The impact of endurance exercise intensity on local and systemic hormonal and cytokine responses in the recreationally active young and old

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Abstract

Propelled by the significant socio-economic issues presented by obesity and an ageing population, research has identified links between physical activity, metabolism and disease, and quality of life. Our overall aim was to implement an empirically informed, palatable acute endurance exercise intervention that elicits beneficial hormonal responses with the potential for improved health/quality of life across the lifespan. Two groups of similar male participants (Study 1: n = 6; age, 28 ± 5 yrs.; BMI, 25 ± 4 kg/m^2 - Study 2: n = 7; age, 26 ± 7 yrs.; BMI, 25 ± 4 kg/m^2) performed equal work (varying duration) moderate (80 % GET) (M) and heavy (30 % Δ) (H) (Study 1), and H and very heavy (60 % Δ) (VH) (Study 2) acute constant work-load cycle ergometer exercise trials, respectively (Chapter 3). Analysis of bioptic material indicated increased mRNA (GAPDH normalised) in skeletal muscle for IL-6: baseline (B) *vs*. M (P = 0.006), H (P < 0.001) and VH (P < 0.001), and M *vs*. VH (P = 0.02); TNFα: B *vs*. VH (P = 0.04) and SOCS3 B *vs*. M (P = 0.02) and VH (P = 0.04). Exercise was without effect in subcutaneous adipose tissue (Chapter 4). The systemic concentrations of IL-6 increased and remained elevated for 24 hrs. in response to exercise. The increase was greatest following M (P = 0.001). IGF-I and cortisol concentrations declined by 60 min post-exercise (P = 0.001 and P = 0.04, respectively) (M, H and VH). GH increased to peak at the end of exercise (P < 0.001) (M, H and VH) (Chapter 5). To investigate the effect of age, groups of male participants (Study 3: 20 - 30 yrs., n = 8; 30 - 40 yrs., n = 10; 40 - 50 yrs., n = 8; 50 - 60 yrs., n = 8) performed a similar 30 min bout of heavy (30 % Δ) domain exercise (Chapter 6). The systemic concentrations of IL-6 displayed a bi-phasic profile in all groups. IL-6 increased during exercise at 10 min and then 60 min post-exercise (P < 0.001). Insulin and leptin declined during exercise in all groups (P < 0.001 and P = 0.002, respectively). Adiponectin was unchanged. GH increased similarly in all groups to peak again at the end of exercise (P < 0.001). IGF-I was unchanged. Concentrations were consistently higher in the 20 – 30 group however (P = 0.001). Cortisol declined similarly post-exercise in all groups (P < 0.001). We suggest that the oxygen uptake dynamics approach used here should be used when investigating physiological phenomenon potentially sensitive to skeletal muscle metabolic threshold events. We conclude that the exercise-induced hormone and cytokine responses studied in recreationally active health males between 20 and 60 yrs. age most likely reflects the requirements of metabolism. Further work should assess the effectiveness of this modified approach against resistance exercise in a training study format.

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If it is true that something easily acquired is not really worth having then right now I feel that this document is probably one of the most valuable items I possess. The ‘journey’ has been long and hard, but ultimately rewarding.

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Authors declaration

I declare that the work in this thesis was carried out in accordance with the regulations of Manchester Metropolitan University. Apart from the help and advice acknowledged, the work within was solely completed and carried out by the author.

Any views expressed in this thesis are those of the author and in no way represent those of Manchester Metropolitan University.

This thesis has not been presented to any other university for examination either in the United Kingdom or overseas. No portion of the work referred to in this research project has been submitted in support of an application for another degree or qualification of this or any other university or institute of learning.

Signed:…… *Dated: ……25th February 2015……*

Dedication

Dear Amy, 2015 is going to be a wonderful year.

Publications and presentations

IRM Symposium Presentation: Stewart, C. E., Saini, A., Sharples, A. P., Moss, A. D., Dimchev, G., Durcan, P., Faulkner, S. H., and Al-Shanti, N. (2010). The Interaction of Growth Factors and Cytokines in the Regulation of Muscle Growth and Atrophy.

Contents

Glossary of abbreviations

5-HT	5-hydroxytryptamine receptors, *alternatively* serotonin receptors
°C	Degrees Celsius
Δ	Delta, e.g. 30 % Δ (30 % of the workload variation between the GET and $\dot{V}O_2$max).
ΔΔ	Delta delta, e.g. ΔΔC(t) (Livak Method for the determination of normalised gene expression)
>	Greater than
<	Less than
$\dot{V}O_2$max	Maximum rate of oxygen uptake (ml/kg/min [relative] or ml/min [absolute])
µIU/ml	Micro-international unit per millilitre
µl	Microlitres
$\dot{V}O_2$peak	Peak rate of oxygen uptake (ml/kg/min [relative] or ml/min [absolute])
% HRmax	Percentage of heart rate at $\dot{V}O_2$max (%)
% $\dot{V}O_2$max	Percentage of the maximum rate of oxygen uptake (%)
% $\dot{V}O_2$peak	Percentage of the peak rate of oxygen uptake (%)
$\dot{V}CO_2$	Rate of carbon dioxide production (ml/min)
$\dot{V}O_2$	Rate of oxygen uptake (ml/min)
$\dot{V}O_2$/HR	Rate of oxygen uptake divided by heart rate (ml/b/min)
$\dot{V}_E$	Ventilatory equivalent, e.g. $\dot{V}_E/\dot{V}O_2$ *or* $\dot{V}_E/\dot{V}CO_2$
α	Alpha
β	Beta
ACC	Acetyl CoA carboxylase
ACL	ATP-citrate lyase
ACSM	American College of Sports Medicine
ACTH	Adrenocorticotropic hormone, *alternatively* Corticotropin
ADAM17	A disintegrin and metalloprotease 17
AdipoR1	Adiponectin receptor 1
AdipoR2	Adiponectin receptor 2
AHA	American Heart Association

ALS	Acid-labile subunit
ANOVA	Analysis of variance; statistical test between population means
AMPK	Adenosine monophosphate-activated protein kinase
AP-1	Activator protein-1
ATBF	Adipose tissue blood flow
ATh	Anaerobic threshold
AVP	Arginine vasopressin, *alternatively* Vasopressin
B	Baseline
b/min	Beats per minute
BASES	The British Association of Sport and Exercise Sciences
BBB	Blood-brain-barrier
BDNF	Brain-derived neurotrophic factor
BGlu	Blood glucose (mmol/l)
BLa	Blood lactate (mmol/l)
BMI	Body mass index (kg/m^2)
BST	Bed nucleus of the stria terminalis
C(t)	Cycle number at which fluorescence yield crosses the threshold line
Ca^{2+}	Calcium ion, positively charged resulting from the loss of 2 electrons
cAMP	Cyclic adenosine monophosphate, *alternatively* cyclic AMP or 3'-5'-cyclic adenosine monophosphate
cm	Centimetres
CNS	Central nervous system
CO_2	Carbon dioxide
CRH	Hypothalamic corticotropic-releaseing hormone
CRP	C-reactive protein
CSA	Cyclosporin A
CUL-5	Cullin-5
CVD	Cardio-vascular disease
CXCL1	C-X-C motif ligand 1
DEXA	Dual-energy X-ray absorptiometry
DNA	Deoxyribonucleic acid
ECC	Excitation-contraction coupling
EIA	Enzyme immunoassay

ELISA	Enzyme-linked immunosorbent assay
ERK1/2	Extracellular signal-regulated protein kinase
EPS	Electrical pulse stimulation
EWGSOP	European working group on sarcopenia in older people
FGF-2	Basic fibroblast growth factor
FGF-21	Fibroblast growth factor 21
FOXO	Forkhead box
g	Gravity
G6Pase	Glucose-6-phosphatase
GABA	Gamma-aminobutyric acid
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
G-CSF	Granulocyte-colony stimulation factor
GET	Gas exchange threshold
GH	Growth hormone
GH/IGF-I	Growth hormone/Insulin-like growth factor-I axis
GLUT4	Glucose transporter type 4
GLP-1	Glucagon-like peptide-1
GP	Glucose production
GP130	Glycoprotein 130, *alternatively* interleukin-6 receptor-beta (IL-6Rβ)
GP130Rβ	Glycoprotein 130 receptor-beta
GRB2	Growth factor receptor-bound protein 2
GS	Glycogen synthase
GSK3	Glycogen synthase kinase 3
GU	Glucose utilisation
H^+	Hydrogen ions
H_2O_2	Hydrogen peroxide
H	Heavy domain exercise
HPA	Hypothalamic-pituitary-adrenal axis
HR	Heart rate (b/min)
HRmax	Heart rate at $\dot{V}O_2$max (b/min)
hr. or hrs.	Hour or hours
HSL	Hormone sensitive lipase
IGFBP-1	Insulin-like growth factor binding protein-1
IGFBP-3	Insulin-like growth factor binding protein-3

IGF-I	Insulin-like growth factor-I, *alternatively* Somatomedin C
IGF-II	Insulin-like growth factor-II
IGF-IR	Insulin-like growth factor-I receptor
IGFs	Insulin-like growth factors
IKK	IKappa-B kinase kinase complex
IL-1	Interleukin-1
IL-1α	Interleukin-1-alpha
IL-1β	Interleukin-1-beta
IL-1ra	Interleukin-1 receptor antagonist
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-6Rα	Interleukin-6 receptor-alpha
IL-7	Interleukin-7
IL-8	Interleukin-8
IL-10	Interleukin-10
IL-12R	Interleukin-12 receptor
IL-15	Interleukin-15
IP3	Inositol trisphosphate
IR	Insulin receptor
IRM	Institute for Biomedical Research into Human Movement and Health
IRS	Insulin receptor substrate
IRS1	Insulin receptor substrate-1
IRS2	Insulin receptor substrate-2
IRS3	Insulin receptor substrate-3
IRS4	Insulin receptor substrate-4
IU/ml	International unit per millilitre
J	Joules
JAK	Janus kinase
JAK2	Janus kinase 2
JNK	c-Jun NH_2-terminal kinase
kDa	Kilodalton
kg	Kilogram
kg/m^2	Kilogram per metre squared

l	Litres
LepRb	Long isoform of the leptin receptor
LIF	Leukemia inhibitory factor
LTh	Lactate threshold
m	Metres
M	Moderate domain exercise
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemoattractant protein-1 (*mouse*) or chemokine ligand 2 (CCL2) (*human*)
MeA	Medial nucleus of the amygdala
MEK1/2	Mitogen-activated protein kinase kinase-1/2, *alternatively* MAP2K, MAPKK
MePO	Median preoptic nucleus
mg	Milligrams
min	Minutes
MIP-1	Macrophage inflammatory protein-1
MIP-1α	Macrophage inflammatory protein-1-alpha
MIP-1β	Macrophage inflammatory protein-1-beta
ml	Millilitres
ml/kg	Millilitres per kilogram
ml/kg/min	Millilitres per kilogram per minute
ml/min	Millilitres per minute
MLSS	Maximal lactate steady state
mM	Millimolar
mmol/l	Millimoles per litre
MMU	Manchester Metropolitan University
MRI	Magnetic resonance imaging
mRNA	Messenger ribonucleic acid
mTOR	Mechanistic, *alternatively*, mammalian target of rapamycin
MuSCs	Muscle satellite cells
n	Number of participants
NCBI	National Center for Biotechnology Information
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells
ng	Nanograms
ng/µl	Nanogram per microlitre

ng/ml	Nanogram per millilitre
nm	Nanometres
NPY	Neuropeptide Y
NTS	Nucleus tractus solitarius
O_2	Oxygen
OVLT	Organum vasculosum of the lamina terminalis, *alternatively* supraoptic crest
P	Expression of statistical significance (probability), e.g. $P < 0.05$
PDH	Pyruvate dehydrogenase
PDK1	Pyruvate dehydrogenase kinase 1
pg/ml	Picogram per millilitre
PGC-1α	Peroxisome proliferator-activated receptor γ coactivator-1α
pH	Logarithm of the molecular concentration of hydrogen ions
PDE3B	Phosphodiesterase 3B
PI3K	Phosphatidylinositol 3-kinase
PKA	Protein kinase A, *alternatively* cAMP-dependent protein kinase
PKB	Protein kinase B, *alternatively* Akt (Ak = *alphabetical classification*, t = thymoma
PKC-λ/ζ	Atypical protein kinase C isoforms λ and ζ
P-MLSS	Power output at maximal lactate steady state (Watts)
PO	Power output (Watts)
POmax	Power output at $\dot{V}O_2$max (Watts)
POMC	Pro-opiomelanocortin
PP2C	Protein phosphatase 2C
PPARα	Peroxisome proliferator-activated receptor-alpha
r	Pearson product-moment correlation coefficient, e.g. $r = 0.514$
RAS	Rat sarcoma
RAF	Rapidly accelerated fibrosarcoma, *alternatively* Proto-oncogene c-RAF, or RAF-1
RER	Respiratory exchange ratio
rhGH	Recombinant growth hormone
RM	Repetition maximum

