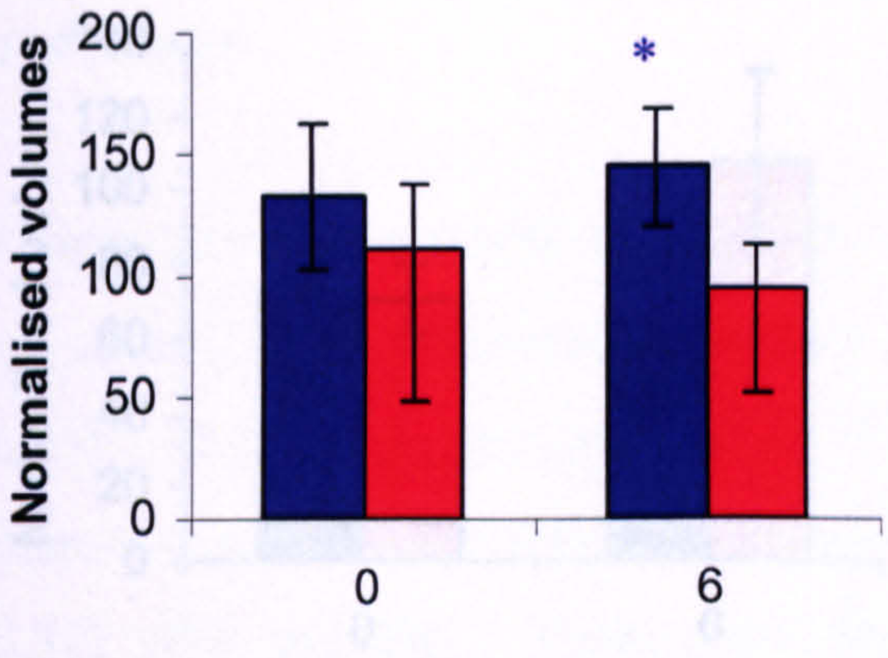
Data are shown as means ± SE for men (■) and women (■) at 0 and 6 weeks of training.

*P < 0.05 statistically significant from pre-exercise training (0) values.

Heat shock proteins are an abundant group of proteins whose expression is increased when the cells are exposed to elevated temperatures or other stress, and can be readily investigated by 2-DE and Coomassie staining. The heat shock protein 70kDa family was identified as 8 spots (M_r 70-71 kDa) p*I* spanning pH 5.28 to 5.62. In males after interval exercise training, the protein isoform 1 (spot 493, P = 0.012, Figures 6.26) increased by 8 %. Heat shock protein Beta 6 (HSP20) was identified as 2 spots (M_r 17 kDa), with an increased expression in the more alkaline spot (433; P = 0.009) after training in men (Figure 6.27).

No changes were apparent in HSP70 isoform 1 (P=0.505) or HSP20 (P=0.607) in females vastus lateralis muscles after training (Figures 6.26 & 6.27). There were also no significant differences at baseline level between the muscle biopsies from the men and women in relation to HSP 70 (P = 0.592) and HSP Beta 6 (P = 0.167). Interestingly, in the females, there was an increase of 35 % in heat shock protein beta 1 (HSP27; spot 559, P = 0.032) after training (Figures 6.28). Four HSP beta 1 proteins were identified (M_r 23 kDa) with a p*I* spanning pH 5 to 7. The phosphorylation of HSP27 has previously been reported in the literature (Lavoie *et al.*, 1993) conveying that the more acidic protein spot is modified. Analysis of the peptide mass of spot 559 showed no additional peak peptide within the theoretical peptide mass, and showed no post-translational modifications. There were also no differences at baseline in HSP Beta 1 (P = 0.829) between the men and women.

In men, a decrease in albumin by 23 % was also observed (spot 465, P = 0.007) after interval training (Figure 6.29).



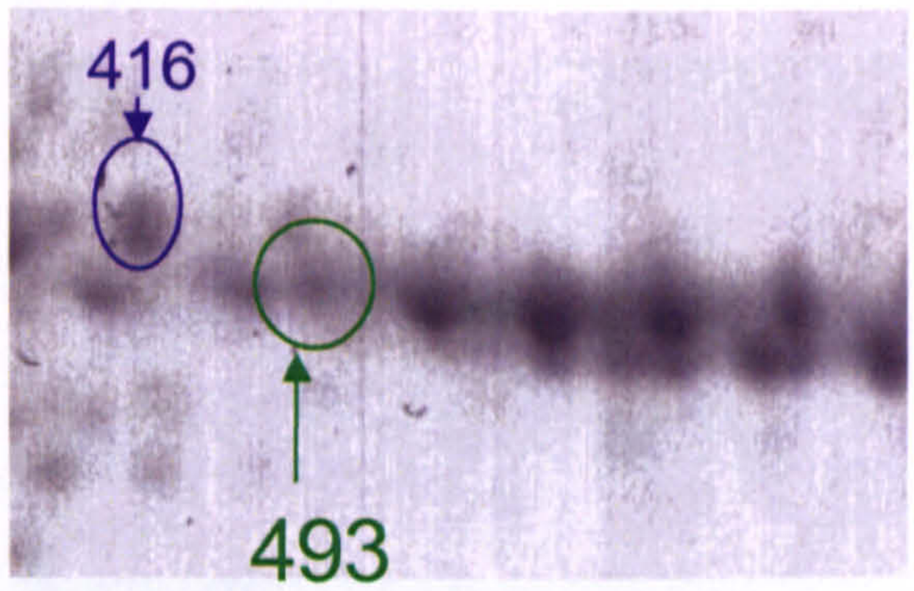
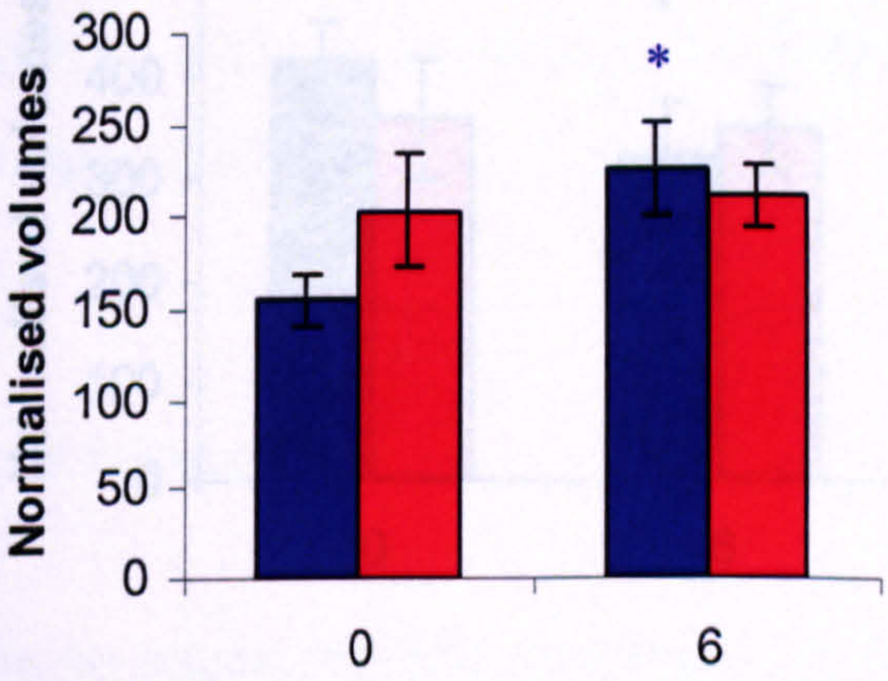


Figure 6.26: Training-induced changes in heat shock protein 70, isoform 1 (spot 493) after interval training.

Data are shown as means ± SE for men (■) and women (■) at 0 and 6 weeks of training.

*P < 0.05 statistically significant from pre-exercise training (0) values.



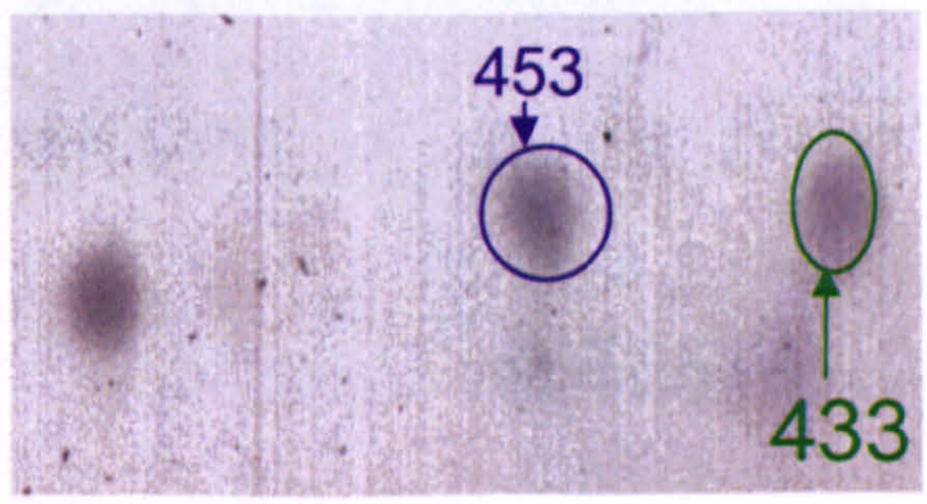
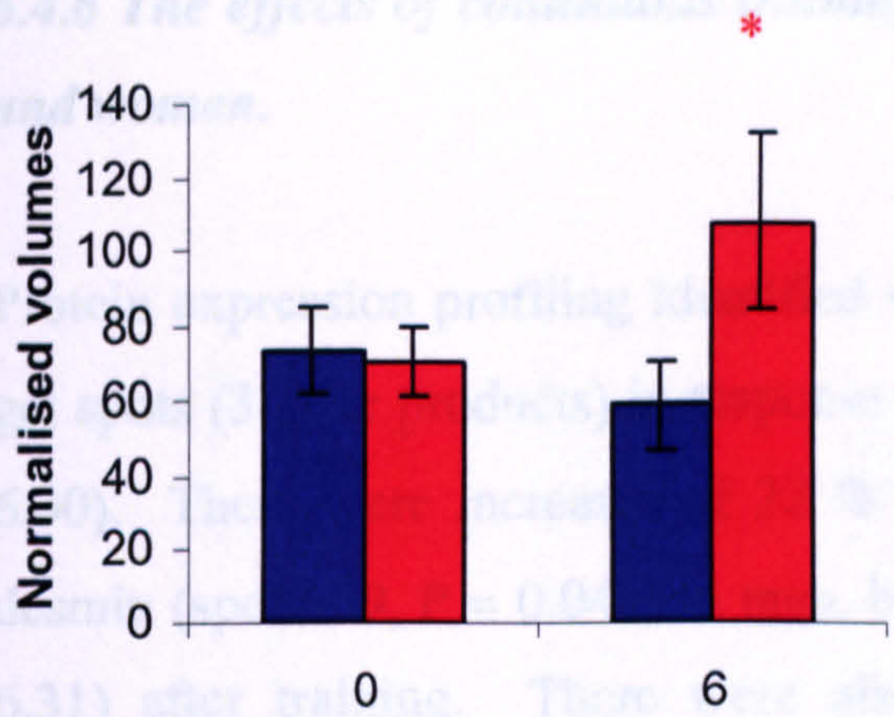


Figure 6.27: Training-induced changes in heat shock protein 20 (spot 433) after interval training.

Data are shown as means ± SE for men (■) and women (■) at 0 and 6 weeks of training.

*P < 0.05 statistically significant from pre-exercise training (0) values.



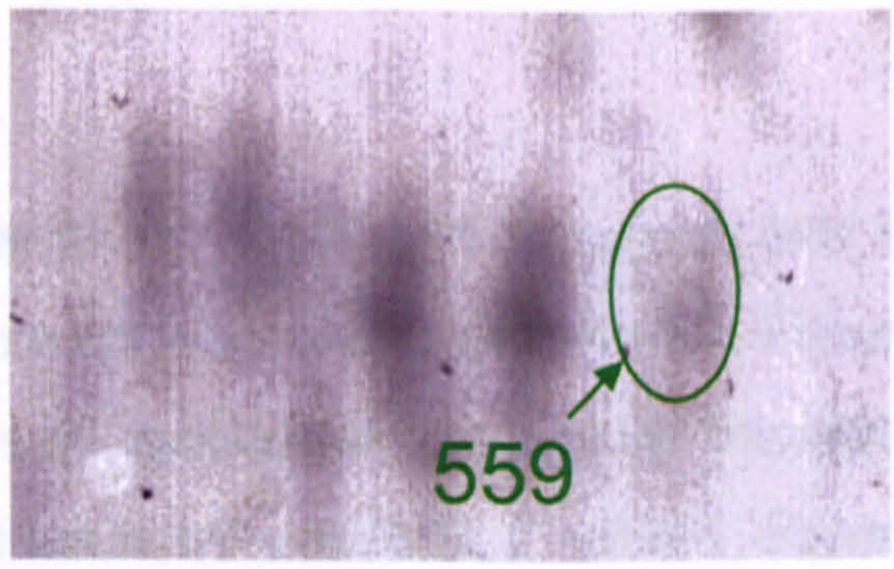
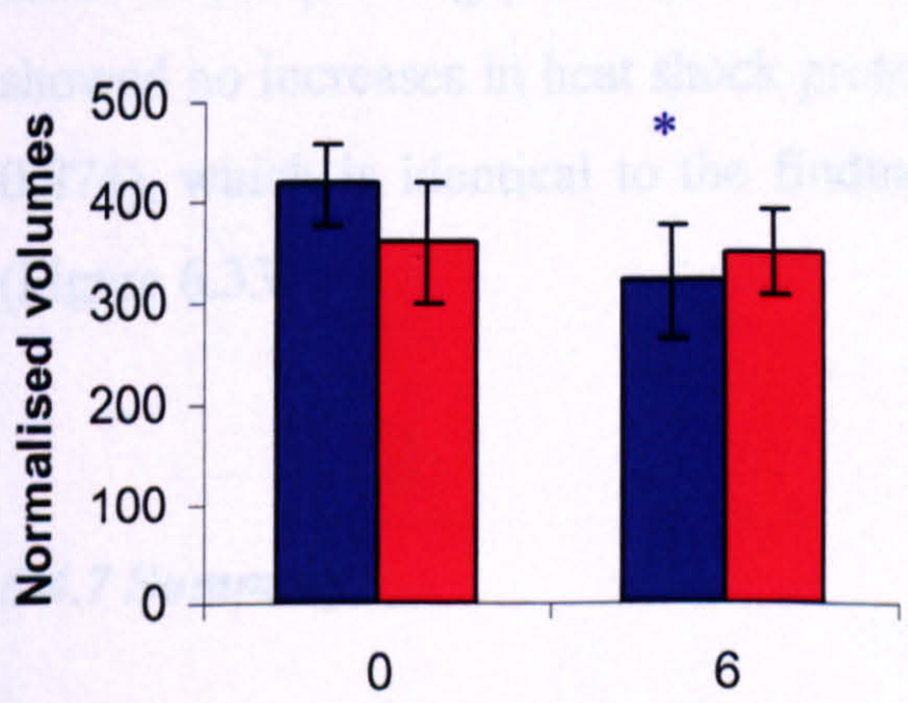


Figure 6.28: Training-induced changes in heat shock protein 27 (spot 559) after interval training.

Data are shown as means ± SE for men (■) and women (■) at 0 and 6 weeks of training.

*P < 0.05 statistically significant from pre-exercise training (0) values.



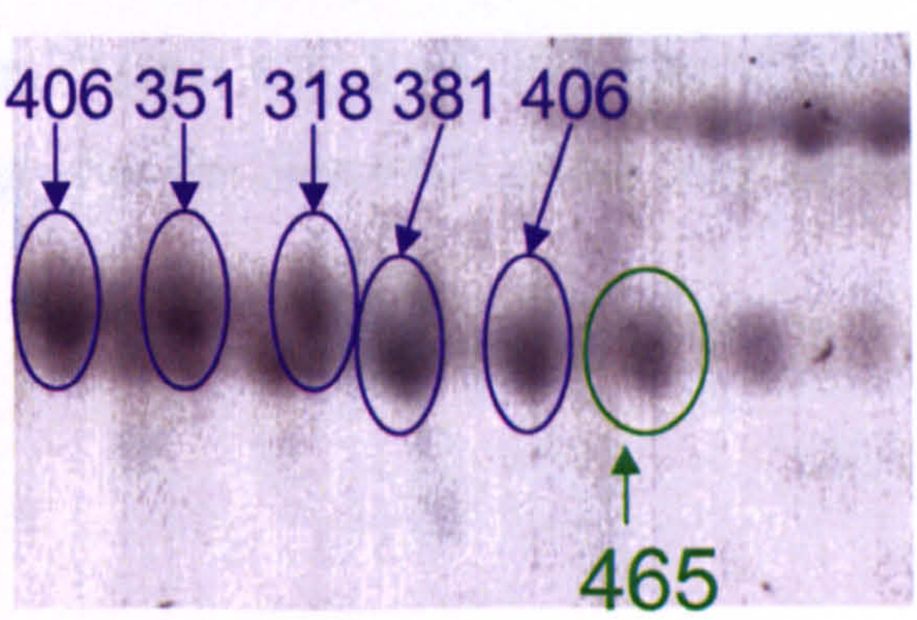


Figure 6.29: Training-induced changes in albumin (spot 465) after interval training.