RNA	Ribonucleic acid
RPE	Rating of perceived exertion
RPM	Revolutions per minute
RTK	Receptor tyrosine kinase
RT-PCR	Reverse transcription-polymerase chain reaction
s	Seconds
SD	Standard deviation
SEM	Standard error of the mean
SOCS	Suppressor of cytokine signalling
SOCS3	Suppressor of cytokine signaling 3
SOS	Son of Sevenless
STAT	Signal transducer and activator of transcription
STAT3	Signal transducer and activator of transcription 3
sTNFrs	Soluble tumour necrosis factor receptors
sTNF-r1	Soluble tumour necrosis factor-receptor 1
sTNF-r2	Soluble tumour necrosis factor-receptor 2
T2DM	Type 2 diabetes mellitus, *alternatively* non-insulin-dependent diabetes mellitus (NIDDM)
T3/T4	Triiodothyronine/Thyroxine, *Thyroid hormones*
TAG	Triacylglyceride(s)
TNFα	Tumour necrosis factor-alpha
TRIM72	Homo sapiens tripartite motif-containing 72, *alternatively* Mitsugumin 53 (MG53)
UCP-1	Uncoupling protein-1
VH	Very heavy domain exercise
vs.	Versus
W	Watts
W/min	Watts per minute
WAT	White adipose tissue
yr. or yrs.	Year or years

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Chapter 1. Literature review

1.1. Age-related skeletal muscle functional decline

The increase in the prevalence of skeletal muscle functional decline with age and the globally increasing numbers of the 'oldest old' is of major socio-economic significance (World Health Organization, 2012). In the United Kingdom alone, the number of people aged 85 and over doubled from 0.7 million to over 1.4 million between 1985 and 2010 and this number is projected to increase to 3.5 million by 2035 (Office for National Statistics, 2012). For this reason there has been a concerted drive to understand the mechanisms behind the skeletal muscle functional decline that occurs with increasing age (Mitchell et al., 2012). Of particular importance are the mechanisms governing the loss of skeletal muscle strength 'Dynapenia' (Clark and Manini, 2008; 2010; 2012) and the contributions of age-related reductions in skeletal muscle mass 'Sarcopenia' (Rosenberg, 1989; 1997), declining levels of physical activity (Amati et al., 2009; Cobley et al., 2015; Wullemset al., 2016), increasing adipose tissue mass (obesity) (Visser, 2011) and inflammation (Franceschi and Campisi, 2014). Nair (2005) suggested that the reduction in skeletal muscle mass/strength, in combination with reduced endurance, is responsible for reduced voluntary physical activity levels in ageing individuals. The result is a reduction in total energy expenditure and weight gain through adipose tissue accumulation (Nair, 2005). Increased adipose tissue mass (subcutaneous, visceral and skeletal muscle infiltrated) is a burden to locomotion, potentially reducing voluntary physical activity further, and sustains skeletal muscle functional decline through infiltrating macrophage mediated-release of pro-inflammatory cytokines (such as Tumour necrosis factor-alpha (TNFα), Interleukin-6 (IL-6), Interleukin-1 (IL-1)) and proteins leptin, adiponectin and resistin (Neels and Olefsky, 2006; Tilg and Moschen, 2006; Antuna-Puente et al., 2008). These processes promote a pathological spiral of skeletal muscle functional decline, adipose tissue gain and inflammation (figure 1.1). Given the current increase in the proportion of older individuals on a worldwide scale, and a high incidence of poor quality of life in old age, it is essential to establish whether, with a better understanding of the mechanisms involved, these detrimental changes can be positively influenced.

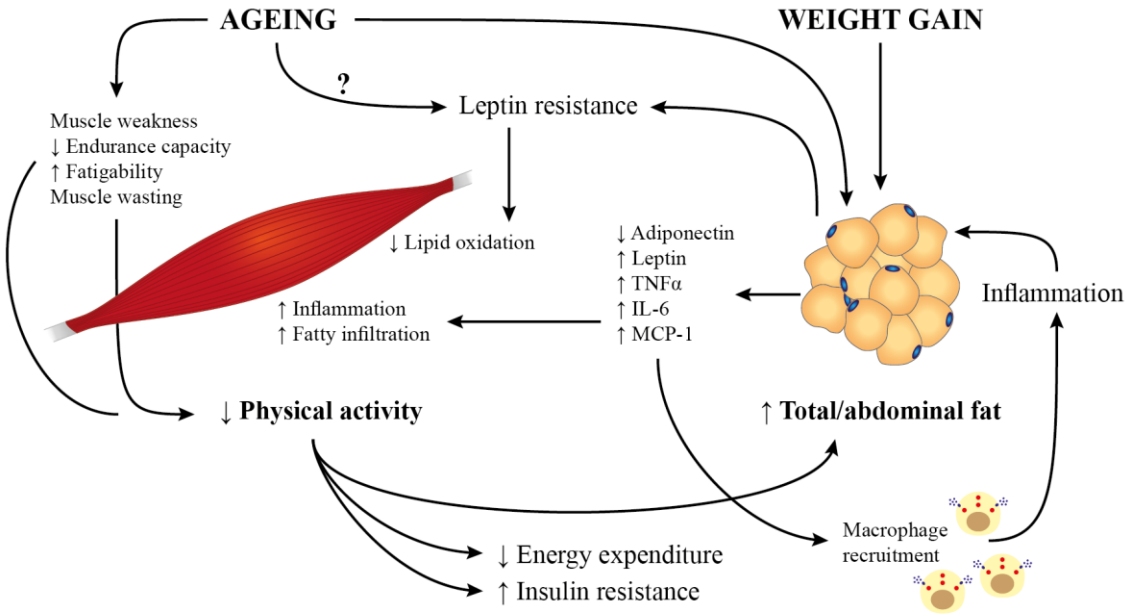


Figure 1.1. Adapted from Zamboni et al., (2008). Inter-relationships between adipose tissue and skeletal muscle with advancing age.

1.1.1. The ageing process: hormone and cytokine adaptations

The natural aging process is accompanied by a decline in endocrine function involving both the responsiveness of tissues and a reduction in the secretion of hormones (Lamberts et al., 1997) such as testosterone, oestrogen, Growth hormone (GH) and Insulin-like growth factor-I (IGF-I) (Baumgartner et al., 1999; Chahal and Drake, 2007; Giovannini et al., 2008). In addition, there maybe increased levels of pro-inflammatory cytokines such as TNFα and IL-6 (Visser et al., 2002). On top of this, modifications in the central mechanisms controlling the temporal organisation of hormone release can result in detrimental rearrangements of circadian hormonal and non-hormonal rhythms (Chahal and Drake, 2007; Aguilera, 2011). An abrupt reduction in oestrogen production (menopause) in women results in an increase in abdominal adipose tissue mass (Cooke and Naaz, 2004) and loss of strength (Lowe et al., 2010). Similar effects are observed in men due to a gradual, but progressive decline in testosterone (andropause) (Gray et al., 1991). In older individuals the amplitude, but not the number of GH pulses per day can

be reduced by as much as 50 % (Veldhuis et al., 2005). This progressive decline in GH secretion (somatopause) has been shown to cause a reduction in protein synthesis, lean body mass, bone mass, and a decline in immune function (Corpas et al., 1993). The considerable influence IGF-I has over skeletal muscle mass through hypertrophy (Phosphatidylinositol 3-kinase (PI3K)/Protein kinase B (PKB)/Mammalian target of rapamycin (mTOR)) and inhibition of atrophy (PI3K/PKB/mTOR/Forkhead box (FOXO)) has been well documented (Latres et al., 2005; Glass, 2010b; Fernandes et al., 2012). The anabolic and lipolytic effects of GH are mediated by circulating or paracrine IGF-I, and IGF-I declines in parallel with GH (Corpas et al., 1993). The Hypothalamic-pituitary-adrenal (HPA) axis and its end product, the pleiotropic glucocorticoid cortisol, mediate the relationship between stressful life experiences and health outcomes (Krøll, 2010; Aguilera, 2011; Lucassen et al., 2013). Data from Van Cauter et al., (1996) suggested daily mean cortisol concentrations increase by 20 – 50 % between the age of 20 and 80 yrs., with the evening nadir in serum cortisol concentrations greater and earlier in older individuals.

Nutrition and anabolism, and the associated physiological adaptations are influenced by a number of hormones including insulin, leptin and adiponectin. These in turn are altered with age. Data from Bryhni et al., (2010) suggest that insulin release is blunted with advancing age, indicated by impaired processing of pro-insulin to insulin, but this is not unequivocal. For instance, a preserved sensitivity to insulin, indicated by a insulin concentrations similar to those in younger adults (Paolisso et al., 1996) is widely acknowledged to be a key factor in the healthy ageing phenotype in centenarians (Arai et al., 2009; van Heemst, 2010). The systemic concentration of leptin declines throughout life independent of Body mass index (BMI) and other endocrine changes (Isidori et al., 2000). Interestingly, women generally have higher systemic leptin concentrations, which decline at a greater rate during ageing, than men. Commonly, a fall in leptin concentration is observed around the time of menopause (Isidori et al., 2000). Conversely, adiponectin concentrations have been shown to increase with age (Adamczak et al., 2005; Isobe et al., 2005; Kizer et al., 2010) but more dramatically in women around menopause (Isobe et al., 2005).

It has been suggested that an increase in the leptin to adiponectin ratio reflects insulin resistance and the risk of vascular disease (Finucane et al., 2009). This is based on the causal relationship between obesity and insulin resistance, and a proportional increase in

systemic leptin concentrations and adipose tissue mass (Lönnqvist et al., 1995), which are associated with a decrease in systemic adiponectin concentrations (Arita et al., 1999). Similarly, a decrease in the ratio of GH to cortisol has been suggested to reflect body compositional changes favouring accumulation of visceral adipose tissue mass in both the young (Misra et al., 2008) and old (Nass and Thorner, 2002). A reduction in lipolytic capacity due to declining systemic GH concentrations is a contributory agent in the age-related increase in adipose tissue mass, as is prolonged exposure to elevated systemic cortisol concentrations (Nass and Thorner, 2002). Given the central role obesity and inflammation play in the pathogenesis of a number of chronic conditions that increase with age, the leptin to adiponectin ratio and GH to cortisol ratio may provide a useful index of age-related dysfunction.

1.1.2. The relationship between Inflammation, inflammageing and increasing adipose tissue mass

Inflammation and the inflammatory response are essential physiological processes, ensuring survival by defending against infection and enabling recovery from injury. The inflammatory response involves a variety of cells, organs and organ systems (Gruys et al., 2005; Serhan et al., 2007). An age-related increase in the production of inflammatory compounds occurs in the immune system (Vescovini et al., 2007; Larbi et al., 2008), brain (Licastro et al., 2003), adipose tissue (Hotamisligil, 2006) and muscle (Greiwe et al., 2001). The up-regulation of the inflammatory state with increasing age has been termed ‘inflammageing’ (Franceschi et al., 2000; Cevenini et al., 2013; Franceschi and Campisi, 2014). The result is a chronic systemic low-grade pro-inflammatory state that appears to be associated with the pathogenesis of cancer (Schottelius and Dinter, 2006; Aggarwal and Gehlot, 2009), the metabolic syndrome (Camera et al., 2008), insulin resistance (Reaven and Chen, 1988; Goodpaster et al., 2000; 2003), Type 2 diabetes mellitus (T2DM) (Pradhan et al., 2001; Festa et al., 2002) and Cardio-vascular disease (CVD) (Libby, 2002; 2006).