Data are shown as means ± SE for men (■) and women (■) at 0 and 6 weeks of training.

*P < 0.05 statistically significant from pre-exercise training (0) values.

6.4.6 The effects of continuous training on muscle protein expressions in men and women.

Protein expression profiling identified statistically significant changes in only 4 gel spots (3 gene products) in response to continuous training (Table 6.2; Figure 6.30). There were increases of 22 % in the cytoskletal intermediate filament, desmin (spot 539, P = 0.046) in men, but not in women (2 %, P = 0.601, Figure 6.31) after training. There were also decreases of 30 % noted in NADH ubiquinone oxidoreductase 75 kDa, (spot 588, P = 0.031) in men (Figure 6.32). This is a component of complex 1 of the electron transport chain. Again, similar changes in expression were not seen in the females (P = 0.205).

One of the 4 differentially expressed gene products (HSP 70 protein 8) was present as multiple proteins. There were decreases (33 %) in heat shock protein 70kDa protein 8 (spot 418, P = 0.045; spot 459, P = 0.03) in men (Figure 6.33). These two spots were similar in molecular weights (M_r ~ 71082) but possessed different p*I* spanning pH 5.5 to 6 in males after training. Consistently, females showed no increases in heat shock protein 70 (spot 418, P = 0.167; spot 459, P = 0.874), which is identical to the findings after the interval training programme (Figure 6.33).

6.4.7 Summary.

In the vastus lateralis muscles of the men, there were changes in the expression levels from slow troponin T to the fast troponin T isoform after interval exercise training. Congruently, there was also a decrease in the slow myosin light chain isoform after interval training. These changes in expression levels suggest a partial conversion from a slower phenotype towards a muscle with faster contractile properties. The increase in creatine kinase after interval training in men suggests an enhancement of anaerobic metabolism (PCr system). Similarly, the decrease in NADH oxidoreductase suggests less aerobic metabolism after continuous training, but in men only. Heat shock protein responses to exercise training appear to be both training-specific and sex-specific.

Table 6.2: Mascot search results (database entry and protein ID) and the normalised volume before (baseline) and after (6 weeks), fold difference and the significance level, after continuous training.

Spot number	Description	Swissprot ID	Baseline (Normalised Volume ± SE)	6 weeks (Normalised Volume ± SE)	Sex	Fold difference	P value
418	Heat shock cognate 71 kDa protein	HSP7C_HUMAN	564 ± 37	432 ± 36	Male	-1.307	0.045
459	Heat shock cognate 71 kDa protein	HSP7C_HUMAN	197 ± 38	112 ± 19	Male	-1.755	0.03
539	Desmin	DESM_HUMAN	348 ± 50	445 ± 51	Male	1.016	0.046
588	NADH-ubiquinone oxidoreductase 75 kDa subunit	NDUS1_HUMAN	44 ± 1	31 ± 4	Male	-1.422	0.031

Values are means ± SE.

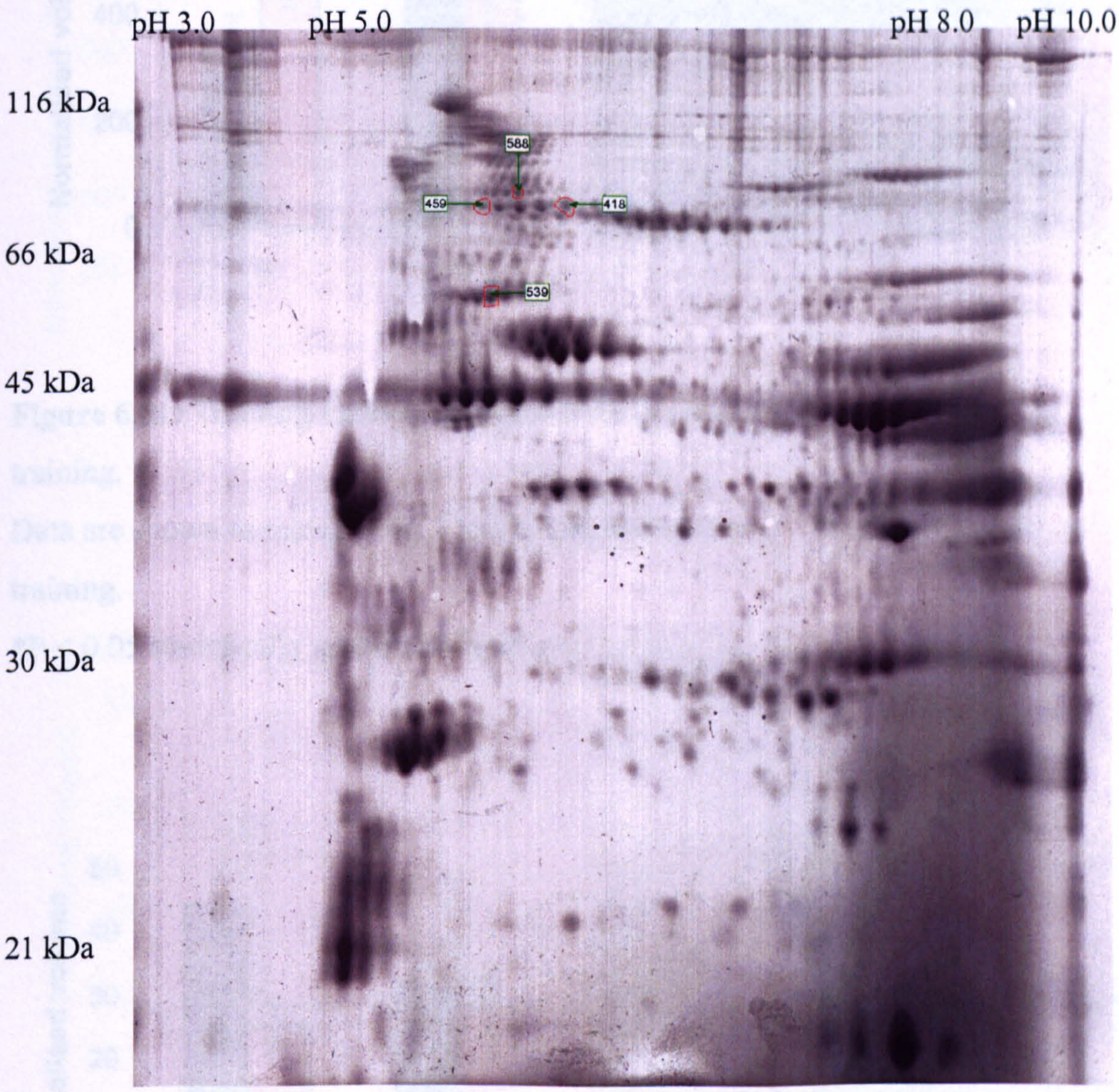
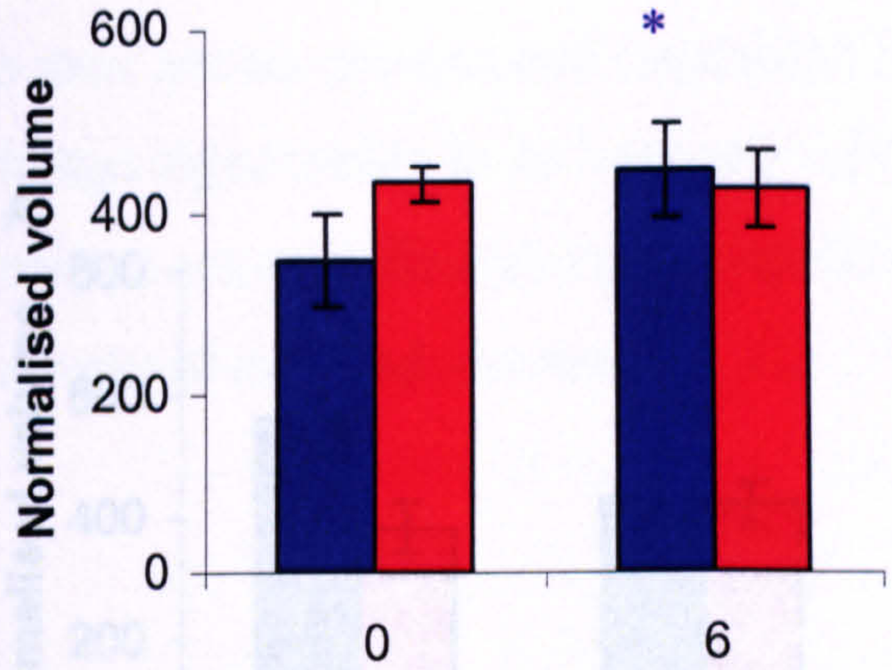


Figure 6.30: Identification of changed protein expressions after continuous training.

The numbers identified on this map correspond to the numbers reported in Table 6.2.

Proteins are separated according to isoelectric point (PI; pH 3-10NL) from left to right, and resolved molecular weight (200 kDa – 20 kDa) from top to bottom.



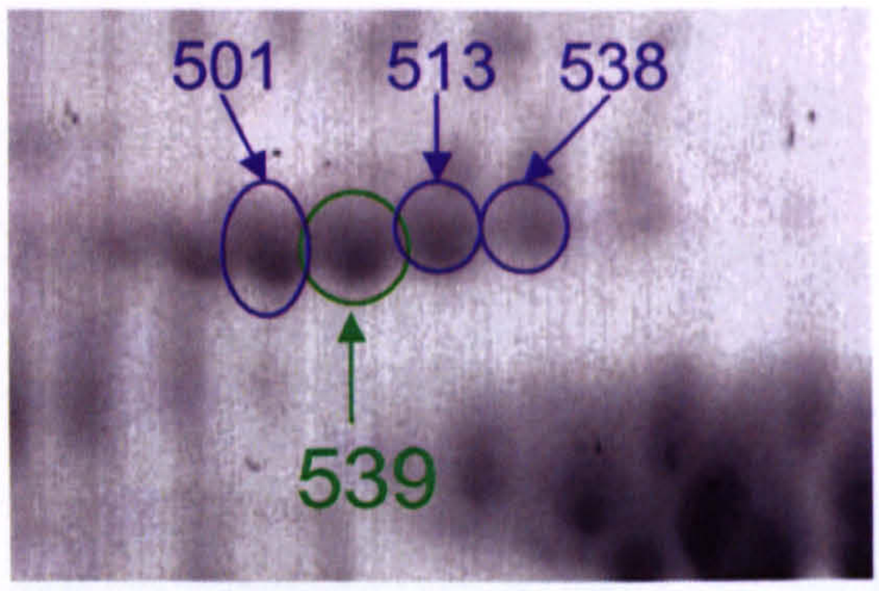
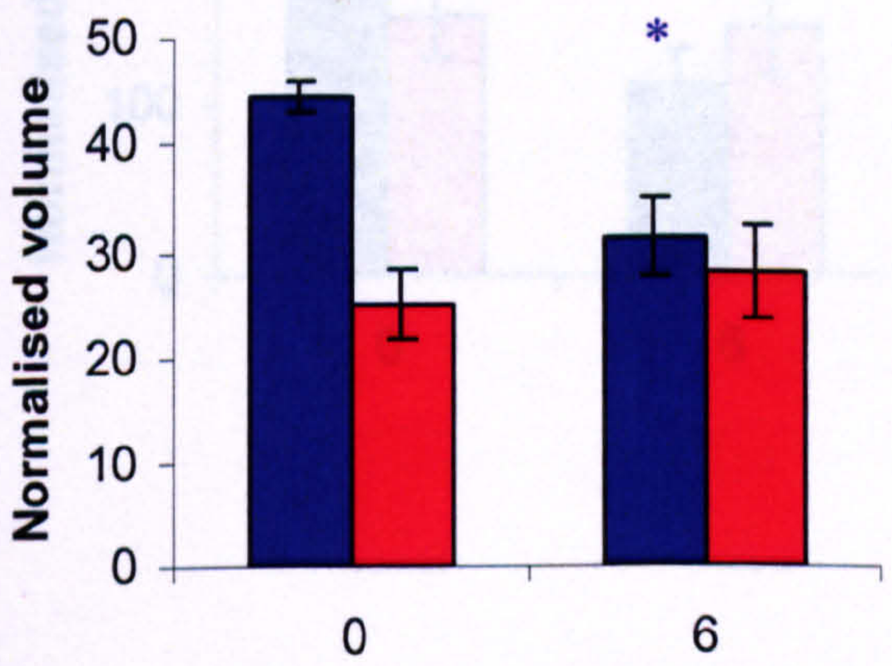


Figure 6.31: Training-induced changes in desmin (spot 539) after continuous training.

Data are shown as means ± SE for men (■) and women (■) at 0 and 6 weeks of training.

*P < 0.05 statistically significant from pre-exercise training (0) values.



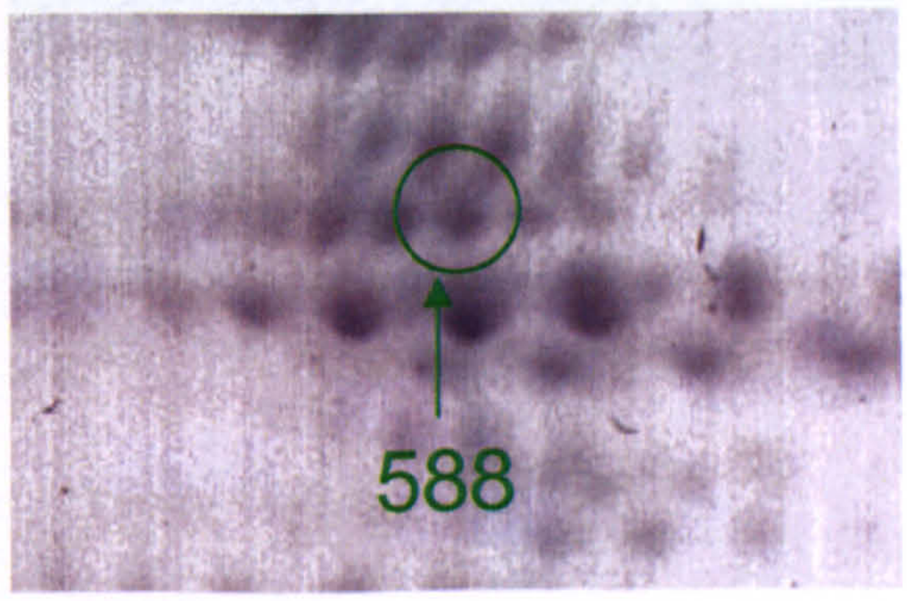


Figure 6.32: Training-induced changes in NADH ubiquinone oxidoreductase 75 kDa (spot 588) after continuous training.

Data are shown as means ± SE for men (■) and women (■) at 0 and 6 weeks of training.

*P < 0.05 statistically significant from pre-exercise training (0) values.

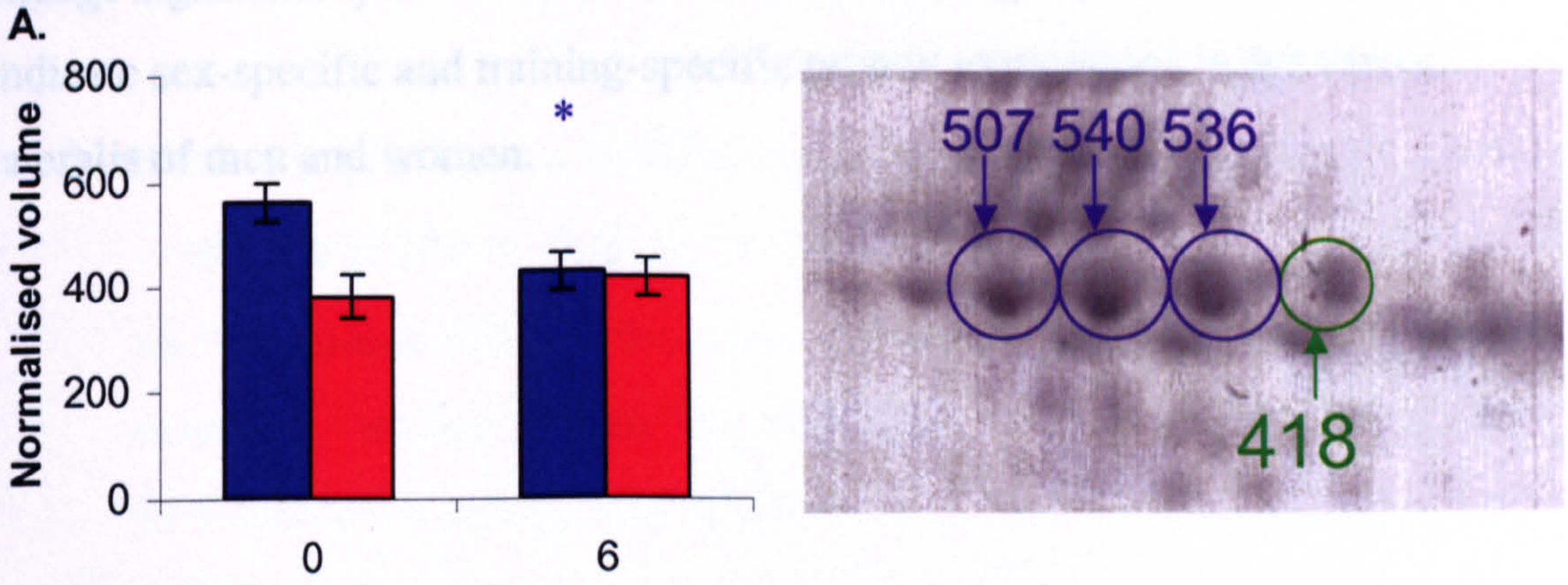


Figure 6.33: Training-induced changes in heat shock protein 70 isoform 8, in spot 418 (A) and spot 459 (B) after continuous training.