Increasing adipose tissue mass is characterised by adipocyte hypertrophy and is often associated with elevated levels of circulating inflammatory markers or chronic inflammation. Increased systemic concentrations of inflammatory markers (leptin, TNFα, IL-6) reflect an ‘overflow’ of these proteins and subsequent health implications (Trayhurn, 2005; Goossens, 2008; Kwon and Pessin, 2013). During adipocyte

hypertrophy the capacity for lipid storage is stretched which may impair further lipid storage, resulting in ectopic fat deposition and insulin resistance as other tissues are exposed to an excessive influx of lipids (Lettner and Roden, 2008). Adipocyte hypertrophy induces relative hypoxia in clusters of adipocytes distant from the capillary network, inducing changes in gene expression with up-regulation of inflammatory proteins, a shift from aerobic to anaerobic metabolism with increased lactate production, and importantly, insulin resistance and the development of adipose tissue fibrosis (Trayhurn, 2013). Adipocyte hypertrophy also induces the expression of chemo-attractant proteins (Cancello et al., 2005) resulting in infiltration of the adipose tissue bed by inflammatory (M1) macrophages (Weisberg et al., 2003; Xu et al., 2003; Lumeng et al., 2007), and a corresponding increase in macrophage secretion of cytokines pro-inflammatory TNFα and IL-6, amongst others, (Manderson et al., 2007) thereby fuelling the inflammatory environment. Pedersen (2009) recently proposed the 'diseasome' of physical inactivity in which chronic diseases including T2DM, CVD and cancer, associated with an enhanced risk of premature morbidity, are linked by visceral and ectopic fat accumulation and therefore systemic and chronic inflammation resulting from physical inactivity.

1.2. Exercise as a therapeutic intervention

Notwithstanding the significant drive to investigate the mechanisms behind the age-related decline in skeletal muscle mass and, more recently, strength, it appears we are still some way from translating research findings into primary care diagnosis and interventions in clinical practice (Sayer, 2010; von Haehling et al., 2010; Sayer, 2014). However, towards this aim, the European working group on sarcopenia in older people (EWGSOP) have produced a diagnostic algorithm based on measurements of performance, skeletal muscle mass and function (Cruz-Jentoft et al., 2010), which has been validated for use in clinical practice (Da Silva Alexandre et al., 2014). From the perspective of interventions, research has shown progressive resistance training is effective in increasing skeletal muscle mass (hypertrophy) and strength in the older individuals (Borst, 2004; Liu and Latham, 2009; Peterson et al., 2010; 2011; Degens, 2012) and has been advocated as an effective intervention for skeletal muscle functional decline by the American College of Sports Medicine (ACSM) (Chodzko-Zajko et al., 2009), American Heart Association (AHA) (Nelson et al., 2007) and others (Marcell, 2003; Abellan van Kan et al., 2009). However, progressive resistance training alone does

not offer a complete solution to address the multifactorial aetiology of skeletal muscle functional decline, especially given the complex influence of co-morbidities such as obesity (Stenholm et al., 2008; Zamboni et al., 2008; Waters and Baumgartner, 2011), or by contrast, the nutritional abnormalities prevalent in older individuals (low calorie and protein intake) (Bartali et al., 2006; Phillips, 2015) against the background of altered hormone and cytokine profiles and low-grade systemic inflammation (inflammageing) (Franceschi et al., 2000; Cevenini et al., 2013; Franceschi and Campisi, 2014) described above. As such, a multimodal lifestyle approach incorporating physical activity (endurance/aerobic and strength/power), nutrition (optimum calorific quantity and nutritional makeup) and key pharmaceutical therapies appears logical (Waters et al., 2010). Evidence suggests that including an endurance exercise component in such an approach might prove effective in reducing visceral and ectopic fat accumulation and therefore may go some way to addressing the systemic and chronic inflammation resulting from physical inactivity. Such an exercise component might also provide a sufficient stimulus for improvement in cardio-respiratory fitness, in addition to minor improvements in or maintenance of skeletal muscle strength and power, potentially promoting functional improvements in the performance of activities of daily living.

Research has identified that skeletal muscle and adipose tissue function as endocrine organs (Trayhurn, 2005; Pedersen and Febbraio, 2008; Pedersen, 2013), collectively secreting hundreds of hormones, cytokines and other proteins that act as chemical messengers governing essential biological processes (Bortoluzzi et al., 2006; Alvarez-Llamas et al., 2007; Lehr et al., 2012). Pedersen and colleagues proposed the term ‘myokines’, for hormones, cytokines and other proteins released by skeletal muscle fibres as a result of contractile activity (Pedersen et al., 2003). Similarly, hormones, cytokines and other proteins secreted by adipose tissue are collectively known as ‘adipokines’ (Trayhurn and Wood, 2004). Recent research suggests that myokines play a role in mediating the beneficial effects of endurance exercise (Pedersen, 2009; Pedersen and Febbraio, 2012). Adipokines, on the other hand, regulate energy metabolism (Yu and Ginsberg, 2005; Ronti et al., 2006), and therefore contribute to inflammatory mechanisms resulting from the storage of excess adipose tissue (Tilg and Moschen, 2006; Arai et al., 2011). A number of proteins such as IL-6 and TNFα are secreted by both skeletal muscle and adipose tissue, and as such have been suggested to be involved in the cross-talk between these tissues, termed ‘the adipose-muscular axis’ (Argilés et al., 2004; Tomas et al., 2004). Myokines and adipokines communicate via autocrine, paracrine (Argilés et al.,

2004; Tomas et al., 2004; Pedersen, 2009) as well as endocrine signalling mechanisms (Pedersen et al., 2003; Calabrò et al., 2009) indicating the complex regulatory system governing adaptations in these tissues. Endurance exercise may be considered as an effective exercise component in a multimodal lifestyle approach addressing age-related skeletal muscle functional decline only if it can be proven that such a component confers positive benefits. It is therefore imperative that we gain a better understanding of how the secretomes of skeletal muscle and adipose tissue respond to this form of exercise, the associated mechanisms, and how systemic concentrations vary as a result.

1.2.1. Skeletal muscle derived 'myokine' interactions

Skeletal muscle primarily functions to contract, which provides stability and facilitates movement, but this tissue also has a major role in glucose metabolism, being responsible for ~ 75 % of whole-body insulin-stimulated glucose uptake (Shulman et al., 1990; Corcoran et al., 2007). Skeletal muscle contractions also stimulate the secretion of myokines. The pleiotropic cytokine IL-6 (Poupart et al., 1987) was the first protein to be designated as a myokine (Pedersen et al., 2003; 2004; 2007) and is the most widely studied. Within 30 min of the onset of endurance exercise the rate of transcription, translation and release of IL-6 from skeletal muscle is dramatically increased, particularly when the exercise is sustained at a high level and muscle glycogen content is reduced (Keller et al., 2001; Steensberg et al., 2002). In skeletal muscle IL-6 acts in an autocrine manner, signalling through Glycoprotein 130 receptor-beta (GP130Rβ)/Interleukin-6 receptor-alpha (IL-6Rα) ('classical' signalling), resulting in activation of the Adenosine monophosphate-activated protein kinase (AMPK) and/or PI3K signalling pathways that result in increased glucose uptake and fat oxidation (Pedersen and Fischer, 2007) (figure 1.2). Interleukin-6 may even have a role in modulating the choice of substrate in skeletal muscle during exercise by altering Pyruvate dehydrogenase (PDH) complex via AMPK (Biensø et al., 2014). Evidence suggests the c-Jun NH_2-terminal kinase (JNK)/Activator protein-1 (AP-1) pathway maybe a primary signalling pathway controlling IL-6 transcription in contracting skeletal muscle (Whitham et al., 2012), and that IL-6 expression depends on an Inositol trisphosphate (IP3)-dependent Calcium (Ca^{2+}) signal, acting as an early step, promoting a positive IL-6 loop via the Janus kinase 2 (JAK2)/Signal transducer and activator of transcription 3 (STAT3) pathway (Bustamante et al., 2014). Paradoxically, IL-6 is also pro-inflammatory. In pro-inflammatory (M1) macrophages within the adipose tissue bed, IL-6 activates IKappa-B kinase kinase

complex (IKK)/Nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB), which leads to the production of inflammatory cytokines, primarily TNFα, but also Interleukin-1-beta (IL-1β) (Weisberg et al., 2003; Xu et al., 2003). Recent evidence suggests IL-6 and TNFα behaviour is mediated by A disintegrin and metalloprotease-17 (ADAM17) (Edwards et al., 2008; Gooz, 2010). Cleavage of the membrane-bound IL-6Rα by ADAM17 constitutes a rapid and irreversible regulatory switch (Düsterhöft et al., 2014) that facilitates IL-6 'trans-signalling' (Rose-John, 2012), a process shown to promote the transition from acute to chronic inflammation (Jones and Rose-John, 2002; Jones et al., 2001; 2005; Rose-John et al., 2006; Rabe et al., 2008a).

Classical IL-6 signalling and activation of JAK2/STAT3 signalling pathway results in the rapid induction of Suppressor of cytokine signalling-3 (SOCS3) (Starr et al., 1997; Hilton, 1999; Babon and Nicola, 2012). Suppressor of cytokine signalling-3 inhibits IL-6 signalling via negative feedback regulation, by high-affinity binding with both JAK and GP130 simultaneously (Babon et al., 2012). The 'SOCS box' domain, present on all Suppressor of cytokine signalling (SOCS) proteins, then facilitates proteasomal degradation of IL-6 through interaction with Cullin-5 (CUL-5) and the formation of an E3 ubiquitin-ligase complex (Starr et al., 1997; Hilton et al., 1998; Babon and Nicola, 2012).

Suppressor of cytokine signalling-3 also regulates the response to cytokines, growth factors, and hormones that signal independently of GP130, namely the Interleukin-12 receptor (IL-12R), Granulocyte-colony stimulation factor (G-CSF), leptin and insulin. SOCS3 is induced by and binds to the leptin receptor, thereby limiting leptin action (Bjørbaek et al., 1998; 1999; 2000; Eyckerman et al., 2000). In obesity, the expression of SOCS3 in the Central nervous system (CNS) is increased. Suppressor of cytokine signalling-3 expression, induced by elevated concentrations of IL-6 and TNFα impairs insulin signalling in peripheral organs by binding to the Insulin receptor (IR) or the Insulin receptor substrate (IRS) proteins Insulin receptor substrate-1 (IRS1) and Insulin receptor substrate-2 (IRS2), leading to their ubiquitination and degradation, or by preventing receptor tyrosine phosphorylation (Rui et al., 2002; Ueki et al., 2004; 2005; Shi et al., 2006).