Data are shown as means ± SE for men (■) and women (■) at 0 and 6 weeks of training.

*$P < 0.05$ statistically significant from pre-exercise training (0) values.

In marked contrast to the men, the female vastus lateralis appeared to be less responsive in adapting to both interval and continuous training. This may be due to their greater pre-exercise expression levels of the very proteins found to change significantly in the men after exercise training. These results therefore indicate sex-specific and training-specific protein expressions in the vastus lateralis of men and women.

6.5 Discussion.

6.5.1 Key findings.

1. Similar improvements in $\dot{V}O_{2\,max}$ were seen after interval (6 repetitions of 1 minutes at 100 % $\dot{V}O_{2\,max}$, interspersed by 4 minutes at 50 % $\dot{V}O_{2\,max}$) and continuous (70 % $\dot{V}O_{2\,max}$ for ~ 30 minutes) exercise training for both men and women. The improvements after interval training were attributed to an increased a-vO_2 difference, but this was not apparent after continuous training in both sexes.
2. Women had higher levels of slow troponin T isoform and slow myosin light chain slow isoform (MLC_{sb}) than men prior to the interval training, suggesting a greater type I muscle fibre ratio. Interval training elicited a conversion in troponin T from slow to fast isoforms and a decreased MLC_{sb} in men, but not in women.
3. In contrast to interval training, continuous exercise training elicited no apparent changes in muscle function, nor in oxidative phosphorylation, with decreased expression of subunit-1 of the electron transport chain.
4. The response of heat shock protein expressions after interval exercise training were sex-specific, with increases in HSP27 in the women, but increases in HSP70 and HSP20 in men. Continuous training showed an opposite response in men through a decrease in HSC70. Heat shock protein expression is therefore training dependant, due to different variants of HSP70 being expressed in men after different modes of training.

6.5.2 Pre-training differences in protein expressions between men and women.

Due to the uniqueness of this study design in regards to directly analysing the vastus lateralis biopsies of men and women, observations in protein expressions

prior to exercise training are also important and could influence the outcome of protein expressions as induced by exercise training.

Females had higher levels of creatine kinase-M by 44 % than men before training commenced. High levels of creatine kinase-M have been negatively associated with aerobic metabolism and therefore have been linked to predominantly glycolytic muscle cells (Eschegaray & Rivera, 2001). Creatine kinase plays numerous roles within muscle cells. The PCr/CK system is seen as a crucial temporal and spatial energy buffer (Bessman & Geiger, 1981, Meyer, 1988). This aids in the prevention of a depletion of ATP and an intracellular ADP surplus, thereby avoiding cellular ATPase inhibition. Since creatine kinase regenerates ATP through ADP and H^+, it can aid in proton buffering and exert an indirect effect on glycogenolysis and thus glycolysis (Dalvuluri *et al.*, 1981). Therefore, the females could have higher levels of CK-M due to having a greater anaerobic metabolism, with less need to adapt to 'faster' protein isoforms during the interval training. However, Simoneau & Bouchard (1989) studied 270 healthy individuals and showed that female muscle had a greater mean proportion of type I fibres and lower glycolytic enzyme concentrations, in the vastus lateralis muscle, compared to males. Therefore, there could be other factors influencing the higher expression in CK-M in females.

In line with Simoneau & Bouchard's (1989) findings, before our training of the females, the vastus lateralis biopsy also showed greater expressions of two slow contractile proteins, i.e. including a 38 % greater expression of troponin T (slow isoform; TnT1; Figure 6.15C) and a 52 % increased expression in myosin light chain (slow component; MLC_{sb}; Figure 6.15D).

Muscle fibres generate tension through cross-bridge cycling, involving both actin and myosin proteins. Human slow skeletal Troponin T (TnT1) and MLC_{sb} are principally specific to type I muscle fibres (Brotto *et al.*, 2006; Schiaffino & Reggiani, 1996). This suggests that higher levels of TnT1 and MLC_{sb} are due to a higher type I muscle fibre content in women, compared to men. As previously stated, the majority of the literature states the females have a higher proportion of type I fibres in the vastus lateralis muscle, compared to males (Carter *et al.*,

2001; Simoneau & Bouchard, 1989; Simoneau *et al.*, 1985). However, prior to exercise training, the women also showed greater expression of troponin T fast isoforms (TnT3) compared to the men. The greater expressions in troponin T slow and fast isoforms in women prior to training, probably suggests the dominance of type IIa fibres within the vastus lateralis muscle. Both slow and fast muscles contain troponin, which have evolved into fast and slow fibre type-specific isoforms (Barany, 1967).

To ensure these proteins were exercise training-induced, rather than being previously training-induced, we need to consider the characteristics of the participants involved in our study. All the females who participated in the study were recreationally active. Activity questionnaires prior to training stated that none of the girls participated in team sports, but exercised in the local gym about once a week. These gym sessions mainly consisted of aerobic or resistance exercise and did not contain any sprint or high-intensity training. The men were recreationally active through playing in a local 5-a-side team once a week. Therefore, the men and women were both performing aerobic exercise once a week, through which you might expect similar aerobic improvements. Also, muscle biopsies were taken from a standardised site within the vastus lateralis, thus reducing variability between different parts of the vastus lateralis muscle. Consequently, the females could have possessed a higher type IIa fibres ratio in the vastus lateralis compared to males, but this cannot be fully ascertained as we did not measure muscle fibre type distribution.

Although the female muscle biopsies showed greater expression in contractile proteins prior to training, the men showed a greater initial expression in heat shock protein 60 (59 %) and 70 (65 %; Figures 6.16A and 6.16B). Both HSP70 and HSP60 are involved in chaperone roles. When new proteins emerge from the ribosomes, HSP70 interacts with them at the N-terminus through the substrate-binding domain, by recognising the hydrophobic amino acid residues. HSP70 then acts as a chaperone by directing new, unfolded proteins to members of the HSP60 family. These in turn aid in the folding of the nascent proteins, and their translocation into cellular compartments. HSP60 is mainly localised in the mitochondria and is expressed in proportion to mitochondrial content (Ornatsky

et al., 1995), suggesting that higher levels are expressed in type I fibres. HSP70 has also been associated with energy pathways by interacting with regulatory proteins involved in glucose metabolism (Liu *et al.*, 2006) and has been found to be more dominant in type I fibres (Locke *et al.*, 1991).

In the vastus lateralis muscle, females had a greater expression of troponin T slow isoforms and MLC_{sb} and males expressed higher levels of HSPs, which are both connected to aerobic pathways. Females also showed a greater expression of fast troponin T isoform, suggesting a greater type IIa fibre distribution and therefore a greater anaerobic capacity in the men. However at a whole body level, baseline $\dot{V}O_{2\,max}$ levels, when scaled to lean body mass, showed no differences in aerobic capacities between men and women. These differences in protein expressions between men and women before training could then have greater consequences on muscle metabolic adaptations after exercise training. As already stated these changes in a limited number of individual proteins would need to be backed up by fibre type profiling to confirm the relative proportions of fibre types between men and women.

6.5.3 The effects on aerobic capacity after interval and continuous training.

It has long been established that increases in $\dot{V}O_{2\,max}$ do not differ between continuous endurance and interval training, and that similar increases in $\dot{V}O_{2\,max}$ can be found regardless of pre-training fitness levels (Bhambhani & Singh, 1985; Branch *et al.*, 2000) or sex differences (Eddy *et al.*, 1977). Our results showed similar increases in $\dot{V}O_{2\,max}$ in both sexes after both training modes, which were carefully matched to ensure the same workloads were undertaken.

Interval training has been shown to improve $\dot{V}O_{2\,max}$ through an increase in maximal cardiac output, whereas continuous training has been ascribed to an increase in maximal a-vO_2 difference (Helgerud *et al.*, 2007; Martin *et al.*, 1987). In contrast, our results show increased a-vO_2 differences in men and women only

after interval training. It has now been more widely accepted that women adapt to exercise via peripheral adaptations. Cunningham *et al.* (1979) showed that 12 weeks of interval and continuous training improved $\dot{V}O_{2\,max}$ through increases in a-vO_2 differences in women. Short-term interval training has also shown increases of 15 % after 7 weeks (Eddy *et al.*, 1977) and 34 % after 8 weeks (Daussin *et al.*, 2007). These improvements in $\dot{V}O_{2\,max}$ were attributed to both central and peripheral indices.

At the fixed intensity of 70 % $\dot{V}O_{2\,max}$, continuous training in both sexes did not improve a-vO_2 differences and only small non-significant changes in cardiac output were seen (Figure 6.11). However, Daussin *et al.* (2007) reported that the improvement in $\dot{V}O_{2\,max}$ after continuous exercise training (exercising at individual lactate thresholds) induced only indices of peripheral function. Spina *et al.* (1992) found that 12 weeks of continuous endurance exercise improved cardiac output by 12 % and stroke volume by 16% in young men and women using similar work intensities. This therefore suggests that a longer training period may be required to induce central changes at the intensity of 70 % $\dot{V}O_{2\,max}$. Therefore, these results indicate that oxygen extraction is enhanced after interval training, to a greater extent than continuous training. This could suggest greater muscle metabolic changes after interval training in both men and women. However, caution must be elicited as these changes in $\dot{V}O_{2\,max}$ refer to the whole body and not to just the vastus lateralis muscle.

6.5.4 The effects of training on contractile proteins in men and women.

With the increased oxygen extractions in men and women after interval training, and the similar increases in aerobic capacities, it was expected that both men and women would show similar changes in muscle metabolic profiles after training. However, proteomic data suggests sex-specific differences for both interval and continuous training in some contractile proteins.

Myosin heavy chain isoforms have been previously used extensively for the classification of muscle fibre types. In our gel, myosin heavy chains (slow) move very slowly and were identified at the extreme top. There was no significant (P = 0.682) change in this protein, but 3 men showed decreases in its expression, contributing to a total average decrease of 30 %. This was a large and totally unexpected finding. However, meaningful conclusions cannot be drawn regarding MHC expression within our results due to technique limitations. Using standard isolation techniques during the sample preparation (i.e. frozen sample ground with pestle and mortar), myosin heavy chains form a rigor complex post mortem, which cannot be readily disassociated in the solvent required for isoelectric focusing. A small proportion of the myosin heavy chains were visible on 2-D gels, but the abundance of these spots were much lower than expected. In addition, the spot sometimes identified as myosin heavy chains in 2-D gels are approximately one-half the M_r (~ 120 kDa, pH 5) expected for the intact protein and therefore cannot be used to determine the relative expression of the different muscle isoforms.

In contrast to the women, the vastus lateralis of the male interval training group showed changes in contractile proteins, which included an increased expression of fast troponin T (TnT3) and a decreased expression in slow troponin T (TnT1) isoforms. Brotto *et al.* (2006) stated that the expressions of TnT isoforms (fast and slow) are highly specific to muscle fibre types and correlate to the contractile features of the fibre type, and can be used as fibre type markers. Therefore, our results may well suggest that the decreased expression of slow TnT isoforms and the increased expression in fast TnT isoforms, indicates an increased conversion of fast-twitch muscle fibres from slow after interval training in men (Figures 6.21 and 6.22). Brotto *et al.* (2006) also found that fibres containing slow troponin isoforms had a higher calcium sensitivity compared to fibres containing fast troponin isoforms, which in turn, showed a higher co-operativity of calcium activation. This suggests that interval training in men increases co-operativity of calcium activation at the cross bridges. Using myofibrillar staining techniques, interval sprint training has been shown to decrease slow twitch fibres by 48-57 % (Jansson *et al.*, 1990). Therefore, the vastus lateralis muscle of the men undergoing interval training may be undergoing conversion to faster fibres.

To support this assumption, the vastus lateralis muscle of interval trained men also decreased their expression of myosin light polypeptide 3 ($MLC1_{sb}$/ $ELC1_{sb}$; Figure 6.24). There are four myosin light chains, a pair of regulatory light chains (MLC2) and a pair of alkali (essential) chains (MLC1, MLC3) which are bound to the two myosin heads. There are five alkali light chains (ELCs) found in human skeletal muscle. ELC_{emb} is mainly embryonic, $ELC1_{sb}$ and ELC 1_{sa} are major and minor slow isoforms respectively, and $ELC1_f$ and $ELC3_f$ are the two fast isoforms. Fast type II muscle fibres only contain fast isoforms, whereas type I fibres can contain both fast and slow isoforms (Jostarndt-Fogen *et al.*, 1998). Myosin light chain polypeptide isoform 3 ($ELC1_{sb}$) is present in both ventricular and slow skeletal muscle. Therefore, the decrease in $ELC1_{sb}$ is consistent with the shift from slow troponin T it's to fast isoforms. Together these strengthen the suggestion of a conversion in muscle fibres to a faster contractile state.

In contrast to the men after interval training, the female muscles decreased their expression in the fast troponin T isoform (spot 553; Figure 6.23). Interestingly, this spot did not correlate in terms of molecular weight or pH with the spots (6, 13) for troponin T expressions in the men. Furthermore, it had a lower molecular weight (~ 25 kDa compared to ~ 34 kDa) and a greater acidity (pH 5 compared to pH 7) than the male protein spots. It is therefore possible that the troponin T spot identified in the females was only a fragment from one of the more dominant troponin T spots. However, it is unclear why this fragmentation would occur only in females and not in males. In addition, the mowse score (probability based scoring system with a significance level set at 29 or higher at the 95 % confidence level) was low (33) for the identification of troponin T fast in the females. MS/MS analysis was performed on the same spot, from 3 different gels, and all were found to have low mowse scores in identifying the spot as troponin T fast. However, there were no changes in slow troponin T isoform expression in the females, it is therefore uncertain that a conversion to a faster muscle contractile state was occurring.

In contrast to the slow-fast isoform conversion after interval training, the male interval training group's muscle biopsies also showed an increased expression in

myosin regulatory light chain 2 (MLC2; Figure 6.25). The majority of MLC2 expression occurs in the heart, but it can also be expressed within skeletal muscle. MLC2 is located at the head-rod junction of myosin molecule and shows a high level of primary sequence homology with proteins like calmodulin, TnC and pavalbumin. The functional significance of RLCs has not yet been determined, but studies have shown that RLCs may affect the development of force in skeletal muscle (Hofmann *et al.*, 1990; Metzger & Moss, 1992). Diffee *et al.* (1995) found that muscle fibres containing a mutant RLC (defective cation binding site) possessed reduced maximum muscle tension and stiffness, suggesting RLCs have a regulatory role, e.g. adjusting the accessibility of cross-bridges to bind to actin.

Cytoskeletal intermediate filaments also play a role in muscle contraction regulation. Desmin is a cytoskeletal protein, encoded by the DES gene, and is part of the type III family of cytoskeletal intermediate filaments (similar to actin and tublins). The function of desmin has been deduced from studies using knock-out mice, but its underlying actions have not been fully elucidated. Desmin has been associated in aiding force transmission of the thin filaments at myotendinous junctions (Tidball, 1992), maintaining sarcometric organisation and integrity (Haubold *et al.*, 2003), and regulating the signaling between the contractile structures (Shah et al., 2004). Mitochondrial function, number, distribution and morphology can change when desmin is not present, therefore it has been suggested that desmin provides the mitochondria with information regarding muscle contraction and the energy needs of the cell, to aid in the regulation of aerobic respiration in the muscle cell (Goldfarb *et al.*, 2004). Continuous training with men did not enhance myofibrillar protein expressions, including slow and fast TnT and MLCs, there was an increased expression in desmin. Which of desmin's many potential functions might have been affected by exercise training cannot be determined from our observations.

Therefore in men, interval training elicits an alteration of slow to fast troponin T isoforms, and a decrease in MLC_{sb}, suggesting a conversion to a faster muscle contractile state. This is indicative of an increase in faster muscle isoforms, which is consistent with the high-intensity aspect of the interval training. With

continuous exercise training not including this high-intensity exercise phase, it is less surprising that no changes in myofibrillar proteins were found. Although the increase in desmin expression was found after continuous training in men, but it may not necessarily aid in increased force transmission at the myotendinous junction, as the role of desmin has not been fully elucidated. Conversely, these changes in protein expressions in the men do not reflect the effects of exercise training in women. The women showed higher baseline levels of some slow and fast myofibrillar protein isoforms prior to training. This may potentially explain the lack of adaptations in their contractile proteins, despite the same high-intensity element of interval training.

6.5.5 The effects of exercise training on aerobic and anaerobic pathways in men and women.

Using myofibrillar staining techniques, interval sprint training has been shown to decrease the number of slow twitch fibres in the vastus lateralis by 48-57 % (Jansson *et al.*, 1990). This suggests that interval training causes a shift to a faster contracting muscle phenotype. Conflicting with this, muscle oxidative capacity has been known to increase after 6-8 weeks of sprint training in men (Jacobs *et al.*, 1987; Krustrup *et al.*, 2004). Recent studies have shown an increase in citrate synthase (commonly used as a marker of oxidative metabolism) after only 2 weeks of sprint training (Parra *et al.*, 2000; Rodas *et al.*, 2000; Burgomaster *et al.*, 2005). Burgomaster *et al.* (2005) also found that aerobic endurance capacity was increased with only 6 sessions of sprint interval training, with an increase of 50 % in exercise time to exhaustion. This increase in aerobic capacity was attributed to an increase in mitochondrial potential. These observations therefore suggest that interval training enhances performance by utilising increased aerobic metabolism.

In contrast, Ogura *et al.* (2006) recently showed that 8 weeks of sprint interval training increased the activity of both anaerobic and aerobic enzymes. Other high intensity sprint training programmes have increased the enzymatic activities relating to glycolysis (PFK, LDH, GP), and concentrations of PCr and glycogen

(Cadefau *et al.*, 1990; Linossier *et al.*, 1993, 1997; Dawson *et al.*, 1998; MacDougall *et al.*, 1998), as well as mitochondrial enzymes (Saltin *et al.*, 1976; Burgomaster *et al.*, 2005). Short periods of high intensity exercise have also increased PCr kinase activity (Thorstensson *et al.*, 1975; Costill *et al.*, 1979). Even longer high-intensity periods have induced a greater glycolytic metabolic response (Cadefau *et al.*, 1990). In support of the above studies, our results showed an increase in creatine kinase-M type after interval training in men (Figure 6.19). Therefore, although endurance training has been shown to induce aerobic changes (Chesley *et al.*, 1996; Green *et al.*, 1992, 1995; Hoppeler *et al.*, 1985), the active and passive phases involved in interval training, appear to enhance both aerobic and anaerobic metabolism.

Creatine kinase is part of the ATP: guanido phosphotransferase family, situated in the cell cytoplasm, and has been determined as the mediator of all energy changes within the cell. The majority of creatine kinase-M type (CK-M) is cytosolic (Nigro *et al.*, 1987) with around 10 % bound to the M-line of the sarcomeres (Turner *et al.*, 1973) and an additional percent of CK-M associated with the I-band of the sarcomere (Wegmann *et al.*, 1991). Rossie *et al.* (1990) also found CK-M bound to the SR membrane in chicken pectoralis muscles. They concluded that CK-M contributes to 24-40 % of the maximal rate of calcium uptake vitro, and may aid in the local regeneration of ATP, near the calcium pump, to aid muscle contraction when the muscle is subjected to extremely heavy work loads. Creatine kinase-M activity has also been found to be two-fold higher in fast twitch muscles (type IIa and IIx), compared to slow twitch muscles (type I; Yamashita & Yoshioka, 1991), suggesting a link to anaerobic metabolism.

In our study in men after interval training, we found increases in CK-M expression in the form of 3 individual protein spots. One of these spots was found to be phosphorylated. The autophosphorylation of creatine kinase is dependant on bivalent cations (i.e. Mg^{2+} or Mn^{2+}) and enzyme conformation. However, autophosphorylation is not dependent on substrate turnover and therefore is not present in the enzymatic reaction of CK (Hemmer *et al.*, 1995) and is not the result of the CK transphosphorylation reaction (Stolz *et al.*, 2002).

The CK autophosphorylation has also been suggested to modulate the reversibility of the CK reaction, although the function of this is unclear (Stolz *et al.*, 2002). Autophosphorylation has been found at several theonine sites of CK-M. The results in our study suggest that the phosphorylation of CK-M occurred at serine 24 or at tyrosine 15 and 21, suggesting that autophosphorylation of CK-M did not occur. Ponticos *et al.* (1998) found that CK-M abundance is regulated by phosphorylation in skeletal muscle. Therefore, the phosphorylation of CK-M (spot 12) can be attributed to an increase in AMPK (protein kinase involved in the response to ATP depletion) which phosphorylated and thereby inactivated CK-M.

Studies using mice have shown that when the CK-M gene is 'knocked-out', then the capacity to synthesise ATP increases, as well as improving endurance performance during low intensity exercise (Van Deursen *et al.*, 1993). Rivera *et al.* (1997) examined the association between CK-M and changes in $\dot{V}O_{2\,max}$ after endurance training. The results showed that CK-M limited $\dot{V}O_{2\,max}$ in response to endurance exercise. Using a rat model, Yamashita & Yoshioka (1992) found that 16 weeks of endurance exercise training produced no differences in CK-M activity between type I, IIa or IIb muscle fibres. However, mice with CK-M deficiency showed an increased mitochondrial size and enzyme concentrations, increased concentration of glycogen, and a 2-fold increase in aerobic capacity in predominantly fast glycolytic muscle fibres. Therefore, the CK-M isoform has been negatively associated with aerobic metabolism.

Conversely, these previously reported studies state that there is no sex-specific response to increased CK-M expression with regards to interval training. However, sex-specific differences were apparent in our results. Amelink & Bar (1986) found that ovariectomised female rats, which were oestrogen deficient, had a higher serum creatine kinase efflux after exercise, compared to female rats with normal estrogen levels, therefore suggesting that CK efflux after exercise is attenuated by higher circulating levels of oestrogen. However, the comparisons between serum and muscle CK must be viewed with caution, as CK-M converts into three different isoforms (MM1, MM2 and MM3), with 3 different isoelectric

points (Wevers *et al.*, 1977), after incubation in plasma. The women involved in our study were all on the combined contraceptive pill, which regulated oestrogen levels throughout non-menstruating weeks. This increased expression in CK-M in our study was not apparent in the women after either interval or continuous training. Therefore, the lack of changes in the expression of CK-M in women is unknown and requires further study.

It has long been known that changes in skeletal muscle substrates are a prominent metabolic adaptation to endurance training (Holloszy & Coyle, 1984). Even short endurance training programmes have increased glycogen availability and decrease glycogen catabolism, resulting in an increased endurance capacity (Chesley *et al.*, 1996; Green *et al.*, 1992, 1995). These changes in substrate metabolism have been attributed to an increase in mitochondria, due to a proliferation of tricarboxylic cycle and electron transport proteins (Saltin & Gollnick, 1983; Holloszy & Coyle, 1984). Conversely, after continuous training in our men, there was a decrease in sub-unit 1 of NADH ubiquinone oxidoreductase expression. NADH ubiquinone oxidoreductase is a multi-subunit complex and is situated as the first complex in the electron transport chain of the mitochondria. This complex transfers electrons from NADH to a ubiquinone carrier (NADH dehydrogenase activity), and is passed along the electron transport chain. It also pumps hydrogen ions into the inter-membrane space of the mitochondria from the mitochondrial matrix, causing an electrochemical proton gradient to aid in the synthesis of ATP (Smeitink *et al.*, 2004).

Larsson *et al.* (1964) reported that intense cycling exercise increased blood lactate concentrations, which were related to low muscle cytoplasmic NADH in maximal exercise. This was due to larger increases in mitochondrial NADH in the TCA cycle, which resulted in a greater concentration gradient, causing cytoplasmic NADH to be transported via the malate-aspartate shuttle, thus increasing NADH ubiquinone oxidoreductase activity (Katz & Sahlin, 1988). However, others have reported changes only after continuous training (Fournier *et al.*, 1982; Gorostiaga *et al.*, 1991). These discrepancies between studies are due to different exercise intensities and different training volumes between interval and continuous training groups. The workloads between the interval and

continuously trained groups within our study were equal. Therefore, with participants working at the same exercise workloads in both training modalities, 6 weeks of continuous training in the men does not seem to increase NADH levels and thus NADH ubiquinone oxidoreductase activity (Figure 6.32). Therefore, a longer training period or a higher intensity may be required to elicit changes in muscle aerobic capacities after continuous training in both men and women.

Overall, the conversion of some myofibrillar proteins from slow to fast isoforms in the men after interval training, are consistent with the increased expression in CK-M. The latter suggesting an up-regulation of anaerobic pathways after interval training in men. The lack of change in either myofibrillar proteins or aerobic pathways after continuous training, suggests that the training period may need to be longer than 6 weeks or more intense to elicit changes. Like TnT and MLC expression levels, the females had higher levels of CK-M prior to exercise training, and this therefore could explain the lack of adaptation of the vastus lateralis to exercise training.

6.5.6 The effects of training on heat shock proteins in men and women.

Heat shock proteins in the muscle have been reported to increase after high-intensity interval training, or in response to metabolic, mechanical and oxidative stress (Liu & Steinacker, 2001; Locke, 1997). The enhancement of muscle contractility after interval training has been correlated to an increase in heat shock protein 70 (HSP70; Locke, 1997).

The heat shock protein 70d kDa family are the most abundant heat shock proteins when induced by stress. Heat shock protein 70 has two essential functions, molecular chaperoning and stress sensing. When HSP70 is not acting as a molecular chaperone, it is bound to ATP (Chappell *et al.*, 1987; Milarski & Morimoto, 1989). HSP70 is also involved in cell homeostasis, proliferation, differentiation, cell death and the protection against thermal and oxidative stress (Matranga *et al.*, 2002).

Our results showed multiple protein spots identified as HSP70, in both men and women prior to exercise training. The more alkaline spots (2 spots) were identified as HSP70 protein 1 (common form), with the more acidic spots (8 spots) being identified as HSP70 protein 8 (HSC 71). The heat shock protein 70-kD family contains constitutively expressed members called heat-shock cognate proteins (for example in this case HSC 71). Dworniczak & Mirault (1987) found that HSC 71 mRNA were present in unstressed cells, but were minimally induced by heat. HSC71 is especially involved in chaperone functions to prevent aggregation of proteins, aid in the re-folding of proteins, and allow translocation of protein chains (Bork *et al.*, 1992). It has therefore been suggested that HSP71-related proteins regulate oligomeric assembly-disassembly processes in proteins, through ATP hydrolysis and the release of ADP and Pi (Flaherty *et al.*, 1991). Our results showed that 6 weeks of continuous training decreased HSP70 (HSP cognate 1; HSC-71) in men (Figure 6.33). After 6 weeks of interval training, an increase was also observed in men in HSP70 protein 1 (Figure 6.26). In the women, neither the interval nor continuous training changed HSP70 or HSC71 expressions.

An increase in heat shock protein 70 has been identified after exercise training. Liu *et al.* (1999) trained 10 male rowers for four weeks at different durations, intensities and forms of rowing exercise, resulting in a 5-fold increase. An increase in HSP70 has been reported following 150 eccentric contractions, accompanied by a decline in expressions of myosin heavy chain and actin. This therefore suggests that HSP70 expression may be linked to myofibrillar proteolysis. Thompson *et al.* (2001) showed that HSP70 and HSC71 increased with eccentric exercise, but was primarily increases in HSP70, rather than HSC71. These observations concur with our results, where the high intensity nature of interval training, causing a greater increase in HSP70 than HSC71. Our results support the conclusion that the expression in HSC71 is not reliant on an increased expression in HSP70 as both heat shock forms were present separately in both exercise modes in the men.

Previous research has shown that muscles containing low levels of HSP70 have the greatest increases in expression after exercise training (Gonzalez *et al.*, 2000; Kelly *et al.*, 1996). This was observed by Milne & Noble (2002) who found a greater expression of HSP70 in the soleus, compared to fast muscle components from the vastus lateralis. This expression pattern suggests that higher levels of HSP70 are found in muscles with higher oxidative capacities. Therefore, the increase in HSP70 after interval training in men may be related to enhanced oxidative capacity. Liu *et al.* (2004) showed that six well-trained male rowers undertaking a high-intensity exercise programme, showed an increase in HSP70. The same subjects then undertook a low intensity endurance programme which caused no change in HSP70 levels. This study proposed that the intensity of the exercise training is important in eliciting increases in HSP70 expressions, suggesting different biochemical and physiological changes lead to HSP70 induction. Acute one-legged cycling (Khassaf *et al.*, 2001; 2003), exhaustive knee extensor exercise (Febbraio *et al.*, 2002) muscle damaging contractions of the biceps (Thompson *et al.*, 2001, 2002, 2003) and moderately damaging running exercise protocols (Morton *et al.*, 2006), have all shown increases in HSP70 content in males.

Although HSP70 expression has been increased in men after interval training, few studies have measured expressions in females after exercise training. Chang *et al.* (1998) found that young women on a treadmill-based moderate exercise protocol (20 minutes at 60 % $\dot{V}O_{2\,max}$) did not elicit changes in HSP70 levels in the blood. In the heart, Voss *et al.* (2003) reported higher levels of HSP72 in male rats (rodent form of HSP70) compared to female rats after exercise. This study also reported that the HSP72 content in fast twitch muscles were not different between sexes, but males had a 65 % greater HSP72 content than females in slow twitch muscle. However, it is unlikely that HSP72 is directly associated with the oxidative capacity of skeletal muscle (Locke *et al.*, 1991; 1994). Paroo *et al.* (2002) also found a sex-specific response to HSP72 expression after exercise in rats. The male rats had a greater induction of HSP72 than the female rats in the vastus muscle after exercise. This response was attributed to oestrogen, since ovariectomised female rats exhibited higher HSP70

levels after exercise. Under normal conditions, oestrogen exerts its effects through intracellular located receptors, Tamoxifen (an oestrogen receptor antagonist) did not aid in protecting skeletal muscle from damage after electrical stimulation (Koot *et al.*, 1991) or contraction-induced damage (Paroo *et al.*, 2002). It has become increasingly apparent that oestrogen may act as an antioxidant (similar to vitamin E) and stabilise cellular membranes (Subbiah *et al.*, 1993). However, the concentrations of oestrogen used in these investigations (40 $\mu g.kg^{-1}.day^{-1}$) were much greater than normal circulating levels at picomolar to nanomolar concentrations. Therefore, it is likely that physiological levels of oestrogen are too low to affect the cellular redox state (Paroo *et al.*, 2002). Prior to this study, no research has been undertaken to simultaneously measure changes in HSP70 expression in men and women. This suggests that the studies described above involving rats, may be indicative of a sex-specific response to exercise training in men and women, thereby signifying sex-specific differences in HSP70 expressions after exercise training.

Interval training also showed an increased expression in the smaller heat shock proteins. In men, there was an increased expression of HSP20 (HSP beta 6; Figure 6.27) and in the women, an increased expression of HSP27, after interval training (Figure 6.28). HSP20 was first described by Kato *et al.* (1992) as the by-product of α β-crystallin and HSP27 and is involved in the formation of hetero-oligomeric complexes. More recently, HSP20 has been suggested to act as a molecular chaperone, as it has been found to prevent the aggregation of insulin (Bukach *et al.*, 2004). HSP20 is known to form both homo- and hetero-oligomers with other small HSPs, especially with HSP27. De Klundert *et al.* (1998) suggested that HSP20 was a poor chaperone, due to its tendency to form dimers. However, recent research has shown that neutral or more alkaline HSP20 have a higher chaperone activity, compared to α-crystallin as a decrease in pH may cause partial unfolding and dissociation of small oligomers formed by HSP20 (Bukach *et al.*, 2004). The increased expression of HSP20 in our study was found in the more alkaline spot out of the three spots identified as HSP20. This suggests an increased chaperone role after interval training in men. Wang & Spector (2000) found that α-crystallin prevented acidification-induced aggregation of creatine kinase. With HSP20 being a by-product of the

purification of α β-crystallin, it is suggestive that the increased expression of HSP20 could have been due to the increase creatine kinase levels in men after interval training. In regards to exercise training, few studies have measured changes in smaller heat shock proteins. In skeletal muscle, HSP20 is more dominant in type I fibres (Inaguma *et al.*, 1996; Kim *et al.*, 2004). However, the functional effects of HSP20 with exercise training have not been fully elucidated. Therefore, these findings suggest that HSP20 has a complex and multifaceted role, which requires further study in regards to exercise training, especially within the human population.

The most significant finding in the vastus lateralis of women after interval training was the increased expression of HSP27. HSP27 kDa, otherwise known as heat shock protein beta 1, is localised in the cytosol and translocates into the nucleus under stressed conditions. HSP27 is known to elicit a variety of functions including molecular chaperoning (Rogella *et al.*, 1999), thermotolerance in vivo (Hinkey *et al.*, 1986), aiding in protein refolding and degradation of denatured proteins (Sarto *et al.*, 2000), cell growth and stress response (Parcellier *et al.*, 2003). The most common function of HSP27 is the prevention of non-covalent filament/filament interaction formation of the intermediate filaments and the protection of actin fragmentation. Studies have shown that HSP27 suppresses the rate of actin polymerisation (Miron *et al.*, 1988, 1991) and is believed to function as an F-actin capping protein, regulated by phosphorylated mitogen-activated protein kinase-activated protein kinases 2/3 (MAPKAPK2/3, a major stress-activated kinase; Laudry *et al.*, 1992). This suggests that HSP27 plays a role in intermediate filament protection and the signal transduction pathways involving MAP kinase.