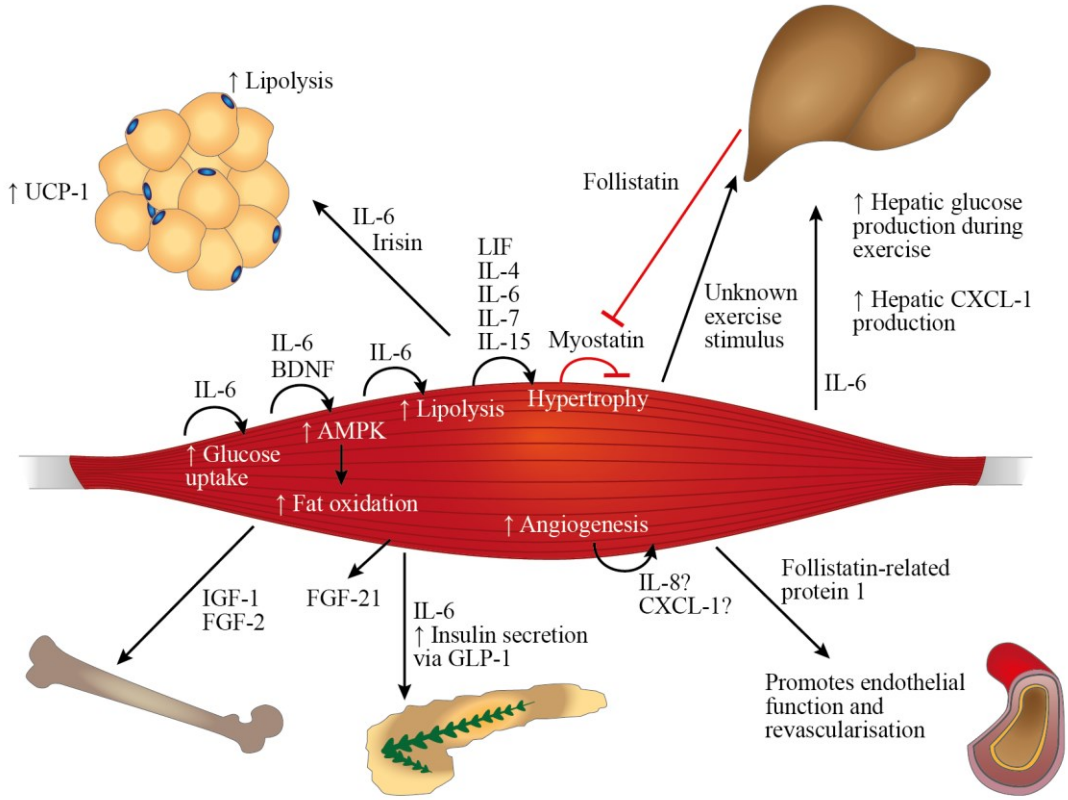


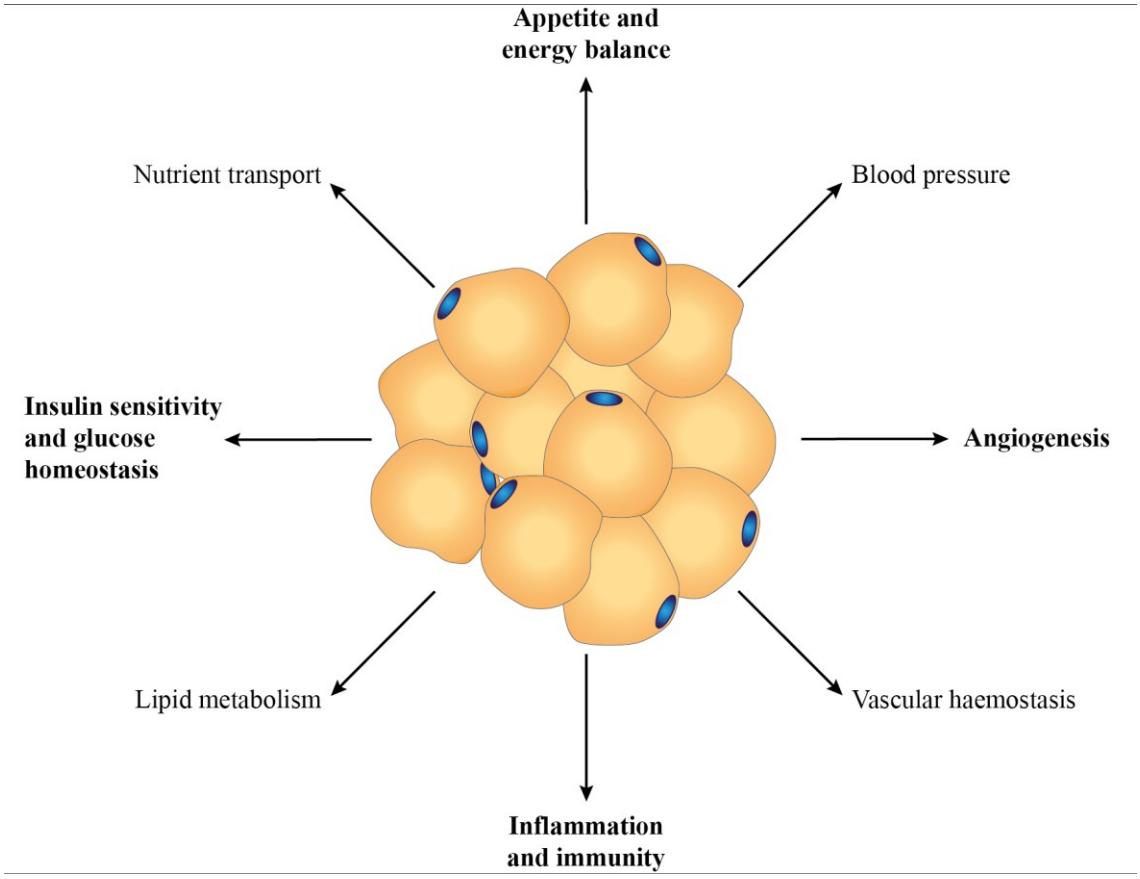
Figure 1.2. Reproduced from Pedersen and Febbraio (2012). Skeletal muscle as an endocrine organ. Interleukin-6 is involved in AMPK-mediated fat oxidation and also enhances insulin-stimulated glucose uptake. Interleukin-6 appears to have systemic effects on the liver and adipose tissue and increases insulin secretion via upregulation of Glucagon-like peptide-1 (GLP-1). Interleukin-6 has been suggested to promote hypertrophy though satellite cell proliferation. Arrows - positive, activating functions. T-lines - inhibitory functions.

1.2.2. Adipose tissue derived 'adipokine' interactions

Adipose tissue insulates the body, stores excess energy in the form of Triacylglyceride (TAG), assists in mediating glucose homeostasis and synthesises and secretes adipokines (figure 1.3). Over 50 adipokines have been identified to date (Trayhurn and Wood, 2004), of which leptin and adiponectin, are the most widely studied. Dysregulation of adipokine actions promote local and systemic inflammation, and co-morbidities including insulin resistance and T2DM (Bays, 2011; Kwon and Pessin, 2013). Mature adipocytes form the major component of adipose tissue, releasing the adipokines leptin and adiponectin (Yadav et al., 2013). The history of leptin can be traced to two murine strains (ob/ob and db/db) discovered in 1950 and 1965 with identical phenotypes including: morbid obesity,

insulin resistance, infertility and lethargy (reviewed by Coleman, 2010). The role of leptin in energy balance/body mass control is mediated by expression of the long isoform of the leptin receptor (Leptin Receptor b (LepRb)) in the hypothalamus (Meister, 2000), and increased leptin is associated with feelings of satiety (Cohen et al., 2001; Villanueva and Myers, 2008). Leptin Messenger ribonucleic acid (mRNA) is expressed in both skeletal muscle (Wang et al., 1998; Guerra et al., 2007) and adipose tissue (Kielar et al., 1998; Lefebvre et al., 1998) and is secreted by adipocytes in proportion to adipose tissue mass, suggesting leptin acts as an 'adipostat', informing the brain of adipose tissue mass, thus regulating energy balance (Zhang et al., 1994; Pelleymounter et al., 1995; Halaas et al., 1995).

Figure 1.3. Adapted from Trayhurn (2013). Major physiological and metabolic processes with which



adipose tissue is involved through the secretion of adipokines from adipocytes.

Leptin binding activates JAK2/STAT3 and PI3K signalling pathways to increase metabolic rate and sympathetic tone and suppress appetite, thereby decreasing body fat mass (Spiegelman and Flier, 2001; Morris and Rui, 2009) (figure 1.4). Adiponectin functions in opposition to leptin, reducing metabolic rate and stimulating appetite

(Kadowaki et al., 2008). Kraus et al., (2002) demonstrated strong differentiation-dependent leptin secretion and PI3K mediated negative autocrine effects on insulin action in highly insulin-responsive SV40T-immortalised brown adipocytes from the FVB strain of mice. Acute leptin treatment of mature adipocytes dose- and time-dependently stimulated phosphorylation of STAT3 and Mitogen-activated protein kinase (MAPK). Acute pretreatment of fully differentiated brown adipocytes with leptin (100 nm) significantly diminished insulin-induced glucose uptake in a time-dependent manner. The response correlated with a ~ 35 % reduction in insulin-stimulated insulin receptor kinase activity, and ~ 60 % and 40 % reductions in insulin-induced IRS1 tyrosine phosphorylation and binding to the regulatory subunit p85 of PI3K, respectively (Kraus et al., 2002). Resistance to the appetite suppressive action of leptin 'leptin resistance' resulting in increased expression of leptin mRNA in adipose tissue (Lönnqvist et al., 1995; Klein et al., 1996) and increased systemic leptin concentrations (Maffei et al., 1995; Considine et al., 1996; Diamond et al., 2004), is a primary risk factor for obesity. Proposed mechanisms for leptin resistance are impaired leptin transport across the Blood-brain-barrier (BBB) (El-Haschimi et al., 2000), hyperleptinaemia-induced SOCS3 (Kievit et al., 2006), defective autophagy (Quan and Lee, 2013) and endoplasmic reticulum stress (Morris and Rui, 2009; Ozcan et al., 2009).

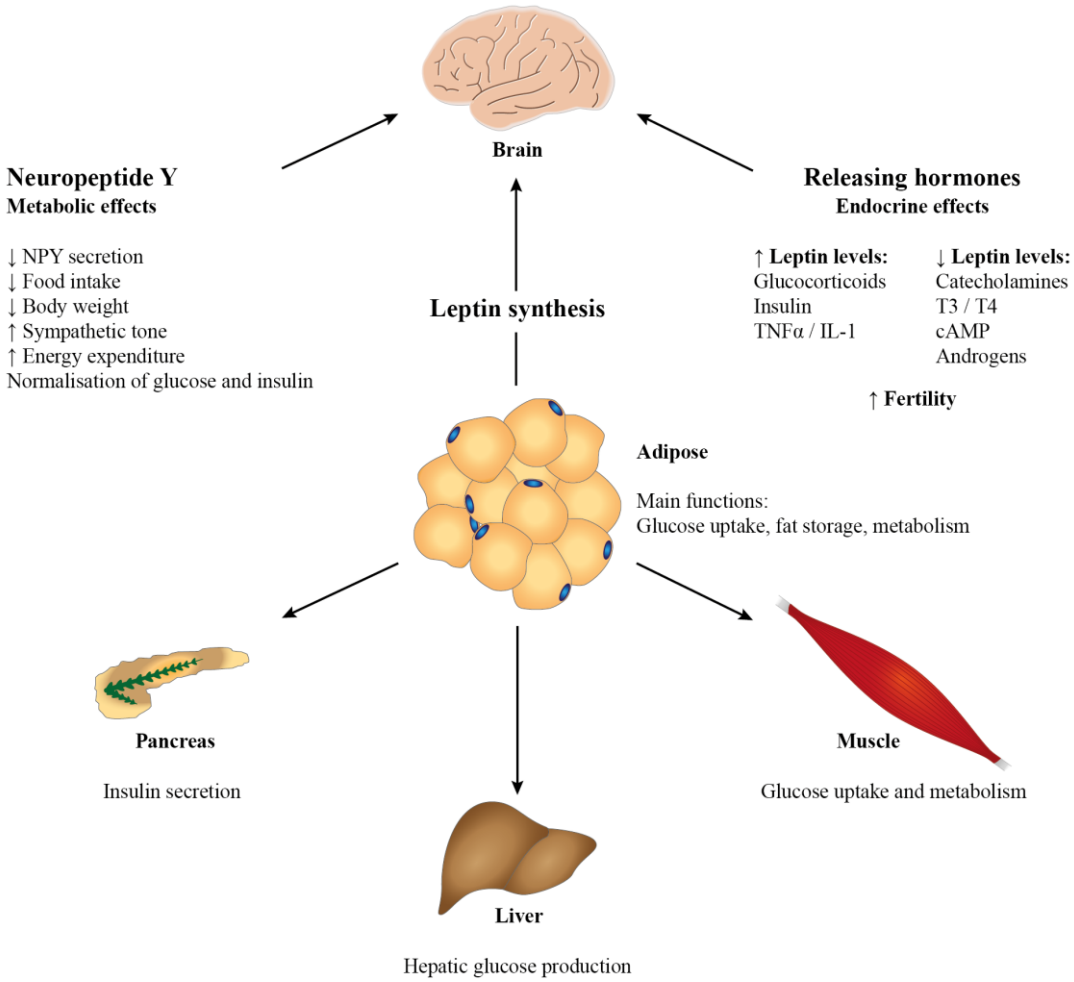


Figure 1.4. Adapted from Meier and Gressner (2004). Action of leptin on the hypothalamus, pancreas, liver and skeletal muscle.