Hinkey *et al.* (1986) first isolated the HSP27 gene, which were similar to alpha-crystallins with about 20 % of the HSP27 residues being phosphorylated by mitogen-activated protein kinase-activated protein kinase 5 (MAPKAPK5). HSP27 is modified post-translationally through the phosphorylation of serine (serine-78 and serine-82), and therefore HSP27 can resolve into several isoelectric points ranging from pH 7 to 5, with the most phosphorylated isoform being the most acidic (Arrigo *et al.*, 1987, 1988; Kim *et al.*, 1984). In our

results, interval training in the women increased the expression of HSP27 at p*I* 5.0, suggesting this was the phosphorylated form, but this could not be confirmed by ms/ms analysis. HSP27 phosphorylation has also been attributed to homeostatic function by stabilising the actin filaments in response to heat shock (Bellomo *et al.,* 1990; Hinshaw *et al.,* 1986, 1988; Lavoie *et al.,* 1993). It is also unclear why the suspected HSP27 phosphorylation occurred in only the females, and not the males, after interval training.

HSP27 has been found to respond to oestrogen. However, Voss *et al.* (2003) found that there were no differences in HSP27 levels between sedentary male and female rats. Our results also showed no differences between the vastus lateralis of the men and women before exercise training, to explain the significantly increased HSP27 spot (spot 559) which occurred after interval training. In men, Morton *et al.* (2007) found no changes in HSP27 levels with exercise-associated hyperthermia and Thompson *et al.* (2003) found that HSP27 levels in the biceps brachi were enhanced by 380 % after 50 high-force eccentric contractions in men. In contrast, HSP27 levels decreased in the vastus lateralis with downhill running. This suggests that HSP27 is both muscle- and exercise-specific in men. In addition, HSP27 has been associated with the remodelling of skeletal muscle after repeated bouts of contractile activity (Neufer *et al,* 1996). In our study, 6 weeks of training may not have been long enough to induce changes in contractile proteins in our female participants possible because of their muscle phenotype before training. Whereas the males may have adapted quicker to training and thus HSP27 expression would have been apparent earlier during the interval training in the men. Therefore, the enhancement of muscle contractile proteins may occur earlier in men compared to women after 6 weeks of interval training.

Although the role of these smaller heat shock proteins are still under review, interval training is known to induce muscle damage through the appearance of alternative markers. Albumin has also been known to be an indicator of muscle damage, and accumulates in damaged muscle fibres (Cornelio & Dones, 1984; Straub *et al.,* 1997), but this decreased in expression after interval training in men. Albumin is a globular unglycosylated monomric serum protein, which

comprises of about one-half of the serum proteins in an aqueous plasma. Over 24 electrophoretic variants of serum albumin had been reported but two primary structures exist, albumin A (common form) and Albumin B (variant form). The function of serum albumin is to transport steroids, fatty acids and thyroid hormones. It also plays a role in stabilising extracellular fluid volume.

Serum albumin concentrations and hence water distribution are associated with muscle strength. Schalk *et al.* (2005) showed that low serum albumin levels were directly associated to weaker muscles. Potential mechanisms for the down-regulation of albumin have been postulated to be due to cytokines (e.g. IL-6) which are involved in protein breakdown (Rothschild *et al.*, 1988; Roubenoff *et al.*, 1994). Studies have shown that at least 75-80 % of muscular albumin is cleared via the lymphatic system (Aukland & Reed, 1993; Reed *et al.*, 1985). Havas *et al.* (2000) showed a 5-fold increase in so called 'intramuscular' albumin clearance after 15 minutes of moderate intensity running. After two hours, albumin levels had declined but were still 2-3 fold higher than baseline values. This pattern in albumin clearance was consistent with opening of muscle capillary beds and adjustments in plasma volume and hence lymph flow. The other explanation of increased albumin expression is contamination of muscle samples with blood during the extraction of the vastus lateralis muscle samples. During the sample preparation, the lysis buffer breaks all protein cellular membranes. However, 4 separate spots were identified as albumin, with one as an albumin precursor protein that was present in all 40 gels. Therefore, the reduction in albumin expression after interval training is more likely to be indicative of changes in blood and lymph flow rather than muscle damage.

Overall, the increased expression of HSP70 in the males after interval training could be connected to the increased expression of myofibrillar proteins. The decrease in HSC71 could be indicative of its involvement in chaperone functions, especially in regulating oligomeric assembly-disassembly processes in proteins to produce ATP. The lack of changes in HSP70/HSC71 expressions in females has been previously attributed to oestrogen levels. With the increase in HSP27 in the female muscle after interval training being related to filament stabilisation, the increased expression could have been indicative of the start of changes in

contractile proteins. Therefore, it is important to note that heat shock protein expressions are not only training specific, but also sex-specific.

6.5.7 Limitations.

6.5.7.1 Training group.

All training sessions were fully supervised, with the treadmill distance and heart rates for each session noted in an exercise diary (in appendix 9.2). However, we also would have liked to measure gait length and muscle fibre-type ratios in the vastus lateralis, to give us a better indication of pre-training differences between men and women. Due to the training supervision, proteomic analysis and cardiovascular tests, there was limited time to measure these indices, with participants being reluctant to undertake more measurements.

6.5.7.2 Gel matching.

No studies have previously used 2D-electrophoresis to assess the effects of two different training modalities on both males and females. Due to the cross-over design, proteomic software was a limiting factor. Ideally, we would have wished to analyse all 40 gels (pre-post, male-female, continuous-interval training) together, but vector alignment was not accurate enough across all 40 gels. Therefore, with the main emphasis of the study was in relation to sex differences, all the interval male and female gels were analysed separately from the continuous trained gels. This resulted in sex-differences being directly compared, but training differences could only be associated. There is no advanced proteomic software currently available which can undertake this complex study design.

6.5.7.3 Triplicate samples.

Most 2D-electrophretic samples are produced in triplicate. To improve subject adhesion to the project, only 2 passes of muscle biopsies were taken from each subject. One muscle biopsy was used for 2D-electrophroesis and the other for western blot analysis (fellow colleague). The average protein content of the muscle samples were 1.2 mg. This was not enough to run in triplicate assays. However, it is contestable that these samples did not in fact need to be run in triplicate. Proteomic techniques used in this study had stringent guidelines, with each gel run identically to ensure minimal variations. Over 200 different isolated protein variants were apparent on each gel, with an average co-efficient of variation of 35 % in normalised spot volume between the 4 groups. The vector alignment analysis also reduced spot-to-spot variations between gels. This low range of variation between gels suggests that technical replicates may not be required for this particular study design.

6.5.8 Summary.

Vastus lateralis muscle metabolism is both training-responsive and sex-specific. In respect to exercise modality, interval training appears to have a greater effect on muscle structure and function than continuous training.

Six weeks of interval training in men, elicited a conversion in contractile proteins from slow to fast isoforms, through the increased expression of fast troponin T isoforms and a concomitant decrease in slow TnT and MLC_{sb} isoform. In men, interval training appears to improve anaerobic capacity, showing an increase in CK-M expression. Interval training also appears to improve aerobic muscle capacity, with markers such as increased RLC and HSP70 response.

As anticipated, improvements in anaerobic capacity were not seen after continuous training, but unexpectedly there were also no changes in aerobic metabolism seen by decreases in electron transport chain complex 1 and HSC71 response. Overall, in men, short-term continuous training does not improve

muscle aerobic capacity, whereas interval training improves both acrobic and anaerobic capacities, and induces a conversion towards a faster contracting phenotype in the vastus lateralis.

The women did not elicit any of these changes in contractile protein after either training modality. However, the increase in HSP27 expression in women is indicative of its role in actin filament stabilisation. The female continuously trained group did not adapt to the training stimulus, with no changes associated with contractile proteins, metabolic enzymes or heat shock proteins. The relatively poor adaptations for both training modalities in women, suggests that a longer training period or a higher intensity may be required to enhance muscle function in females. As in men, interval training may still be considered a more superior training method for women, but has yet to be verified.

Chapter 7

Conclusions

The aims of this thesis were to primarily investigate potential sex-specific differences in overall cardiac function, by subjecting healthy, but sedentary men and women to a long-term endurance training programme. No studies to date have assessed overall cardiac function (CPO), using a long-term endurance training programme, in both older men and women. Our results have shown that 30 weeks of an endurance training programme in 55-65 year old men and women, improved $\dot{V}O_{2\,max}$, but did not improve cardiac function (study 1, chapter 3). In contrast to some previous studies (Spina *et al.*, 1993, 1996a), the main effect of this training in both the men and women was an increase in a-vO_2 differences and not a cardiac adaptation. Although Makrides *et al.* (1990) showed increases in maximal cardiac output after short-term, high-intensity training in both young and older men, even at the highest intensity (75 % HRR) within our training programme, no cardiac adaptations were detected in either sex.

Secondly, given the recent interest in the benefits of interval verses continuous endurance training, we designed a novel training programme which incorporated both interval and continuous training, using the same maintained work loads, in both men and women (study 3, chapter 5). Interestingly, both the men and women showed significant improvements in $\dot{V}O_{2\,max}$ which were related to increases in either the delivery to, or extraction of, oxygen by the working muscles and not a central cardiac adaptation. These peripheral changes in oxygen extraction and delivery, after the interval and continuous training, were probably due to increases in oxidative enzymes (Burgomaster *et al.*, 2005), muscle capillary density (Laursen and Jenkins, 2002), mitochondrial density (Zierath & Hawley, 2004) and/or changes in muscle fibre type expression (Dawson *et al.*, 1998).

A unique aspect of this thesis was the opportunity to examine some of these reported peripheral changes in more detail by examining cellular/molecular adaptations of the vastus lateralis muscle to training in both men and women. Although 6 weeks of continuous training had little effect on muscle protein expressions, interval training in the men did induce changes in gene expression, with the increased expression of fast troponin T isoforms and a concomitant

decrease in slow TnT and MLC_{sb} isoform. These changes support the concept of a gradual conversion towards a faster contracting phenotype in the vastus lateralis of men. In contrast, the vastus lateralis of women did not show any of these changes in protein profiles after either training protocol.

Clearly a more active lifestyle is beneficial for peoples' health. Based on these findings, exercise prescription must be carefully considered regarding attempts to improve cardiac function. The 30-week training programme did start at a low intensity (30 % HRR), which was clearly insufficient to affect cardiac adaptations, as previous studies have only shown changes in cardiac function with an exercise intensity over 60 % HRR. Based on that observation, our study should have shown improvements in cardiac function during the last 12 weeks of training at 60-75 % HRR. However, this was not apparent in either sex. This therefore suggests that either an exercise intensity in excess of 70 % HRR is needed or that the training period was not long enough to induce changes in cardiac function. It may also indicate that although exercise at an older age is still beneficial, overall cardiac function may be more difficult to improve by exercise training. However, a low intensity exercise programme will give health benefits (e.g. countering atherosclerosis), to both men and women, particularly in respect to improving peripheral blood flow. We can conclude that aerobic exercise also reaps greater health benefits, compared to doing no exercise.

These results also show that both exercise intensity and duration affect the degree of central and peripheral improvements to exercise training in younger men and women. The 6-week interval and continuous training programmes may have not been long enough to induce cardiac changes. However, central cardiac improvements have been seen after 2 weeks of high-intensity interval training (Burgomaster *et al.*, 2005; Smith & Wenger, 1981) and increases in maximal $\dot{Q}$, SV and EDV have been found after 6 weeks of interval and continuous training in men (Warburton *et al.*, 2004). It is also apparent within the literature that a higher-intensity programme elicits greater changes in $\dot{V}O_{2\,max}$ and maximal cardiac output (Daussin *et al.*, 2007; Helgerud *et al.*, 2007). Although the work phase within our interval training programme was set at an intensity of 100 % $\dot{V}O_{2\,max}$, a longer 'work phase', at the expense of the 'rest phase', may have

induced cardiac changes or further changes in skeletal muscle protein expressions. However, with muscle proteomes being dynamic rather than static in nature, it is hard to ascertain what the ideal training intensity or time period is needed to induce further changes in the vastus lateralis muscle.

Because of the importance of exercise in relation to public health in preventing or delaying various diseases, including coronary heart and cardiovascular disease, the prescription of exercise needs to be based on strong scientific evidence derived from well design and executed studies. This has been a principle aim of this thesis, which was designed to provide an insight into 'whole body' responses to exercise training and to allow the comparison of different systems and tissues to different modalities of exercise training. In regards to extending the current work, it would be interesting to ascertain whether older men and women can adapt to high-intensity interval training in regards to improving their cardiac function. This would therefore allow better guidance on which modality of exercise training is more appropriate for older men and women to undertake. In regards to younger subjects, using the same maintained workloads between training modalities is important in clarifying the widespread discrepancies within the literature concerning the benefits of interval and continuous training. In utilising this study design, further research can be initiated to ascertain the minimum exercise duration and intensity of interval and continuous training required to improve cardiac function. It is also important to continue to analyse men and women simultaneously to enable a more accurate exercise prescription for both men and women.

Although the proteomics field has been well developed using animal models (particularly rats and chickens), more sophisticated and accurate measurement techniques now allow it to be utilised using human muscle samples in assessing the impact of exercise training on the muscle proteome. Therefore, with proteomics currently being the most powerful tool for analysing numerous proteins within a particular muscle sample, it is advisable that this method should be used in future research to examine sex-specific differences with exercise training. It would also be interesting to measure protein changes in other active muscles i.e. soleus or gastrocnemus muscles in response to various exercise

training programmes. This information can therefore be used to enable elite athletes to train appropriately to gain maximal muscle physiological benefits.

Although we measured cardiac function and skeletal muscle protein expression in regards to exercise training, vascular adaptations to training were not measured. Therefore, studies should examine a wider variety of physiological indices to gain a better 'whole body' overview in response to exercise training. This may alleviate discrepancies within the literature between different physiological system adaptations in regards to exercise training.

Chapter 8

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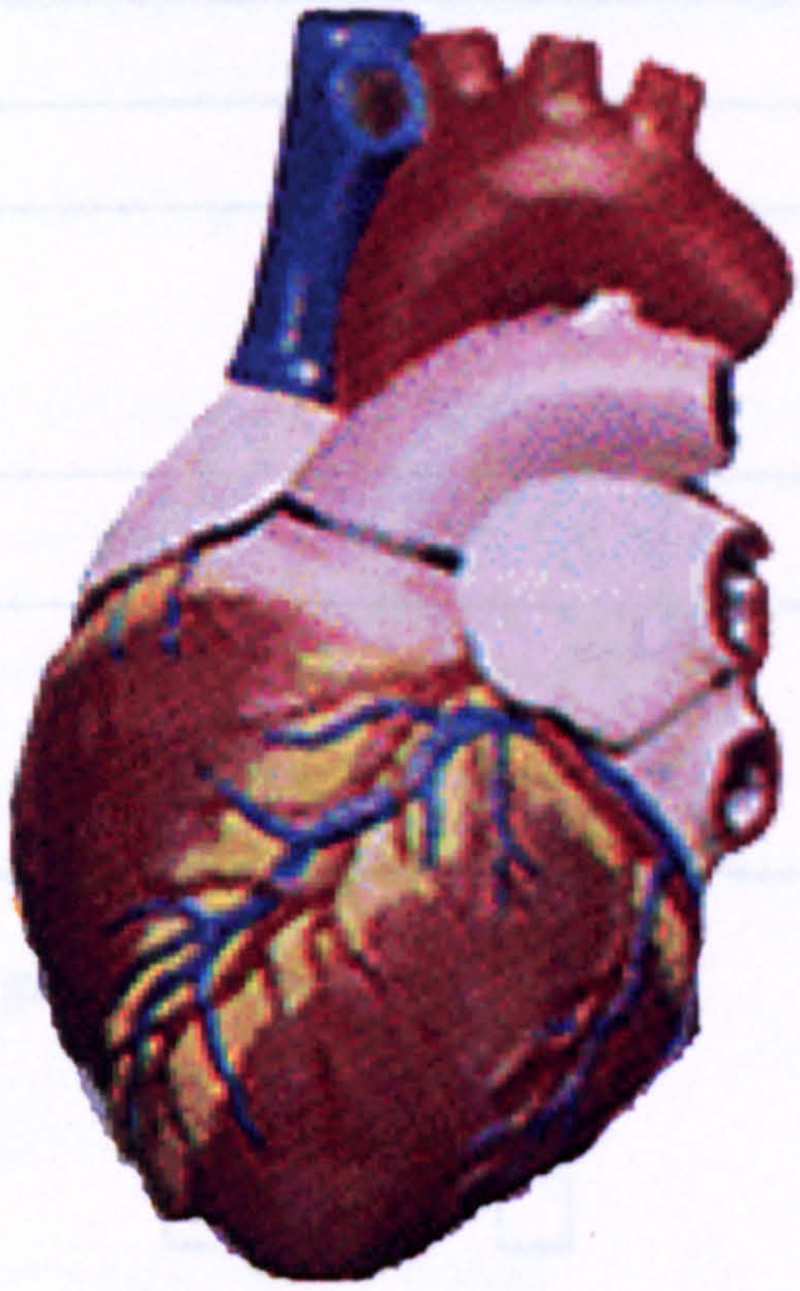
Chapter 9