Adiponectin was originally discovered by four distinct groups (Scherer et al., 1995; Hu et al., 1996; Maeda et al., 1996; Nakano et al., 1996). Similar to leptin, the adipostat functions of adiponectin are primarily achieved by regulating neuronal activity in the hypothalamus via binding to the adiponectin receptors (Adiopnectin receptor 1 (AdipoR1) and Adiponectin receptor 2 (AdipoR2)) (Guillod-Maximin et al., 2009). In addition to insulin and IGF-I mediated expression in adipose tissue (Halleux et al., 2001), adiponectin is also expressed in skeletal muscle (Krause et al., 2008). Adiponectin signals through AMPK and Peroxisome proliferator-activated receptor-alpha (PPARα) by binding to AdipoR1 in skeletal muscle and AdipoR2 in the liver (Yamauchi et al., 2003a; Civitarese et al., 2004) (figure 1.5), primarily to improve insulin sensitivity, increase fuel oxidation and reduce TAG content (Yamauchi et al., 2001; 2003b). Adiponectin has been shown to exert significant and diverse autocrine/paracrine effects, including regulating

its own expression (Wu et al., 2003; Fu et al., 2005; Lin and Li, 2012). Adiponectin gene expression in murine 3T3-L1 fibroblasts promotes cell proliferation and differentiation from pre-adipocytes into adipocytes, augments programmed gene expression responsible for adipogenesis, and increases lipid content and insulin responsiveness of the glucose transport system in adipocytes (Fu et al., 2005). Wu et al. (2003) incubated isolated epididymal rat adipocytes with the globular domain of recombinant adiponectin, which increased Thr-172 phosphorylation and catalytic activity of AMPK and enhanced the Ser-79 phosphorylation of Acetyl CoA carboxylase (ACC). Glucose uptake was increased in adipocytes without stimulating tyrosine phosphorylation of the IR or IRS1, and without enhancing phosphorylation of PKB on Ser-473. Further, adiponectin enhanced insulin-stimulated glucose uptake at sub-maximal insulin concentrations and reversed the inhibitory effect of TNFα. These findings suggest normal leptin and adiponectin function is essential in regulating the accumulation of body fat and sensitivity to insulin. Although it is becoming evident that exercise can have a positive effect on lipid metabolism, the impact that it has on the adipokines adiponectin and leptin is less clear. It has been suggested that the positive benefits of exercise on systemic concentrations of both leptin (decrease) and adiponectin (increase) are realised principally through a reduction in adipose tissue mass (Fatouros et al., 2005; Blüher et al., 2006; Simpson and Singh, 2008). This is clearly an avenue worthy of further investigation.

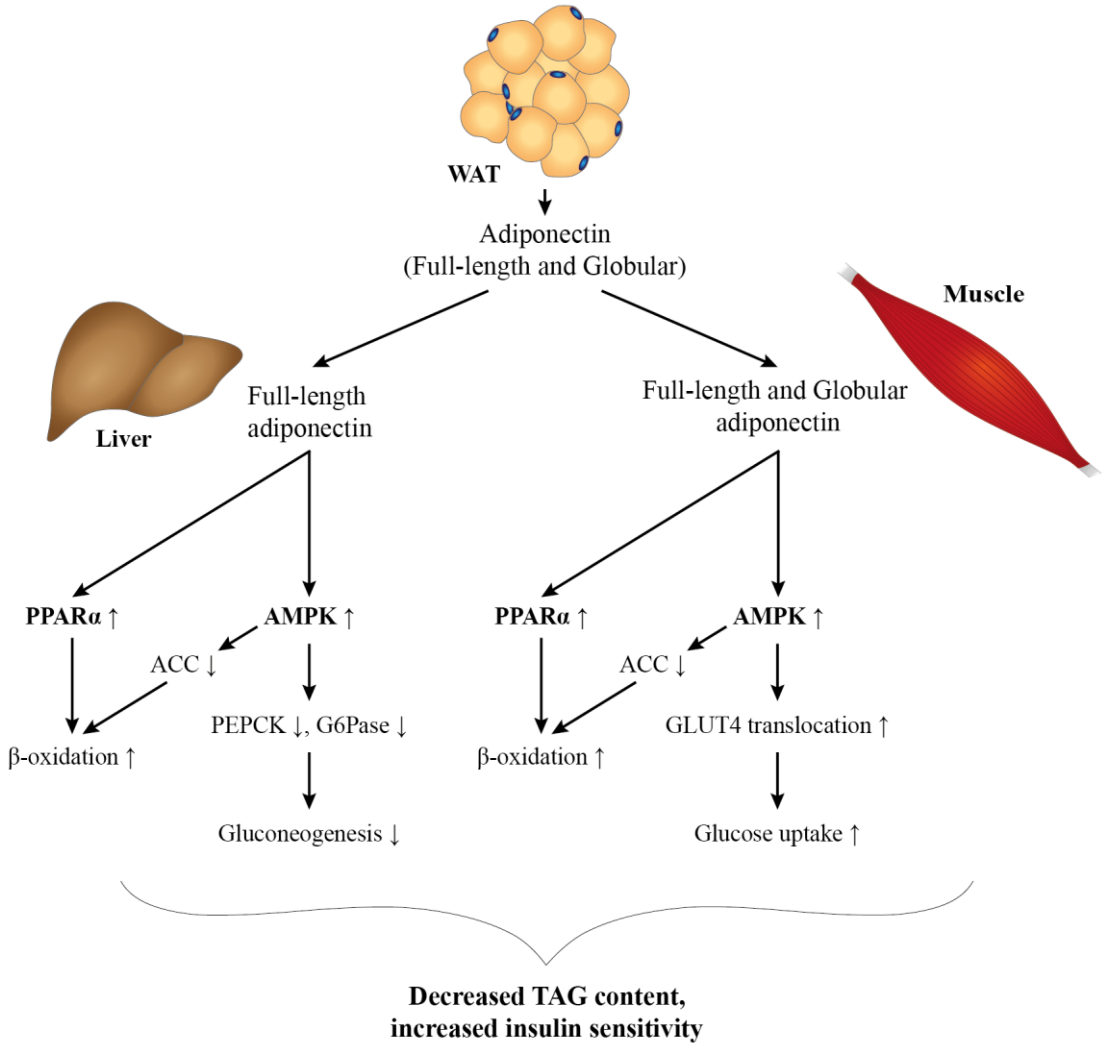


Figure 1.5. Adapted from Kadowaki and Yamauchi (2005). Adiponectin activation of Adenosine monophosphate-activated protein kinase (AMPK) and Peroxisome proliferator-activated receptor-alpha (PPARα) in the liver and skeletal muscle.

1.2.3. The influence of endurance exercise on systemic protein abundance

Ostrowski et al., (1998a; 1998b; 1999; 2001) identified the exercise-induced systemic abundance and skeletal muscle expression kinetics of TNFα, IL-1β, Interleukin-10 (IL-10), IL-6, Interleukin-15 (IL-15), Interleukin-1 receptor-antagonist (IL-1ra), Soluble tumour necrosis factor receptor-1 (sTNFr1), Soluble tumour necrosis factor receptor-2 (sTNFr2), Interleukin-8 (IL-8), Macrophage inflammatory protein-1-alpha (MIP-1α) and Macrophage inflammatory protein-1-beta (MIP-1β) during and after bouts of acute endurance running. The response appeared analogous to the acute phase response to trauma. Research focused on exercise-induced skeletal muscle fibre damage as the stimulus for the response (Pedersen et al., 1998; Croisier et al., 1999; Malm, 2001) and

the possibility that exercise might provide a model with which to study inflammation (Pedersen and Nieman, 1998; Friedland et al., 1992; Shephard, 2001). Further investigation identified skeletal muscle contractile activity as the source of increased systemic concentrations of proteins such as IL-6 (Steensberg et al., 2000). Differences between the cytokine cascade induced by exercise and infection were identified. Typically, exercise does not induce a rise in the systemic concentrations of pro-inflammatory cytokines TNFα and IL-1β (Pedersen and Febbraio, 2008). Interleukin-6 was designated a pleotropic cytokine (Pedersen et al., 2001) with a pro-inflammatory role in disease and an anti-inflammatory role as a myokine induced through exercise (Pedersen et al., 2003; 2004; 2007). Interleukin-6 is thought to be beneficial during endurance exercise, due to its ability to diminish systemic concentrations of TNFα (Starkie et al., 2003) and to increase lipolysis in adipose tissue (van Hall et al., 2003), thus sparing glycogen as an energy source and, over time, potentially reducing inflammation via a reduction in adipose tissue mass. Further, systemic anti-inflammatory effects of IL-6 have been observed (Tilg et al., 1997; Steensberg et al., 2003). Fischer (2006) suggested that a reduction in basal IL-6 is a characteristic of the normal adaptation to training, whereas high basal systemic IL-6 concentrations are closely associated with physical inactivity. Indeed, life-long endurance activity has been shown to be associated with significantly lower basal inflammatory markers C-reactive protein (CRP) and IL-6 (Mikkelsen et al., 2013).

1.2.3.1. Insulin

Insulin was first successfully isolated in 1922 (Banting and Best, 1922) and was the first protein to have its entire primary amino-acid structure sequenced (Sanger, 1949; Ryle et al., 1955). Between 1961 and 1967 significant steps were made in the synthesis of biologically active forms of the protein (Du et al., 1961; Kung et al., 1963; Wang et al., 1965; Katsoyannis, 1966; 1967). The primary sequence of human insulin shares 50 % amino-acid identity in the Alpha (α) and Beta (β) domains with IGF-I and Insulin-like growth factor-II (IGF-II) (Rinderknecht and Humbel, 1976; 1978). Insulin action is initiated by binding to the IR (Kahn, 1985; Freychet et al., 1971; Cuatrecasas, 1972). Both the IR and the IGF-I receptor (IGF-IR) belong to the Receptor tyrosine kinase (RTK) family (Hubbard and Till, 2000). The IR is ubiquitously expressed in human tissues, with the highest expression levels in adipose tissue, liver and skeletal muscle (Kahn et al., 1981).

Insulin is secreted by clusters of pancreatic β-cells located in the islets of Langerhans in response to elevated concentrations of glucose in the blood (Newsholme et al., 2014). Glucose-stimulated insulin secretion is a biphasic response comprising an initial phase which develops rapidly but lasts only a few minutes, followed by a second sustained phase (Luzi and DeFronzo, 1989; Curry et al., 1968; Cerasi and Luft, 1967). The insulin response promotes glucose uptake in skeletal muscle, adipose tissue and the liver, therefore acting towards maintaining a constant concentration in the blood. The binding of insulin with its receptor results in auto-phosphorylation of tyrosine kinase residues (Kasuga et al., 1982) that in turn lead to recruitment and phosphorylation of IRS1, phosphorylation and activation of PI3K, PKB and Atypical protein kinase C isoforms λ and ζ (PKC-λ/ζ), which result in Glucose transporter type 4 (GLUT4) translocation and glucose transport into the cell (Taniguchi et al., 2006; Thirone et al., 2006) (figure 1.6). In adipose tissue, insulin has an anti-lipolytic effect, stimulating free fatty acids uptake and lipogenesis (Duncan et al., 2007). Insulin reduces hepatic glycogenolysis (Wahren and Ekberg, 2007) and promotes amino-acid transport, lipid metabolism, glycogen synthesis, gene transcription and Messenger ribonucleic acid (mRNA) turnover, and protein and Deoxyribonucleic acid (DNA) synthesis in liver and muscle (Cheatham and Kahn, 1995). Insulin resistance is a condition in which skeletal muscle, liver and adipose tissue demonstrate reduced responsiveness to insulin. Insulin resistance is a key factor in the pathogenesis of T2DM and the metabolic syndrome (Facchini et al., 2001). A recent report suggests three distinct mechanisms linking obesity to insulin resistance and a predisposition to T2DM: 1) increased production of adipokines/cytokines and a reduction in adiponectin concentration, 2) ectopic fat deposition in the liver and skeletal muscle, and 3) mitochondrial dysfunction and compromised β-cell function (Eckel et al., 2011).