Appendices

Male Participant Questionnaire

Liverpool John Moores University
Research Institute for
Sport and Exercise Sciences

Project: Effects of Ageing on the Power Output of the Heart



Personal Details
&
Medical, Lifestyle and Dietary Assessment Questionnaires

Please Read Carefully

The main purpose of this questionnaire is to find out about your health status and lifestyle habits. Information that you provide will be used to determine your suitability to participate safely and effectively in this study

Please note: This questionnaire is an important part of the study. We request that you answer all questions as accurately and as honestly as possible. Most questions can be answered by either placing a circle around the appropriate response, a tick in the box provided, or a short written response

Section One

Personal Information

1. Name:_____

2. DOB: Age:

3. Height: Weight:

4. Address _____

_____

_____

5. Telephone number:

Home: _____

Mobile:_____

6. Email:_____

7. What is your ethnic group (please tick box)

Caucasian	Hispanic	Black	Asian	Chinese	Other
☐	☐	☐	☐	☐	☐

Section Two

Personal Medical History Assessment **(circle answer)**

8. Has your doctor ever said that you have had a heart condition? Yes No

If yes, please give details, including dates _____

_____

_____

9. Have you ever been instructed to perform physical activity only recommended by a doctor? Yes No

If yes, please give details, including dates _____

_____

10. Have you ever had a real, or suspected, heart attack? Yes No

If yes, when did it occur _____

11. Have you ever experienced rapid heart beating or palpitations? Yes No

If so, please give details, including what you were doing at the time _____

_____

_____

12. Have you ever had angina or a sharp heavy pain in your chest as the result of physical activity? Yes No

If so, please circle level of activity: low moderate strenuous

13. Do you have reduced eye sight or had an eye operation? Yes No

If yes, is that because

It is hard to read a textbook up close	D
It is hard to see clear in the distance (short sightedness)	D
You are colour blind	D
Other than the previous	D

Do you wear glasses for this? Yes No

If yes, is there a difference in the level of correction for both eyes? Yes No

14. Do you have reduced hearing ability? Yes No

If yes, has this been diagnosed by your doctor? Yes No

15. Do you sometimes lose your balance due to

Dizziness	Yes No
Stumbling over an object	Yes No
Walking up/down stairs, pavement, sloping ground…	Yes No
Unexpected obstacle	Yes No
Other than the previous	Yes No

If you sometimes lose your balance, has this ever led to a fall (even without injury)? Yes No

16. Do you ever lose consciousness? Yes No

17. Have you ever had a resting or exercise ECG taken? Yes No

If yes, was the ECG normal? Yes No

18. Have you ever been severely breathless as a result of low/moderate level exercise? Yes No

19. Do you suffer from high or low blood pressure? Yes No

If yes, which one? Low High

20. Are you currently taking prescribed medication to control your blood pressure? Yes No

If yes, give name and dosage _____

21. Have you ever been told your blood cholesterol is too high? Yes No

If yes, please state your cholesterol level (if known) _____

22. Are you currently taking prescribed medication to control your cholesterol ? Yes No

If yes, state name and dosage _____

23. Do you suffer from any kidney problems now or in the past? Yes No

If yes please specify condition and medication _____

_____

24. Do you suffer from diabetes? Yes No

If yes, how is it controlled **(please tick)**

a) Dietary means ☐ b) Insulin injection ☐

c) Oral medication ☐ c) Uncontrolled ☐

25. Do you suffer from asthma, or any respiratory disorders? Yes No

Please give details of condition and any medication taken including inhaler ____

_____

_____

Is the breathing condition made worse by exercise? Yes No

If yes, what level of exercise (please circle) low moderate strenuous

26. Do you have any musculo-skeletal problems that could be made worse by a change in physical activity? Yes No

If so, please give details of condition _____

What level of exercise can you do without making your condition worse? (please circle) low moderate strenuous

27. Do you know of any other reason why you should not undertake physical activity? Yes No

If yes, why _____

28. Do you suffer from any of the following: -

HIV/AIDS	Yes	No
Hepatitis B or C	Yes	No
Or any other disease transmitted by blood	Yes	No
Haemophiliac	Yes	No
Chron's disease	Yes	No
Thyroid Problems	Yes	No
Adrenal Problems	Yes	No
Pituitary Problems	Yes	No

29.Do you smoke? Yes No

What do you smoke (please circle) cigarettes cigars pipe

If yes,

How long have you smoked for? _____

How many per day? _____

Have you ever smoked? Yes No

If yes,

How long did you smoke for? _____

How many per day? _____

When did you stop? _____

Section Three

Physical Activity Assessment

30. Considering a typical **7-day period** (week), how **many times** do you do the following kinds of exercise for during your free time (write on each line the appropriate number).

	Times Per Week	**Duration (to the nearest 5mins)**
a) Strenuous Exercise (Heart beats rapidly)		
(e.g running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous longer distance cycling)	_____	_____
b) Moderate Exercise (Not Exhausting)		
(e.g. fast walking, baseball, tennis, easy cycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)	_____	_____
c) Mild Exercise (Minimal Effort)		
(e.g. yoga, archery, fishing from river bed, bowling, horseshoes, golf, easy walk)	_____	_____

31. Considering a typical **7-day period** (week), during your leisure time, how often to do you engage in regular activity long enough to work up a sweat with your heart beating rapidly?

OFTEN	SOMETIMES	NEVER/RARELY
☐	☐	☐

32. Are you currently engaged in moderate or intense training? Yes No

If yes, please detail training schedule including, type of activity, intensity, number of sessions per week and duration of each session on the attached sheet at the end of the questionnaire.

33. Have you ever previously engaged in moderate or intense training? Yes No

If yes, please give details of your schedule:

Intensity	**Number of times per week**	**Duration of each session (to nearest 5mins)**

What year did you start training? _____

How long ago did you stop training? _____

Section four

Diet Assessment
(please circle)

34. Are you a vegetarian Yes No

35. During a typical day what do you eat/drink

_____

_____

_____

36. Do you take any of the following food supplements:

Co-enzyme Q10	Calcium	Iron	Vitamins
Glucosamine/Chrondroitin	Folic Acid	Garlic	Magnesium
Evening primrose oil	Omega 3		

Other (please state): _____

37. Please detail any further information you would like to tell us _____

Participant signature: _____

Thank you for completing this questionnaire

Question 35 continued.
Please give details of current training schedule.

Once complete please return to:-

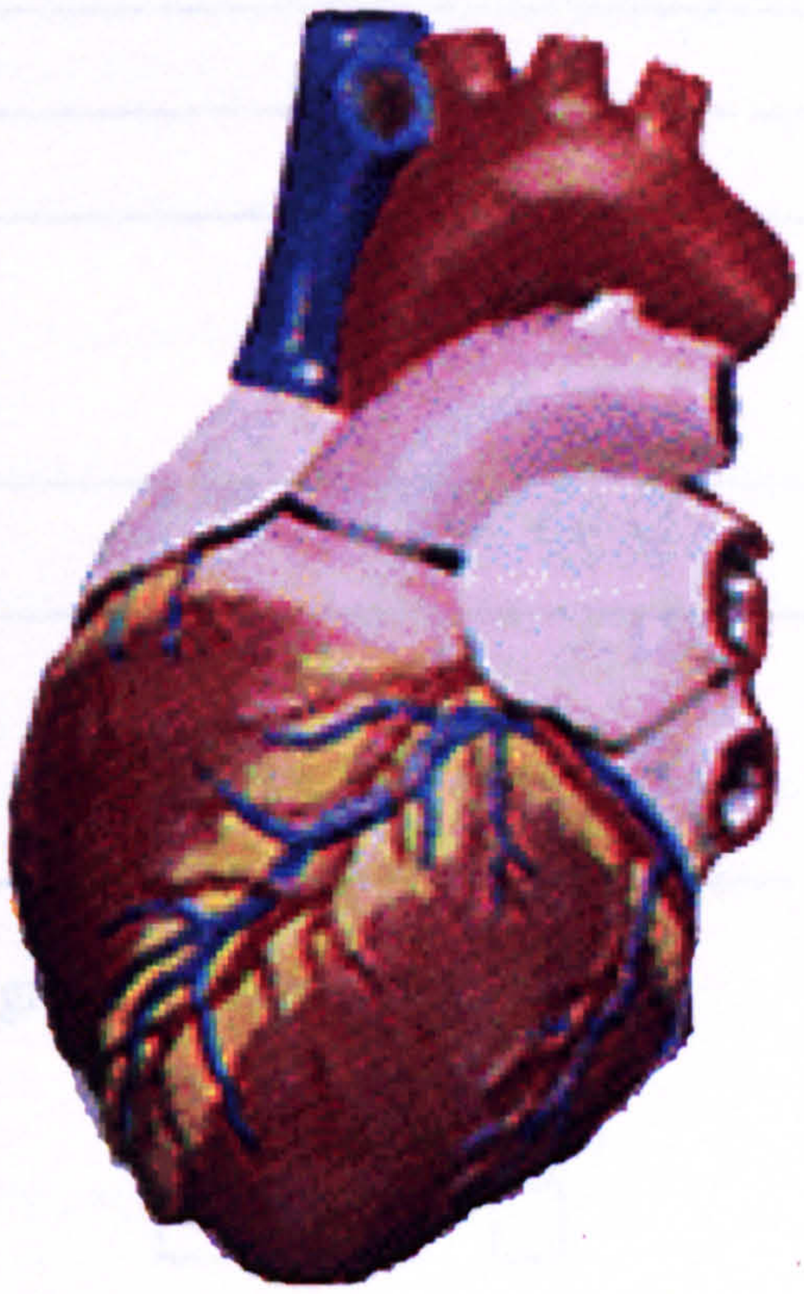
Paul Woods,
Research Institute for Sport & Exercise Sciences,
Liverpool John Moores University,
Henry Cotton Campus,
15-21 Webster Street,
Liverpool,
L3 2ET.

Tel: 07968 422 618
E-Mail: P.R.Woods@ljmu.ac.uk

Female Participant Questionnaire

Liverpool John Moores University
Research Institute for
Sport and Exercise Sciences

Project: Effects of Ageing on the Power Output of the Heart



Personal Details
&
Medical, Lifestyle and Dietary Assessment Questionnaires

Please Read Carefully

The main purpose of this questionnaire is to find out about your health status and lifestyle habits. Information that you provide will be used to determine your suitability to participate safely and effectively in this study

Please note: This questionnaire is an important part of the study. We request that you answer all questions as accurately and as honestly as possible. Most questions can be answered by either placing a circle around the appropriate response, a tick in the box provided, or a short written response

Section One

Personal Information

1. Name:_____

2. DOB: Age:

3. Height: Weight:

4. Address _____

_____

_____

5. Telephone number:

Home: _____

Mobile:_____

6. Email:_____

7. What is your ethnic group (please tick box)

Caucasian	Hispanic	Black	Asian	Chinese	Other
☐	☐	☐	☐	☐	☐

Section Two

Personal Medical History Assessment **(circle answer)**

8. Has your doctor ever said that you have had a heart condition? Yes No

If yes, please give details, including dates _____

_____

_____

9. Have you ever been instructed to perform physical activity only recommended by a doctor? Yes No

If yes, please give details, including dates _____

_____

10. Have you ever had a real, or suspected, heart attack? Yes No

If yes, when did it occur _____

11. Have you ever experienced rapid heart beating or palpitations? Yes No

If so, please give details, including what you were doing at the time _____

_____

_____

12. Have you ever had angina or a sharp heavy pain in your chest as the result of physical activity? Yes No

If so, please circle level of activity: low moderate strenuous

13. Do you have reduced eye sight or had an eye operation? Yes No

If yes, is that because

It is hard to read a textbook up close	D
It is hard to see clear in the distance (short sightedness)	D
You are colour blind	D
Other than the previous	D

Do you wear glasses for this? Yes No

If yes, is there a difference in the level of correction for both eyes? Yes No

14. Do you have reduced hearing ability? Yes No

If yes, has this been diagnosed by your doctor? Yes No

15. Do you sometimes lose your balance due to

Dizziness	Yes	No
Stumbling over an object	Yes	No
Walking up/down stairs, pavement, sloping ground…	Yes	No
Unexpected obstacle	Yes	No
Other than the previous	Yes	No

If you sometimes lose your balance, has this ever led to a fall (even without injury)? Yes No

16. Do you ever lose consciousness? Yes No

17. Have you ever had a resting or exercise ECG taken? Yes No

If yes, was the ECG normal? Yes No

18. Have you ever been severely breathless as a result of low/moderate level exercise? Yes No

19. Do you suffer from high or low blood pressure? Yes No
If yes, which one? Low High

20. Are you currently taking prescribed medication to control your blood pressure? Yes No

If yes, give name and dosage _____

21. Have you ever been told your blood cholesterol is too high? Yes No

If yes, please state your cholesterol level (if known) _____

22. Are you currently taking prescribed medication to control your cholesterol ? Yes No

If yes, state name and dosage _____

23. Do you suffer from any kidney problems now or in the past? Yes No

If yes please specify condition and medication _____

_____

24. Do you suffer from diabetes? Yes No

If yes, how is it controlled **(please tick)**

a) Dietary means ☐ b) Insulin injection ☐

c) Oral medication ☐ c) Uncontrolled ☐

25. Do you suffer from asthma, or any respiratory disorders? Yes No

Please give details of condition and any medication taken including inhaler ____

_____

_____

Is the breathing condition made worse by exercise? Yes No

If yes, what level of exercise (please circle) low moderate strenuous

26. Do you have any musculo-skeletal problems that could be made worse by a change in physical activity? Yes No

If so, please give details of condition _____

What level of exercise can you do without making your condition worse? (please circle) low moderate strenuous

27. Do you know of any other reason why you should not undertake physical activity? Yes No

If yes, why _____

28. Do you suffer from any of the following: -

HIV/AIDS	Yes	No
Hepatitis B or C	Yes	No
Or any other disease transmitted by blood	Yes	No
Haemophiliac	Yes	No
Chron's disease	Yes	No
Thyroid Problems	Yes	No
Adrenal Problems	Yes	No
Pituitary Problems	Yes	No

29. Do you smoke? Yes No

What do you smoke (please circle) cigarettes cigars pipe

If yes,

How long have you smoked for? _____
How many per day? _____

Have you ever smoked? Yes No

If yes,

How long did you smoke for? _____
How many per day? _____
When did you stop? _____

Section 3.

Hormonal Status.

30. Have you reached the menopause?

- ☐ Yes, surgical hysterectomy.
- ☐ Yes, naturally, confirmed by my doctor.
- ☐ Yes, naturally, unconfirmed by my doctor.
- ☐ Unsure.
- ☐ No.

If YES, please continue.
If NO, go to section 3.

31. At what age did you reach the menopause? _____

32. Are you taking any Hormone Replacement Therapy (HRT)? Yes No

If NO, go to question 44.
If YES, please continue.

33. Please list what HRT name, hormonal contents and dosage _____

_____

_____

34. Why do you use HRT?

- ☐ Treatment of short term symptoms (hot flushes, night sweats, vaginal dryness)
- ☐ Prevention or treatment of osteoporosis.
- ☐ Prevention of heart disease.
- ☐ Other. Please describe. _____

35. How long have you been taking HRT?

- ☐ Less than 1 year
- ☐ 1-2 years
- ☐ 3-5 years
- ☐ Longer than 5 years , please state how long _____

36. If you are not taking HRT now, have you ever used it in the past? Yes No

If YES, please continue.
If NO, go to section 4.

37. When did you stop taking HRT?

- ☐ Less than 1 year ago
- ☐ 1-2 years ago
- ☐ 3-5 years ago
- ☐ Longer than 5 years ago, please state how long _____

38. How long did you take HRT for?

- ☐ Less than 1 year
- ☐ 1-2 years
- ☐ 3-5 years
- ☐ Longer than 5 years, please state how long _____

39. Why did you stop taking HRT?

- ☐ Side effects (headache, acne, bloating, breast discomfort)
- ☐ Worried about health risks (cancer, hypertension, blood clots, etc.)
- ☐ No longer needed.
- ☐ Other. Please describe. _____

Section Three

Physical Activity Assessment

40. Considering a typical **7-day period** (week), how **many times** do you do the following kinds of exercise for during your free time (write on each line the appropriate number).

	Times Per Week	**Duration (to the nearest 5mins)**
a) Strenuous Exercise (Heart beats rapidly) (e.g running, jogging, hockey, football, soccer, squash, basketball, cross country skiing, judo, roller skating, vigorous swimming, vigorous longer distance cycling)	_____	_____
b) Moderate Exercise (Not Exhausting) (e.g. fast walking, baseball, tennis, easy cycling, volleyball, badminton, easy swimming, alpine skiing, popular and folk dancing)	_____	_____
c) Mild Exercise (Minimal Effort) (e.g. yoga, archery, fishing from river bed, bowling, horseshoes, golf, easy walk)	_____	_____

41. Considering a typical **7-day period** (week), during your leisure time, how often to do you engage in regular activity long enough to work up a sweat with your heart beating rapidly?

OFTEN	SOMETIMES	NEVER/RARELY
☐	☐	☐

42. Are you currently engaged in moderate or intense training? Yes No

If yes, please detail training schedule including, type of activity, intensity, number of sessions per week and duration of each session on the attached sheet at the end of the questionnaire.

43. Have you ever previously engaged in moderate or intense training? Yes No

If yes, please give details of your schedule:

Intensity	**Number of times per week**	**Duration of each session (to nearest 5mins)**
_____	_____	_____

What year did you start training? _____

How long ago did you stop training? _____

Section four

Diet Assessment
(please circle)

44. Are you a vegetarian Yes No

45. During a typical day what do you eat/drink

_____

_____

_____

46. Do you take any of the following food supplements:

Co-enzyme Q10	Calcium	Iron	Vitamins
Glucosamine/Chrondroitin	Folic Acid	Garlic	Magnesium
Evening primrose oil	Omega 3		

Other (please state): _____

_____

47. Please detail any further information you would like to tell us _____

Participant signature: _____

Thank you for completing this questionnaire

Any other information which may be relevant

Once complete please return to:-

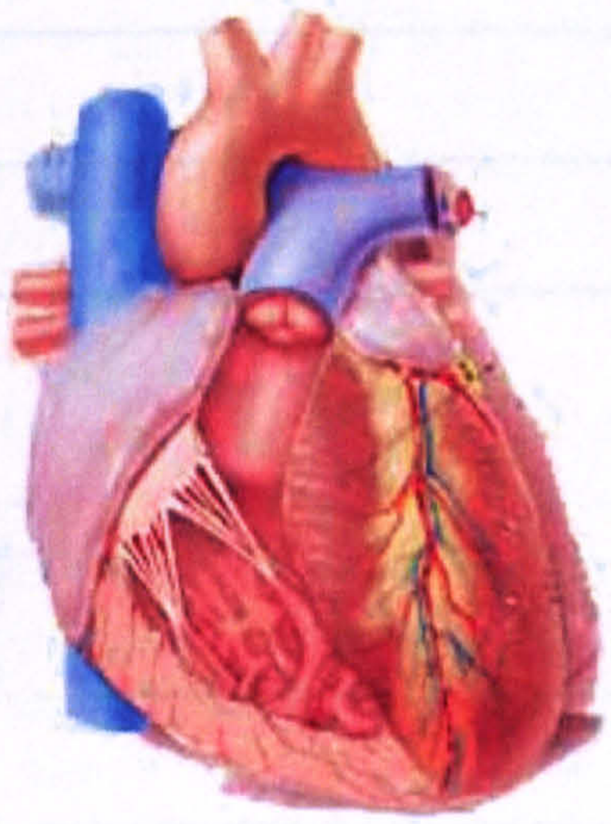
Paul Woods,
Research Institute for Sport & Exercise Sciences,
Liverpool John Moores University,
Henry Cotton Campus,
15-21 Webster Street,
Liverpool,
L3 2ET.

Tel: 07968 422 618
E-Mail: P.R.Woods@ljmu.ac.uk

British Heart Foundation

School of Sport and Exercise Sciences

Liverpool John Moores University
Research Institute for Sport and Exercise Sciences



Personal Details and Exercise Diary

Please Read Carefully

The purpose of this diary is to monitor your weekly exercise sessions. The information you provide will act as a record for your activity sessions and will determine the achievement of your training goals, therefore monitoring your progress throughout the year.

Important: This diary is an important aspect of our study. Therefore, we request you to answer the questions as honestly and accurately as possible.

Personal Information

Name:_____

Date of Birth:____/____/_____ Age: _____

Address: _____

_____

_____

_____

Telephone Number:

Home _____

Work _____

Mobile _____

E-mail:_____

Next of kin contact details:

Name _____

Relationship to you _____

Telephone number _____

Mobile _____

Introduction to your exercise:

We would like to take this opportunity to once again thank you for participating in the training programme. This unique research is supported by the British Heart Foundation, which gives you the opportunity to improve your health. The next 12 months are going to be busy, but we hope they are also enjoyable and beneficial to you. Our team here at the Ageing Research Unit are 100% committed to providing the highest level of support and professionalism. However, to make this project successful we need you also to be committed to the programme as the completion of the 12-month programme is vital.

For the next 6 weeks, we require you to exercise 3 times a week for 30 minutes. This exercise will be set at 30% heart rate reserve (you will be notified what that is on your first training session). We ask that on your first visit you attend our own university gym so that we can explain the protocol and how the equipment works. Following that we will require you to come in to our gym at least once a week so check on your progress. Otherwise you are welcome to go to any of the 13 Lifestyle Gyms around Liverpool. The session will consist of 10 minute walk, followed by a 10 minute cycling, followed by a 10 minute walk. Each exercise session will be supervised by one of the team at the university gym, or a qualified gym instructor at the lifestyle gyms.

After the initial 6 weeks, the frequency of your exercise will increase to 5 times a week. Those 2 extra sessions can involve other activities outside the gym (walking, cycling etc) but must not exceed 30 minutes or your heart rate reserve value. To enable you to measure your heart rate outside the gym, we provide heart rate monitors, which will be given to you and explained in your introductory session. It must be noted that these heart rate monitors are yours during the 12 month programme and we cannot guarantee replacing them if they are lost or damaged.

Please do not exercise more or less than required as it may seriously jeopardise our results and make them invalid.

If you have any queries regarding this information or general queries regarding the programme, then please don't hesitate to contact us. The contact details are below.

Thank you once again.

Contact details:

Mark Black	07968422616
Kat Spaull	07968422617
Paul Woods	07968422618
Shelina Skyrme	07968422

Exercise record

30% HRR = bpm

Week 1:		**Peak Heart Rate during Exercise**		
	Date	**Walk 1**	**Bike 1**	**Walk 2**
Session 1	___/___/___	_____bpm	_____bpm	_____bpm
Session 2	___/___/___	_____bpm	_____bpm	_____bpm
Session 3	___/___/___	_____bpm	_____bpm	_____bpm

Additional comments (i.e. hard, tiring)

Week 2:		**Peak Heart Rate during Exercise**		
	Date	**Walk 1**	**Bike 1**	**Walk 2**
Session 1	___/___/___	_____bpm	_____bpm	_____bpm
Session 2	___/___/___	_____bpm	_____bpm	_____bpm
Session 3	___/___/___	_____bpm	_____bpm	_____bpm

Additional Comments (i.e. hard, tiring)

Lifestyle Gyms

Gym address	Telephone No.	Opening times
Lifestyles Austin Rawlinson ParkLands Conleach Road Speke Liverpool L24 0TR	0151 233 2100/2102	Mon- Fri 8.30am-9.30pm Sat-Sun 8.30am-3.30pm
Lifestyles Cardinal Heenan Honeysgreen Lane Liverpool L12 9HZ	0151 233 2345	Mon-Fri 4.00pm-10.00pm Sat- Sun 10.00am-4.00pm
Lifestyles Croxteth Altcross Road Liverpool L11 OBS	0151 548 3421	Mon-Fri 2.00pm-10.30pm Sat –Sun 8.30am-4.30pm
Lifestyles Everton Great Homer Street Liverpool L5 5PH	0151 207 1921	Mon-Fri 8.00am-10.30pm Sat 8.00am-4.00pm Sun 9.00am-4.00pm
Garston Leisure Centre Long Lane Garston Liverpool L19 6PE	0151 233 5700/5702 answerphone 0151 233 5701	Monday - Friday 7.15am - 10pm Saturday 7.15am - 4pm Sunday 8.15 am -4pm
Lifestyles Millennium Ground Floor Millennium House Victoria Street Liverpool L1 6DL	0151 233 4123	Mon - Fri 6.30am - 9.00pm Sat - Sun 7.30am - 4.00pm
Park Road Sports Centre Steble Street Liverpool L8 6QH	0151 233 3600	Monday - Friday 8.30-9pm Saturday and Sunday 8.30 - 4pm
Peter Lloyd Leisure Centre Bankfield Road Liverpool L13 OBQ	0151 254 1968	Monday - Wednesday 8am -10pm Thursday 9am - 10pm Friday 8am - 10pm Saturday - Sunday 9am - 4pm
Walton Sports Centre Walton Hall Avenue Liverpool L4 9XP	0151 523 3472	Monday - Friday 9am - 10pm Saturday & Sunday 9am - 3pm

Name:
Weight kg
1 min sprint = Speed km/h
4 min jog = Speed km/h

Week 1:

	Date	Average heart rate	Distance covered (km)
1			
2			
3			

Extra Exercise:

Date	Exercise Type	Duration (mins)	Intensity *

*1 = high, 2 = moderate, 3 = low

Week 2:

	Date	Average heart rate	Distance covered (km)
1			
2			
3			

Extra Exercise:

Date	Exercise Type	Duration (mins)	Intensity *

* 1 = high, 2 = moderate, 3 = low

Week 3:

	Date	Average heart rate	Distance covered (km)
1			
2			
3			

Extra Exercise:

Date	Exercise Type	Duration (mins)	Intensity *

* 1 = high, 2 = moderate, 3 = low

Week 4:

	Date	Average heart rate	Distance covered (km)
1			
2			
3			

Extra Exercise:

Date	Exercise Type	Duration (mins)	Intensity *

* 1 = high, 2 = moderate, 3 = low

Week 5:

	Date	Average heart rate	Distance covered (km)
1			
2			
3			

Extra Exercise:

Date	Exercise Type	Duration (mins)	Intensity *

* 1 = high, 2 = moderate, 3 = low

Week 6:

	Date	Average heart rate	Distance covered (km)
1			
2			
3			

Extra Exercise:

Date	Exercise Type	Duration (mins)	Intensity *

* 1 = high, 2 = moderate, 3 = low

Name:
Weight kg
Speed km/h

Week 1:

	Date	Average heart rate	Distance covered (km)
1			
2			
3			

Extra Exercise:

Date	Exercise Type	Duration (mins)	Intensity *

*1 = high, 2 = moderate, 3 = low

Week 2:

	Date	Average heart rate	Distance covered (km)
1			
2			
3			

Extra Exercise:

Date	Exercise Type	Duration (mins)	Intensity *

* 1 = high, 2 = moderate, 3 = low

Week 3:

	Date	Average heart rate	Distance covered (km)
1			
2			
3			

Extra Exercise:

Date	Exercise Type	Duration (mins)	Intensity *

* 1 = high, 2 = moderate, 3 = low

Week 4:

	Date	Average heart rate	Distance covered (km)
1			
2			
3			

Extra Exercise:

Date	Exercise Type	Duration (mins)	Intensity *

* 1 = high, 2 = moderate, 3 = low

Week 5:

	Date	Average heart rate	Distance covered (km)
1			
2			
3			

Extra Exercise:

Date	Exercise Type	Duration (mins)	Intensity *

* 1 = high, 2 = moderate, 3 = low

Week 6:

	Date	Average heart rate	Distance covered (km)
1			
2			
3			

Extra Exercise:

Date	Exercise Type	Duration (mins)	Intensity *

* 1 = high, 2 = moderate, 3 = low

Table 9.3: Mascot outputs (database entry, Mowse score, number of peptides matched) and the observed M_r and p*I* for each gel spot identified.

PMF Analysis.

Reference number	Full description	Swiss-Prot ID	Score (<59 sig)	Expect	% sequence coverage	no. of peptides	M_r	pI
10	Troponin T, fast skeletal muscle (TnTf) (Fast skeletal muscle troponinT) (fTnT) (Beta TnTF).	TNNT3_HUMAN	89	4.20E-04	2	8	31824	5.71
12	Creatine kinase M-type (EC 2.7.3.2) (Creatine kinase M chain) (M-CK).	KCRM_HUMAN	232	9.90E-12	18	44	43302	6.77
13	Troponin T, fast skeletal muscle (TnTf) (Fast skeletal muscle troponinT) (fTnT) (Beta TnTF).	TNNT3_HUMAN	47	0.00066	1	5	31824	5.71
15	Troponin T, fast skeletal muscle (TnTf) (Fast skeletal muscle troponinT) (fTnT) (Beta TnTF).	TNNT3_HUMAN	109	0.00015	8	2	31824	5.71
18	Creatine kinase M-type (EC 2.7.3.2) (Creatine kinase M chain) (M-CK).	KCRM_HUMAN	120	1.60E-08	13	20	43302	6.77
24	Beta-enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase)(Muscle-specific enolase) (MSE) (Skeletal muscle enolase) (Enolase 3).	ENOB_HUMAN	56	0.042	13	7	47299	7.59
25	60 kDa heat shock protein, mitochondrial precursor (Heat shock protein60) (HSP-60) (Hsp60) (60 kDa chaperonin) (Chaperonin 60) (CPN60)(Mitochondrial matrix protein P1) (P60 lymphocyte protein) (HuCHA60).	CH60_HUMAN	107	3.10E-07	22	12	61187	5.7
26	Adenylate kinase isoenzyme 1 (EC 2.7.4.3) (ATP-AMP transphosphorylase)(AK1) (Myokinase).	KADI_HUMAN	70	0.0017	32	8	21735	8.73
29	Aconitate hydratase, mitochondrial precursor (EC 4.2.1.3) (Citratehydro-lyase) (Aconitase).	ACON_HUMAN	131	1.50E-08	17	15	86113	7.36
31	Creatine kinase M-type (EC 2.7.3.2) (Creatine kinase M chain) (M-CK).	KCRM_HUMAN	234	7.60E-19	49	19	43302	6.77

32	Myosin light polypeptide 3 (Myosin light chain 1, slow-twitch muscleB/ventricular isoform) (MLC1SB) (Ventricular/slow twitch myosin alkalilight chain) (Cardiac myosin light chain-1) (CMLC1).	MYL3_HUMAN	67	0.041	35	6	22117	5.03
284	Troponin T, slow skeletal muscle (TnTs) (Slow skeletal muscle troponinT) (sTnT).	TNNT1_HUMAN	69	0.021	23	8	32985	5.86
302	Myosin light chain 1, skeletal muscle isoform (MLC1F) (A1 catalytic)(Alkali myosin light chain 1).	MLE1_HUMAN	100	1.60E-06	51	9	21189	4.97
315	Aconitate hydratase, mitochondrial precursor (EC 4.2.1.3) (Citratehydro-lyase) (Aconitase).	ACON_HUMAN	68	3.00E-02	12	10	86113	7.36
318	Serum albumin precursor.	ALBU_HUMAN	78	0.00027	18	12	71317	5.92
321	Serum albumin precursor.	ALBU_HUMAN	78	0.00027	18	12	71317	5.92
332	Actin, alpha skeletal muscle (Alpha-actin-1)	ACTS_HUMAN	68	0.028	16	7	38142	5.39
338	Aconitate hydratase, mitochondrial precursor (EC 4.2.1.3) (Citratehydro-lyase) (Aconitase).	ACON_HUMAN	131	1.50E-08	17	15	86113	7.36
339	Heat shock cognate 71 kDa protein (Heat shock 70 kDa protein 8).	HSP7C_HUMAN	99	2.20E-06	19	12	71082	5.37
345	Actin, alpha skeletal muscle (Alpha-actin-1).	ACTS_HUMAN	130	1.90E-08	36	13	42366	5.23
351	Serum albumin precursor.	ALBU_HUMAN	60	1.70E-02	19	10	71317	5.92
352	Myosin light polypeptide 3 (Myosin light chain 1, slow-twitch muscleB/ventricular isoform) (MLC1SB) (Ventricular/slow twitch myosin alkalilight chain) (Cardiac myosin light chain-1) (CMLC1).	MYL3_HUMAN	90	1.50E-05	51	8	22089	5.03
356	ATP synthase subunit beta (EC 3.6.3.14) (Fragment).Homo sapiens (Human).	QOQEN7_HUMAN	133	9.60E-09	33	15	48083	4.95
360	Myosin-7 (Myosin heavy chain 7) (Myosin heavy chain, cardiac musclebeta isoform) (MyHC-beta) (Myosin heavy chain slow isoform) (MyHC-slow).	MYH7	70	0.017	11	12	134206	5.24

361	Actin, alpha cardiac muscle 1 (Alpha-cardiac actin).	ACTC_HUMAN	110	1.90E-06	29	12	42334	5.23
371	Aconitate hydratase, mitochondrial precursor (EC 4.2.1.3) (Citratehydro-lyase) (Aconitase).	ACON_HUMAN	81	0.0012	15	12	86113	7.36
381	Serum albumin precursor.	ALBU_HUMAN	66	3.90E-03	14	9	71317	5.92
385	Troponin T, slow skeletal muscle (TnTs) (Slow skeletal muscle troponinT) (sTnT).	TNNT1_HUMAN	75	0.00049	27	9	32985	5.86
387	ATP synthase subunit alpha, mitochondrial precursor.	ATPA_HUMAN	118	3.00E-07	26	14	59828	9.16
394	Myosin light polypeptide 6B (Smooth muscle and nonmuscle myosin lightchain alkali 6B) (Myosin light chain 1 slow-twitch muscle A isoform)(MLC1sa).	MYL6B_HUMAN	82	0.00011	32	8	22864	5.56
405	Heat shock protein beta-1 (HspB1) (Heat shock 27 kDa protein) (HSP 27)(Stress-responsive protein 27) (SRP27) (Estrogen-regulated 24 kDaprotein) (28 kDa heat shock protein).	HSPB1_HUMAN	58	0.025	26	5	22826	5.98
406	Serum albumin precursor.	ALBU_HUMAN	81	1.20E-04	18	10	71317	5.92
407	Serotransferrin precursor (Transferrin) (Siderophilin) (Beta-1-metal-binding globulin).	TRFE_HUMAN	75	6.30E-03	14	10	79280	6.81
409	Glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) (GAPDH).	G3P_HUMAN	89	1.80E-05	34	8	36201	8.57
412	Myosin regulatory light chain 2, ventricular/cardiac muscle isoform(MLC-2) (MLC-2v).	MLRV_HUMAN	189	2.00E-13	71	14	18777	4.92
415	Myosin regulatory light chain 2, ventricular/cardiac muscle isoform(MLC-2) (MLC-2v).	MLRV_HUMAN	95	4.90E-05	48	11	18777	4.92
416	Heat shock 70 kDa protein 1 (HSP70.1) (HSP70-1/HSP70-2).	HSP71_HUMAN	138	2.50E-10	21	14	70280	5.48
418	Heat shock cognate 71 kDa protein (Heat shock 70 kDa protein 8).	HSP7C_HUMAN	156	3.9-012	24	16	71082	5.37