Systemic concentrations of insulin have been shown to increase following acute bouts of resistance exercise (Kraemer and Castracane, 2014). By contrast, during prolonged endurance exercise, circulating insulin levels decline with time in healthy individuals (Hansen et al., 2012). It has been suggested that the concentration of insulin circulating in the blood is related to the intensity and duration of exercise, determined by the rate of glucose production and utilisation (Sigal et al., 1994; 2000), mediated by the catecholamine response during intense exercise (Marliss et al., 1991; 1992; Sigal et al., 1996; Marliss and Vranic, 2002).

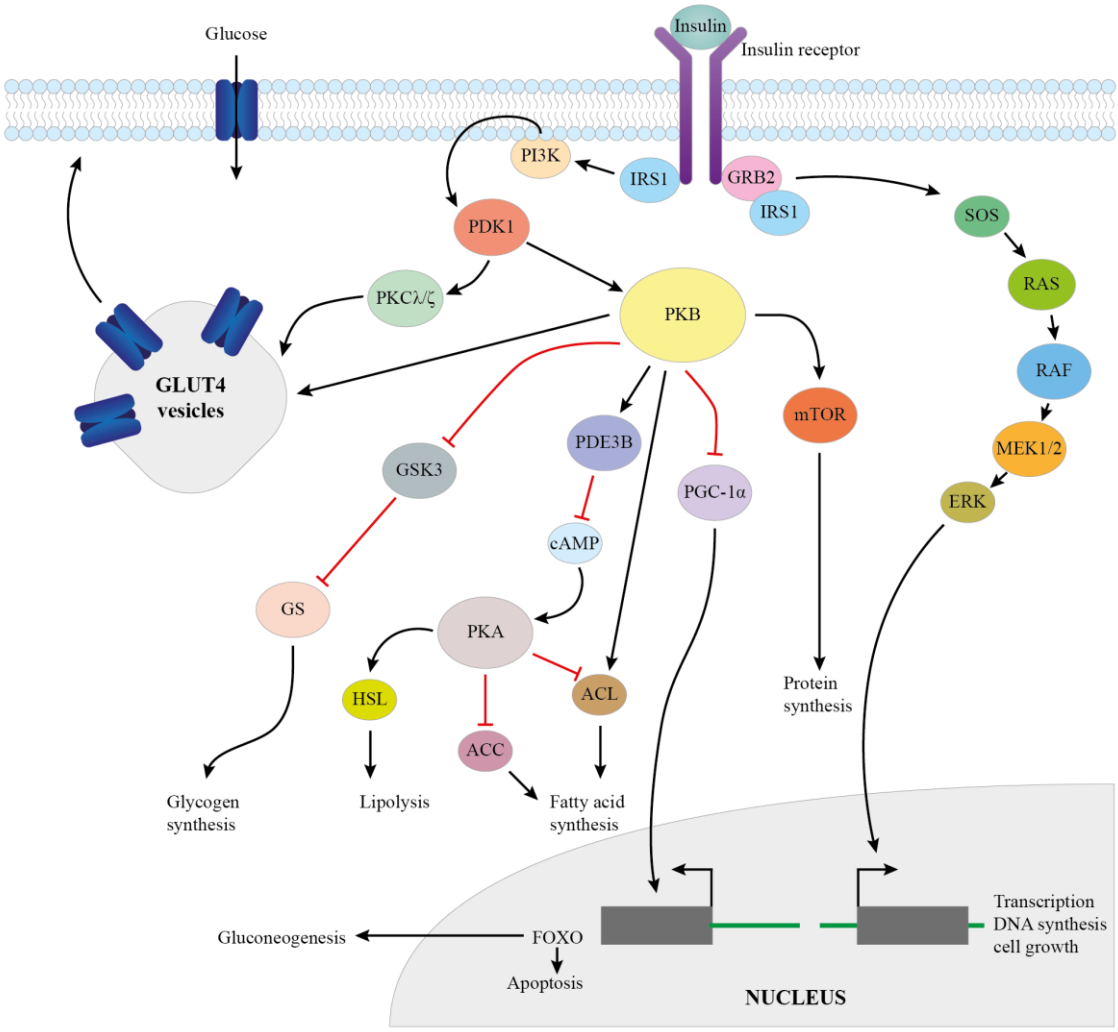


Figure 1.6. Adapted from The Medical Biochemistry Page (no date). The insulin-signalling system affects numerous intracellular processes. Arrows - positive, activating functions. T-lines - inhibitory functions.

1.2.3.2. Insulin-like growth factor-I

Insulin-like growth factor-I was initially termed Somatomedin C (Daughaday et al., 1972). The name insulin-like growth factor was proposed in 1976 following the discovery of the proteins' non-suppressible insulin-like activity (Rinderknecht and Humbel, 1976). Insulin-like growth factor-I is an essential mediator of normal postnatal growth, cell proliferation, differentiation and survival. Its constitutive expression is tissue-specific (liver and skeletal muscle) and influenced by hormonal (e.g. insulin and growth hormone), nutritional (glycaemic levels) and developmental regulators (figure 1.7). It mediates its effects through binding to its cell surface receptor, a 350 kDa protein similar to the insulin receptor, composed of two ligand-binding α-subunits and two trans-

membrane β-subunits with integral tyrosine kinase activity. Additionally, IGF-I can bind to the insulin receptor and vice versa, albeit with lower affinity. Insulin-like growth factor-I is an important metabolic biomarker associated with a variety of health- and exercise-related outcomes (Nindl, 2010). Insulin-like growth factor-I regulates glucose homeostasis by stimulating glucose uptake in skeletal muscle (Berg and Bang, 2004). In contrast, GH antagonises the hepatic and peripheral effects of insulin on glucose metabolism (Møller and Jørgensen, 2009). Insulin-like growth factor-I and GH are tightly linked. In adults GH exerts a potent anabolic influence through IGF-I, known as the Growth hormone/Insulin-like growth factor-I (GH/IGF-I) axis (Butler and Le Roith, 2001). Growth hormone regulates the hepatic synthesis of IGF-I and Insulin-like growth factor binding protein-3 (IGFBP-3), the major IGF carrier and modulator of systemic IGF-I action. Insulin-like growth factor-I, in turn, regulates GH secretion via negative feedback (Frystyk, 2004; 2010). The main mechanisms through which IGF-I exerts its considerable influence over skeletal muscle mass is hypertrophy (Glass, 2010a) and inhibition of atrophy (Latres et al., 2005; Fernandes et al., 2012). Insulin-like growth factor-I secreted locally i.e. by skeletal muscle, and not systemic IGF-I is suggested to be primarily responsible for the anabolic response to exercise (LeRoith et al., 2001; Velloso, 2008). The relationship between local and systemic IGF-I in mediating the outcomes of exercise are still not fully understood, as investigations into the effect of exercise on systemic concentrations of IGF-I has yielded inconsistent results (Frystyk, 2010; Nindl and Pierce, 2010), but local IGF-I may augment systemic concentrations (Adams, 2002; Berg and Bang, 2004; Nindl, 2010). Nemet et al., (2002; 2006) described a biphasic systemic IGF-I response to exercise, with a small but significant increase during the first 10 - 20 min followed by a decline as exercise progresses. Data from high and low intensity short duration (10 min) exercise (Schulze et al., 2002) and longer duration (30 min) heavy exercise bouts (Zaldivar et al., 2006) support this, as do data resulting from bouts of short duration maximal exercise (De Palo et al., 2008; Zebrowska et al., 2009).

1.2.3.3. Growth hormone

Growth hormone is secreted in a pulsatile fashion by the anterior pituitary gland (Hindmarsh et al., 1997) and has a key regulatory role in controlling the hepatic release of the IGF-II and IGF-II (Rodriguez et al., 2007), co-ordinating the postnatal growth of multiple target tissues, including skeletal muscle (Florini et al., 1996) (figure 1.7). Growth hormone deficiency is associated with impairment of growth, e.g. Laron Dwarfism (Godowski et al., 1989), a cluster of cardio-vascular risk factors such as altered body composition with increased body fat, insulin resistance, adverse lipid profile, reduced physical performance, reduced bone mineral density and impaired quality of life (Papadogias et al., 2003), whilst excessive GH secretion early in life typically results in pituitary gigantism, and later somatotroph adenomas and acromegaly (Lindholm, 2006). Systemic concentrations of GH are augmented by a variety of stimuli, of which endurance exercise has been shown to be extremely potent (Sutton and Lazarus, 1976; Felsing et al., 1992; Pritzlaff et al., 1999). Increases in systemic GH concentrations have been observed after as little as 10 min of cycle ergometer exercise (Felsing et al., 1992). Felsing et al., (1992) hypothesised that systemic GH concentrations would only increase if a threshold of work intensity corresponding to the Anaerobic threshold (ATh) was exceeded. Indeed, data illustrated that GH was significantly elevated after only 5 min of high intensity exercise, whilst 10 min of low intensity exercise was without effect (Felsing et al., 1992). The exercise-induced increase in systemic GH concentration is suggested to spare blood glucose by increasing gluconeogenesis and mobilising free fatty acids from adipose tissue (Hunter et al., 1965a; 1965b). However, it is thought that the latter maybe restricted during very strenuous exercise due to reduced blood flow to adipose tissue depots. Interestingly, ageing and obesity result in a reduction in both basal and exercise-induced GH concentrations (Chahal and Drake, 2007; Hansen et al., 2012). These findings highlight the importance of identifying an endurance exercise component that promotes increases in systemic GH concentrations to maximise benefits in target groups.

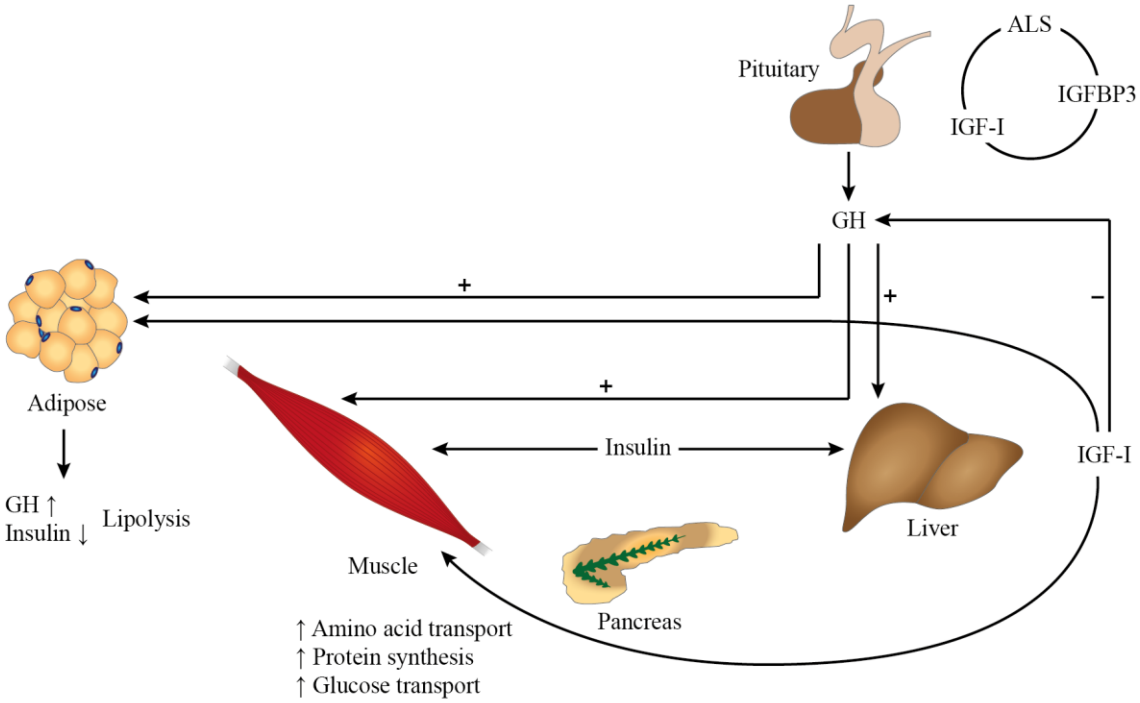


Figure 1.7. Adapted from Clemmons (2006a). Schematic diagram of insulin, growth hormone and insulin-like growth factor-I regulation. In the liver, insulin enables increased IGF-I synthesis in response to GH. Insulin-like growth factor-I exerts negative feedback on GH production in the pituitary. In muscle, insulin and growth hormone increase protein synthesis, and transport of amino acids; insulin also increases transport of glucose. + variables that stimulate GH secretion, - variables that inhibit GH secretion.