422	PCI domain-containing protein 2 (CSN12-like protein).	PCID2_HUMAN	68	3.10E-02	21	9	52738	8.73
425	Trimethyllysine dioxygenase, mitochondrial precursor (EC 1.14.11.8)(Epsilon-trimethyllysine 2-oxoglutarate dioxygenase) (TML-alpha-ketoglutarate dioxygenase) (TML hydroxylase) (TML dioxygenase) (TMLD).	TMLH_HUMAN	67	0.042	22	9	44534	7.04
430	Tropomyosin 1 alpha variant 6.Homo sapiens (Human).	QIZYL5_HUMAN	67	0.003	26	10	28492	4.75
433	Heat shock protein beta-6 (HspB6) (Heat shock 20 kDa-like proteinp20).	HSPB6_HUMAN	57	3.40E-02	30	5	17182	5.95
434	Actin, alpha cardiac muscle 1 (Alpha-cardiac actin).	ACTC_HUMAN	110	1.90E-06	29	12	42334	5.23
435	Peroxiredoxin-2 (EC 1.11.1.15) (Thioredoxin peroxidase 1)(Thioredoxin-dependent peroxide reductase 1) (Thiol-specificantioxidant protein) (TSA) (PRP) (Natural killer cell-enhancing factorB) (NKEF-B).	PRDX2_HUMAN	71	0.0014	29	7	22049	5.66
436	Actin, alpha skeletal muscle (Alpha-actin-1).	ACTS_HUMAN	99	2.40E-05	30	11	42366	5.23
439	Myosin light polypeptide 3 (Myosin light chain 1, slow-twitch muscleB/ventricular isoform) (MLC1SB) (Ventricular/slow twitch myosin alkalilight chain) (Cardiac myosin light chain-1) (CMLC1).	MYL3_HUMAN	93	8.10E-06	9	44	22089	5.03
442	Glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) (GAPDH).	G3P_HUMAN	82	1.20E-03	25	10	36201	8.57
443	Myosin light chain 1, skeletal muscle isoform (MLC1F) (A1 catalytic)(Alkali myosin light chain 1).	MLE1_HUMAN	102	1.20E-05	62	10	21189	4.97
445	Peroxiredoxin-2 (EC 1.11.1.15) (Thioredoxin peroxidase 1)(Thioredoxin-dependent peroxide reductase 1) (Thiol-specificantioxidant protein) (TSA) (PRP) (Natural killer cell-enhancing factorB) (NKEF-B).	PRDX2_HUMAN	66	0.0042	37	6	22049	5.66

448	Troponin T, fast skeletal muscle (TnTf) (Fast skeletal muscle troponinT) (fTnT) (Beta TnTF).	TNNT3_HUMAN	83	7.90E-05	9	29	31805	5.71
449	Myoglobin.	MYG_HUMAN	67	0.0034	34	7	17230	7.14
452	Pyruvate kinase isozymes M1/M2 (EC 2.7.1.40) (Pyruvate kinase muscleisozyme) (Pyruvate kinase 2/3) (Cytosolic thyroid hormone-bindingprotein) (CTHBP) (THBP1).	KPYM_HUMAN	110	1.90E-06	19	14	62125	7.59
453	Heat shock protein beta-6 (HspB6) (Heat shock 20 kDa-like proteinp20).	HSPB6_HUMAN	78	0.0033	41	7	16884	5.95
458	Heat shock protein beta-1 (HspB1) (Heat shock 27 kDa protein) (HSP 27)(Stress-responsive protein 27) (SRP27) (Estrogen-regulated 24 kDaprotein) (28 kDa heat shock protein).	HSPB1_HUMAN	115	6.10E-07	38	10	22427	7.83
459	Heat shock cognate 71 kDa protein (Heat shock 70 kDa protein 8).	HSP7C_HUMAN	107	3.10E-07	18	12	71082	5.37
460	Myosin regulatory light chain 2, skeletal muscle isoform (Fastskeletal myosin light chain 2) (MLC2B).	MLRS_HUMAN	78	0.0033	47	9	19116	4.91
462	Triosephosphate isomerase (EC 5.3.1.1) (TIM) (Triose-phosphateisomerase).	TPIS_HUMAN	164	7.60E-12	50	13	26807	6.51
464	Pyruvate kinase isozymes M1/M2 (EC 2.7.1.40) (Pyruvate kinase muscleisozyme) (Pyruvate kinase 2/3) (Cytosolic thyroid hormone-bindingprotein) (CTHBP) (THBP1).	KPYM_HUMAN	94	7.10E-05	17	10	58538	7.6
465	Serum albumin precursor.	ALBU_HUMAN	98	3.00E-06	19	12	71317	5.92
466	Pyruvate kinase isozymes M1/M2 (EC 2.7.1.40) (Pyruvate kinase muscleisozyme) (Pyruvate kinase 2/3) (Cytosolic thyroid hormone-bindingprotein) (CTHBP) (THBP1).	KPYM_HUMAN	132	1.20E-08	19	15	43302	6.77
468	Creatine kinase M-type (EC 2.7.3.2) (Creatine kinase M chain) (M-CK).	KCRM_HUMAN	80	0.00017	21	10	43302	6.77
472	Serum albumin precursor.	ALBU_HUMAN	69	2.50E-02	13	8	71317	5.92

474	Beta-enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase)(Muscle-specific enolase) (MSE) (Skeletal muscle enolase) (Enolase 3).	ENOB_HUMAN	67	0.042	15	9	47285	7.59
475	Tropomyosin beta chain (Tropomyosin-2) (Beta-tropomyosin).	TPM2_HUMAN	71	0.014	28	9	32851	4.66
477	Alpha-crystallin B chain (Alpha(B)-crystallin) (Rosenthal fibercomponent) (Heat shock protein beta-5) (HspB5) (Renal carcinomaantigen NY-REN-27).	CRYAB_HUMAN	64	6.70E-03	42	7	48083	4.95
479	ATP synthase subunit beta (EC 3.6.3.14) (Fragment).Homo sapiens (Human).	Q0QEN7_HUMAN	83	1.10E-03	21	8	26807	6.51
482	Triosephosphate isomerase (EC 5.3.1.1) (TIM) (Triose-phosphateisomerase).	TPIS_HUMAN	113	9.6-e007	36	11	29707	6.94
484	Carbonic anhydrase 7 (EC 4.2.1.1) (Carbonic anhydrase VII) (Carbonatedehydratase VII) (CA-VII).	CAH7_HUMAN	67	0.036	24	7	29707	6.94
485	Carbonic anhydrase 7 (EC 4.2.1.1) (Carbonic anhydrase VII) (Carbonatedehydratase VII) (CA-VII).	CAH7_HUMAN	154	7.60E-11	59	12	26939	6.92
486	Peroxiredoxin-2 (EC 1.11.1.15) (Thioredoxin peroxidase 1)(Thioredoxin-dependent peroxide reductase 1) (Thiol-specificantioxidant protein) (TSA) (PRP) (Natural killer cell-enhancing factorB) (NKEF-B).	PRDX2_HUMAN	55	0.047	25	5	22049	5.66
487	Tropomyosin alpha-3 chain (Tropomyosin-3) (Tropomyosin gamma) (hTM5).	TPM3_HUMAN	71	0.015	33	9	42381	7.36
491	Creatine kinase M-type (EC 2.7.3.2) (Creatine kinase M chain) (M-CK).	KCRM_HUMAN	118	3.00E-07	30	13	43302	6.77
493	Heat shock 70 kDa protein 1 (HSP70.1) (HSP70-1/HSP70-2).	HSP71_HUMAN	132	9.90E-10	21	13	70280	5.48
501	Desmin.	DESM_HUMAN	61	0.012	21	8	53772	5.21
502	Heat shock protein beta-1 (HspB1) (Heat shock 27 kDa protein) (HSP 27)(Stress-responsive protein 27) (SRP27) (Estrogen-regulated 24 kDaprotein) (28 kDa heat shock protein).	HSPB1_HUMAN	164	3.40E-06	38	7	33005	5.86

504	Beta-enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase)(Muscle-specific enolase) (MSE) (Skeletal muscle enolase) (Enolase 3).	ENOB_HUMAN	67	0.042	15	9	47285	7.59
506	Troponin T, slow skeletal muscle (TnTs) (Slow skeletal muscle troponinT) (sTnT).	TNNT1_HUMAN	87	2.90E-05	29	10	32985	5.86
507	Heat shock cognate 71 kDa protein (Heat shock 70 kDa protein 8).	HSP7C_HUMAN	155	5.00E-12	24	16	71082	5.37
508	Beta-enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase)(Muscle-specific enolase) (MSE) (Skeletal muscle enolase) (Enolase 3).	ENOB_HUMAN	67	0.042	15	9	26807	6.51
510	Triosephosphate isomerase (EC 5.3.1.1) (TIM) (Triose-phosphateisomerase).	TPIS_HUMAN	93	9.60E-05	36	9	20146	6.76
511	Alpha beta crystallin Homo sapiens (Human).	CRYAB_HUMAN	70	0.0015	27	7	48083	4.95
513	Desmin.	DESM_HUMAN	98	2.30E-06	25	12	53372	5.21
514	ATP synthase subunit beta, mitochondrial precursor (EC 3.6.3.14).	ATPB_HUMAN	116	4.80E-07	30	13	43302	6.77
518	Creatine kinase M-type (EC 2.7.3.2) (Creatine kinase M chain) (M-CK).	KCRM_HUMAN	213	7.90E-18	46	16	43302	6.77
521	Creatine kinase M-type (EC 2.7.3.2) (Creatine kinase M chain) (M-CK).	KCRM_HUMAN	144	6.30E-11	35	13	43302	6.77
523	Troponin T, fast skeletal muscle (TnTf) (Fast skeletal muscle troponinT) (fTnT) (Beta TnTF).	TNNT3_HUMAN	109	1.50E-04	8	2	31824	5.71
526	Myosin light polypeptide 3 (Myosin light chain 1, slow-twitch muscleB/ventricular isoform) (MLC1SB) (Ventricular/slow twitch myosin alkalilight chain) (Cardiac myosin light chain-1) (CMLC1).	MYL3_HUMAN	67	0.041	35	6	22117	5.03
531	Alpha-crystallin B chain (Alpha(B)-crystallin) (Rosenthal fibercomponent) (Heat shock protein beta-5) (HspB5) (Renal carcinomaantigen NY-REN-27).	CRYAB_HUMAN	167	3.80E-12	53	14	53580	5.62
536	Heat shock 70kDa protein 8 isoform 2 variant (Fragment).Homo sapiens (Human).	Q53HF2_HUMAN	131	1.50E-05	33	14	42366	5.23

537	Actin, alpha skeletal muscle (Alpha-actin-1).	ACTS_HUMAN	124	7.60E-08	37	12	42366	5.23
538	Desmin.	DESM_HUMAN	152	9.90E-12	33	14	53544	5.21
539	Desmin.	DESM_HUMAN	101	1.50E-05	26	12	53544	5.21
540	Heat shock 70kDa protein 8 isoform 1 variant (Fragment).Homo sapiens (Human).	Q53GZ6_HUMAN	112	1.20E-06	20	13	43302	6.77
541	Creatine kinase M-type (EC 2.7.3.2) (Creatine kinase M chain) (M-CK).	KCRM_HUMAN	152	1.20E-10	48	14	42381	5.23
542	Actin, aortic smooth muscle (Alpha-actin-2) (Cell growth-inhibitinggene 46 protein).	ACTA_HUMAN	56	0.035	17	9	41748	5.24
547	Triosephosphate isomerase (EC 5.3.1.1) (TIM) (Triose-phosphateisomerase).	TPIS_HUMAN	226	4.80E-18	67	16	42019	6.51
549	Beta-enolase (EC 4.2.1.11) (2-phospho-D-glycerate hydro-lyase)(Muscle-specific enolase) (MSE) (Skeletal muscle enolase) (Enolase 3).	ENOB_HUMAN	73	9.20E-03	24	10	42334	5.23
550	Actin, alpha cardiac muscle 1 (Alpha-cardiac actin).	ACTC_HUMAN	149	2.40E-10	33	15	41757	5.23
551	Actin, alpha cardiac muscle 1 (Alpha-cardiac actin).	ACTC_HUMAN	126	4.80E-08	29	13	42019	5.23
551	Adenylate kinase isoenzyme 1 (EC 2.7.4.3) (ATP-AMP transphosphorylase) (AK1) (Myokinase)	KAD1_HUMAN	70	1.70E-03	32	8	21819	8.73
556	Myosin regulatory light chain 2, ventricular/cardiac muscle isoform(MLC-2) (MLC-2v).	MLRV_HUMAN	168	2.50E-13	71	14	18777	4.92
559	Heat shock protein beta-1 (HspB1) (Heat shock 27 kDa protein) (HSP 27)(Stress-responsive protein 27) (SRP27) (Estrogen-regulated 24 kDaprotein) (28 kDa heat shock protein).	HSPB1_HUMAN	115	1.60E-07	33	7	22826	5.98
570	Actin, aortic smooth muscle (Alpha-actin-2) (Cell growth-inhibitinggene 46 protein).	ACTA_HUMAN	50	0.097	7	2	42009	5.24
576	Actin, alpha skeletal muscle (Alpha-actin-1).	ACTS_HUMAN	99	2.40E-05	30	11	42366	5.23

588	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial precursor(EC 1.6.5.3) (EC 1.6.99.3) (Complex I-75kD) (CI-75kD).	NDUS1_HUMAN	108	2.50E-07	21	12	47285	7.59
589	Serum albumin precursor.	ALBU_HUMAN	66	4.30E-02	19	9	71317	5.92
613	Alpha-actinin-2 (Alpha-actinin skeletal muscle isoform 2) (F-actincross-linking protein).	ACTN2_HUMAN	85	5.80E-04	13	13	86113	5.31
614	Aconitate hydratase, mitochondrial precursor (EC 4.2.1.3) (Citratehydro-lyase) (Aconitase).	ACON_HUMAN	114	7.60E-07	16	14	86113	7.36
619	Glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) (GAPDH).	G3P_HUMAN	81	0.0014	24	9	36201	8.57
625	Myoglobin.	MYG_HUMAN	125	6.10E-08	64	12	17099	7.29
641	Adenylate kinase isoenzyme 1 (EC 2.7.4.3) (ATP-AMP transphosphorylase) (AK1) (Myokinase)	KAD1_HUMAN	65	0.051	43	7	21819	8.73

MS/MS Analysis.

Reference number	Description	Swiss-Prot ID	Score (<29 sig)	Expect	% sequence coverage	Mr	pI	Observed m/z	Residues	Sequence
6	Troponin T, fast skeletal muscle (TnTf) (Fast skeletal muscle troponinT) (fTnT) (Beta TnTF).	TNNT3_HUMAN	47	0.00076	5	31824	5.71	1633.8083	59-72	K.IPEGEKVDFDDIQK.K
8	Troponin T, slow skeletal muscle (TnTs) (Slow skeletal muscle troponinT) (sTnT).	TNNT1_HUMAN	30	0.032	4	33005	5.86	1184.96	249-257	K.YEINVLYNR.I
20	Troponin T, slow skeletal muscle (TnTs) (Slow skeletal muscle troponinT) (sTnT).	TNNT1_HUMAN	48	0.00063	3	33005	5.86	1184.24	249-257	K.YEINVLYNR.I
329	Troponin T, slow skeletal muscle (TnTs) (Slow skeletal muscle troponinT) (sTnT).	TNNT1_HUMAN	44	1.30E-03	3	33005	5.86	1184.28	249-257	K.YEINVLYNR.I
553	Troponin T, fast skeletal muscle (TnTf) (Fast skeletal muscle troponinT) (fTnT) (Beta TnTF).	TNNT3_HUMAN	33	0.017	5	31824	5.71	1633.9471	59-72	K.IPEGEKVDFDDIQK.K
621	Troponin T, fast skeletal muscle (TnTf) (Fast skeletal muscle troponinT) (fTnT) (Beta TnTF).	TNNT3_HUMAN	59	4.10E-05	5	31824	5.71	1633.93	48-61	K.IPEGEKVDFDDIQK.K