1.2.3.4. Cortisol

The glucocorticoid cortisol is secreted following stimulation of the Hypothalamic-pituitary-adrenal (HPA) axis in a tightly controlled manner (Lightman et al., 2008; Russell et al., 2010) (figure 1.8). Systemic concentrations reflect a combination of the normal diurnal pulsatile cortisol secretion and that secreted through transient activation of the HPA axis during times of perceived or actual physical or psychological stress: the fight or flight response, an essential mechanism that promotes adaptation and survival via responses of the neural, cardio-vascular, autonomic, immune and metabolic systems (Charmandari et al., 2005). Once released, cortisol is taken up by skeletal muscle, adipose tissue and the liver. In these tissues cortisol mediates critical physiological processes that aid exercise and recovery, e.g. skeletal muscle proteolysis and Amino-acid metabolism, and the adipose tissue hydrolysis of TAG into free fatty acids and glycerol (Symonds and Lomax, 1992; McMurray, and Hackney 2000; Viru and Viru, 2004; Hackney, 2006).

High circulating levels of cortisol may stimulate gluconeogenesis in the liver, providing additional carbohydrate for energy production (Viru and Viru, 2004). Excessive cortisol secretion (hypercortisolism) has been implicated in the muscle weakness and increased central adiposity/obesity characteristic of Cushing's Disease (Medline Plus, no date). Further, Cree et al., (2010) found that physical inactivity and induced hypercortisolism increased intramuscular TAG content and skeletal muscle insulin resistance in previously healthy males. Investigation into the effect of exercise on systemic concentrations of cortisol has yielded inconsistent results due, it is been suggested, to confounding variables such as time of day, circadian rhythms, psychological stress and training (Howlett, 1987; Hill et al., 2008). However, data suggest endurance exercise at an intensity greater than ~ 50 - 60 % $\dot{V}O_2$max results in cortisol release proportional to exercise intensity (Luger et al., 1987; Hill et al., 2008; Shojaei et al., 2011), while exercise below this level promotes a decline in systemic cortisol concentrations (Davies and Few, 1973; Bloom et al., 1976), potentially reflecting the underlying circadian rhythm (Howlett, 1987). Interestingly, studies by Rosa et al., (Rosa et al., 2011; 2012) indicate that systemic cortisol concentrations are reduced following an acute bout of 'concurrent' training, a combination of endurance [cycling] and resistance training.

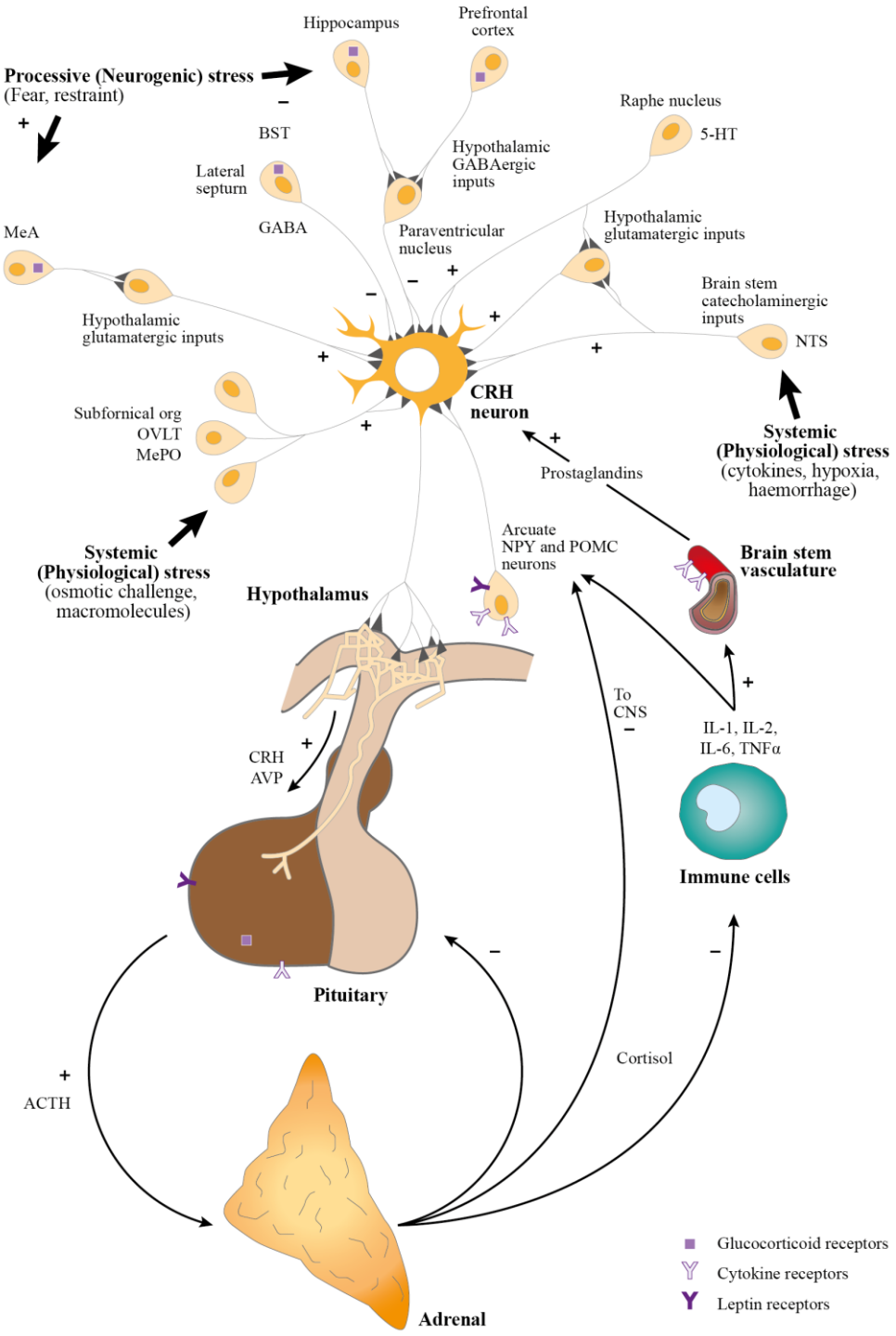


Figure 1.8. Adapted from Inder and Wittert (2005). Regulation of the hypothalamic-pituitary-adrenal axis. Neuronal and neuro-transmitter input to Hypothalamic corticotropic-releaseing hormone (CRH) and Arginine vasopressin (AVP)-containing neurons result in release of these hormones into the hypothalamic-hypophysial portal system. This causes an increase in secretion of Adrenocorticotropic hormone (ACTH) from the pituitary, which in turn stimulates cortisol release from the adrenal cortex. Cortisol acts at both the pituitary and hypothalamic level via negative feedback to inhibit ACTH, CRH and AVP.

1.3. Overall aim, goal and chapter objectives

Research propelled by the significant socio-economic issues presented by an ageing population has identified links between physical inactivity, metabolism and disease. Less active lifestyles and over-nutrition, result in dysregulation of metabolism and the progression of a chronic inflammatory state (immunometabolism). As we age a similar inflammatory process (inflammageing) contributes to the decline in the quality and quantity of skeletal muscle (dynapenia/sarcopenia), functional capability and quality of life. Our overall aim is to implement an empirically informed, palatable acute endurance exercise intervention that elicits beneficial hormonal responses with the potential for improved health/quality of life across the lifespan.

It is clear that exercise recommendations shuld be based on sound molecular and biochemical analyses. The goal of this thesis is therefore to clarify hormone and cytokine responses to endurance exercise with regard to skeletal muscle metabolic threshold events, by using an 'exercise-domain' based approach, thereby improving our understanding of the mechanisms behind the responses, and refining the potential for endurance exercise to deliver health benefits. Our objectives are: 1) to compare data from a cycle ergometer maximum rate of oxygen uptake ($\dot{V}O_2max$) test, conducted using principles and techniques associated with oxygen uptake dynamics research, with that from three acute 'domain-based' constant work-load cycle ergometer exercise interventions of varying intensity, but equal total work done (Chapter 3), 2) to establish the changes in the mRNA expression of selected hormones and cytokines that occur locally in skeletal muscle and subcutaneous adipose tissue (Chapter 4) and 3) systemically (Chapter 5), as a function of our acute 'domain-based' constant work-load cycle ergometer exercise interventions in recreationally active young males, and 4) to establish the the impact of age (20 - 60 yrs.) on the systemic hormone and cytokine response to an acute heavy domain constant work-load cycle ergometer exercise intervention in recreationally active males (Chapter 6).

Chapter 2. General methods

Information supplementary to these methods is present in Appendixes (A - E).

2.1. Laboratory

Experimental work was undertaken in the laboratories of Manchester Metropolitan University (MMU). Experimental work performed between September 2005 and 2008 was performed in the Laboratories housed collectively within the Old Physiology Laboratory, Department of Exercise and Sport Science, Alsager Campus, MMU Cheshire. Following relocation of the Institute for Biomedical Research into Human Movement and Health (IRM) to MMU in Central Manchester in 2008, further experimental work was performed in the Laboratories housed within the IRM facility on 1st floor, John Dalton Tower, All Saints Campus, MMU.

2.1.1 Health and safety

Experimental work conformed to the MMU Heath and Safety guidelines. Procedure documents Health and Safety Procedures for the Exercise Physiology Laboratories, and Health and Safety Procedures for the Biochemistry, Molecular Biology, Cell and Tissue Culture Laboratories and Imaging Facilities, local to the Department of Exercise & Sport Science, were consulted.

2.1.2. Environmental conditions

Data collated from the portable Cardio-pulmonary exercise testing system employed during each exercise test suggested ambient laboratory conditions during exercise testing varied as follows: Temperature 21 ± 1 (range 19 – 25) °C, Relative humidity 44 ± 8 (range 22 – 56) % and Barometric Pressure 746 ± 10 (range 726 - 764) mmHg. Integrated building management and air conditioning systems ensured appropriate ambient conditions were maintained in other laboratory areas used (data not available).

2.2. Participants

2.2.1. Ethical approval

The MMU Department of Exercise & Sport Science Ethics Committee granted ethical approval for each of the studies performed. The procedures described herein therefore conform to the ethical regulations for the use of humans in research as laid out by the committee.

2.2.2. Participant recruitment

Participants were recruited by word of mouth, and through e-mail and poster advertisements targeting male members of the MMU staff/student body. Potential participants were invited to visit the laboratory. During this initial visit each participant was verbally informed of the requirements of the study for which they wished to volunteer, and their attention was drawn to relevant local ethical committee approval documentation. Participants who did not satisfy any of the exclusion criteria, and were willing to participate, were asked to sign the relevant informed consent document. Participants received additional written information for reference purposes and a copy of their signed informed consent form.

2.2.2.1. Exclusion criteria

- Female
- History of smoking
- Age < 18 or > 40 *(Chapters 3, 4 and 5),* Age < 20 or > 60 *(Chapter 6).*

Having any of the following:

- Inflammatory disease
- Chronic disease with regular clinical treatment
- Muscle or bone disease
- Bone fractures within the last twelve months
- Metabolic or hormonal disorders
- Blood clotting disorders
- Epilepsy

2.2.3. Initial measurements: body mass, stature and body mass index

The body mass and stature of each participant was measured following informed consent. Body mass was measured using precision scales (SECA beam balance 709, SECA, Hamburg, Germany) with the participant in minimal clothing and without shoes. Stature was measured using a wall-mounted stadiometer (Harpenden with Veeder-Root high speed counter, Holtain Ltd, Crymych, Wales). The participant was positioned bare-foot with their back against the stadiometer, heals pressed firmly against the heal stop and head in the Frankfort horizontal plane. The participant was instructed to breathe in, following which the stature value was read from the counter mechanism. Body mass index (BMI) was calculated subsequently from the body mass and stature values using the formula BMI = body mass (kg)/stature $(m)^2$ (Eknoyan, 2008).

2.2.4. Participant preparation

Participants were asked to comply with a series of requests prior to visiting the laboratory for data collection. The requests were made to ensure all participants were in a similar physiological state. Participants were asked to abstain from exercise, caffeine and alcohol consumption during the 24 hr. period immediately prior to visiting the laboratory, and to fast during the 12 hr. period immediately prior to visiting the laboratory. In addition, participants were asked to consume only a prescribed volume of water within five minutes of waking, prior to visiting the laboratory. To facilitate this each participant was provided with their own individual plastic water bottle on which was placed a mark corresponding to a water volume of 5 ml/kg body mass. Participants were asked to refill and reuse the bottle prior to each subsequent laboratory visit.

2.3. Electronically-braked cycle ergometry

An electronically-braked cycle ergometer (Ergoline ER800, Jaeger, GmbH, Würzburg, Germany) was used for all exercise tests. The resistance applied to the flywheel is independent of cadence, thus providing a constant work-load regardless of small variations in pedal frequency. Saddle and handlebar height, and handlebar reach were adjusted to ensure an ergonomic fit between each participant and the ergometer. These

data were recorded to ensure replication of the position on subsequent visits. Participants were asked to remain seated at all times while pedalling.

2.3.1. Maximal oxygen uptake determination protocol

A maximum rate of oxygen uptake ($\dot{V}O_2$max) test was completed by all participants to enable exercise trial prescription at specific work-loads. The test commenced following a 60 s resting baseline expired gas sample and 4 min of cycling at 20 W/min (the lowest resistance available on the ergometer) to establish the resting and exercising baseline rate of oxygen uptake ($\dot{V}O_2$). Participants cycled at 60 RPM unless otherwise specified. A ramp rate of 30 W/min was employed, administered by increasing the resistance by 5 W/min every 10 s.

2.3.1.1. Methodological considerations: maximal incremental exercise testing

Since $\dot{V}O_2$max may differ during different types of exercise, the $\dot{V}O_2$max test should be performed within the same mode of exercise and using the same equipment e.g. treadmill, cycle ergometer, that will be used during subsequent periods of data collection. For optimal inter-individual comparisons participants should perform the test in a similar physiological state, e.g. rested, fasted, euhydrated and at the same time of day to avoid circadian effects. Consideration of these conditions is particularly important if the goal of the research is to characterise the effects of an exercise stimulus on local and systemic hormone and cytokine responses (Baldwin et al., 2000; Starkie et al., 2001).

2.3.2. Criteria for a successful $\dot{V}O_2$max test

The following were used as guidelines to determine a successful $\dot{V}O_2$max test. A plateau in the $\dot{V}O_2$ data with increasing work-load was taken as the primary criterion, indicating a successful test. A re-test was performed if a plateau in the $\dot{V}O_2$ data with increasing work-load was not observed and two out of the three secondary criteria were not met.

- A plateau in the $\dot{V}O_2$ data with increasing work-load (Taylor et al., 1955).
- Attainment of age predicted Heart rate at $\dot{V}O_2$max (HRmax) within 10 b/min, predicted using the equation 205.8 – 0.685 x age (Inbar et al., 1994).

- Respiratory exchange ratio (RER) of 1 or greater (Issekutz et al., 1962).
- Rating of perceived exertion (RPE) of 18 or greater (Borg, 1970; 1982).

2.3.2.1. Methodological considerations: criteria for a successful $\dot{V}O_2$max test

A major source of error in maximal exercise testing results from participant motivation, and failure to produce a truly maximum effort. The British Association of Sport and Exercise Sciences (BASES) and the American College of Sports Medicine (ACSM) provide guidelines on maximal exercise testing, which include assessment criteria for determining whether a participant reached a true maximum. In the literature a plateau in the $\dot{V}O_2$ profile with increasing work-load has been the primary criterion by which attainment of $\dot{V}O_2$max has been assessed (Taylor et al., 1955; Astrand, 1967). The plateau has been defined as an increase in oxygen uptake of < 1.5 ml/min/kg (Foster et al., 1986) or < 2.0 ml/min/kg (Astrand, 1960). Secondary criteria such as an RER > 1.0 (Drinkwater, 1984; Cress et al., 1991) to > 1.15 (Shephard et al., 1968), an exercise Heart rate (HR) of at least 85 % of age-predicted maximum (Borg, 1982) or a HR within 10 b/min of age-predicted maximum (Howley et al., 1995), a Blood lactate (BLa) concentration of > 5.5 mM (von Dôbeln et al., 1967) or > 7.9 mM (Astrand 1952), and Borg's RPE scale (Borg, 1982; 1970) have also been used (Tanaka et al., 1997). More recently the validity of criteria such as these has been questioned (Howley et al., 1995; Midgley et al., 2007; Poole et al., 2008; Midgley et al., 2009). Given that the outcome of a $\dot{V}O_2$max test is dependent on so many factors it is surprising that the exercise literature often does not provide sufficient information pertaining to the protocol followed to determine $\dot{V}O_2$max. Similarly, it is often unclear how exercise trial intensities based on $\dot{V}O_2$max are set. Where details are available, considerable variation exists in the protocols used, and there is also considerable inter-individual variation in $\dot{V}O_2$max (Shephard, 1984). In cycle ergometry this issue is further compounded by differences in cadence.

2.4. Heart rate monitoring

Heart rate was monitored during exercise using a short-range telemetry heart rate monitor (Accurex Plus, Polar Electro Oy, Kempele, Finland). Additionally, HR data corresponding with breath-by-breath expired gas analysis data were recorded using the portable cardio-pulmonary testing system described in the following section.

2.5. Expired gas sampling

Breath-by-breath expired gas samples were analysed during exercise using a portable cardio-pulmonary testing system (K4 b^2, COSMED, Rome, Italy). In accordance with the operating instructions, the system was allowed to warm up for 45 min before calibration and use. A volume calibration, performed using a precision 3 l syringe, and a respiratory delay calibration were performed before use. Additionally, a calibration step was performed with room air and precision gases of known Oxygen (O_2) and Carbon dioxide (CO_2) gas concentrations (British Oxygen Company (BOC) Ltd, Guildford, UK) immediately prior to each individual test. Data from the K4 b^2 unit were downloaded to a standard personal computer using appropriate software (Version 8.0b, K4 b^2, COSMED, Rome, Italy). Data were analysed using Microsoft® Excel® 2004 for Macintosh (version 11.2.5, Microsoft Corporation, Redmond, WA, USA).

2.5.1. Methodological considerations: an oxygen uptake dynamics approach

The following paragraphs summarise terms and methodological aspects of the oxygen uptake dynamics approach to exercise testing employed in this thesis. The reader is referred to Jones and Poole (2005) for a general introduction to the area of oxygen uptake dynamics research.

2.5.1.1. The $\dot{V}O_2$ response to constant work-load exercise

Following the onset of constant work-load exercise, $\dot{V}O_2$ increases in an exponential manner, incorporating three distinct phases (Whipp et al., 1982), illustrated in figure 2.1. The cardio-dynamic component phase, during which time $\dot{V}O_2$ rises rapidly, lasts ~ 10 – 30 s. During the primary component phase $\dot{V}O_2$ rises exponentially. When the exercise work-load is below the anaerobic threshold (ATh), and the exercise duration is

sufficiently long, the final steady state phase can be observed, within which the rise in $\dot{V}O_2$ appears to plateau. If the exercise work-load is above the Anaerobic threshold (ATh) a steady state component maybe delayed or absent as $\dot{V}O_2$ continues to rise, a response termed the $\dot{V}O_2$ slow component (Whipp and Wasserman, 1972).

Figure 2.1. Schematic representation of the phases of the oxygen uptake response to constant work-load exercise. Initial (cardio-dynamic component) phase, second (primary component) phase, third (steady state component) phase, and the $\dot{V}O_2$ slow component.

2.5.1.2. The gas exchange threshold

The term Gas exchange threshold (GET) refers to the point at which there is a disproportionate increase in the rate of carbon dioxide production ($\dot{V}CO_2$) with respect to $\dot{V}O_2$ during a maximal incremental exercise test (Beaver et al., 1986b). Increasing exercise intensity beyond a certain work-load threshold results in the accumulation of lactate as a result of anaerobic energy metabolism. Accompanying this increase in lactate is an approximately equal reduction in blood bicarbonate concentration (Wasserman et al., 1967; Beaver et al., 1986a). The resulting increase in CO_2 production can be observed in the expired CO_2 profile (Wasserman et al., 1973; Wasserman, 1984). The point at which lactate starts to increase in the blood has been termed the ATh, the Lactate threshold (LTh) when reference is made to acid-base status, or the GET when observed through monitoring of $\dot{V}O_2$ and $\dot{V}CO_2$ respiratory profiles.

2.5.1.3. Maximal lactate steady state

The term Maximal lactate steady state (MLSS) refers to the highest constant work-load (Power output at maximal lactate steady state (P-MLSS)) at which BLa concentration, whilst elevated above that at rest, is maintained at a constant level. (Beneke and von Duvillard, 1996; Jones and Doust, 1998). Pringle and Jones (2002) suggested that the MLSS represents a clear demarcation in the $\dot{V}O_2$ response to constant work-load cycle exercise; cycling below and at the P-MLSS results in a steady state $\dot{V}O_2$ and the ventilatory equivalent ($\dot{V}_E$), while cycling just above P-MLSS resulted in a progressive increase in $\dot{V}CO_2$ and $\dot{V}O_2$ and subsequent termination of exercise due to fatigue.

2.5.1.4. The concept of exercise domains

Whipp et al., (2005) originally explained the concept of exercise domains and the physiological characteristics that demarcate the domain boundaries, which are summarised in figure 2.2. Exercise of moderate intensity is characterised by the absence of metabolic acidosis. The upper boundary of this domain is marked by the GET. During moderate intensity, constant work-load exercise $\dot{V}O_2$ increases as a two-phase exponential response attaining steady state within ~ 2 - 3 min (Whipp and Wasserman, 1972). Exercise intensities within this domain are usually expressed as a percentage of

GET, e.g. 80 % GET. Heavy intensity exercise, bordering moderate, is indicated by sustained metabolic acidosis with increased arterial blood lactate and decreased pH. In this domain these parameters may become stable or return to baseline. Stepping up in intensity is very heavy exercise. The point denoted by MLSS indicates the boundary between heavy and very heavy exercise (Pringle and Jones, 2002), where increasing arterial blood lactate and decreasing pH ultimately culminate in voluntary cessation of exercise (volitional exhaustion). This point generally coincides with $\dot{V}O_2max$. The exercise intensities that fall within the heavy and very heavy domains are usually expressed as delta (Δ) values, e.g. 30 % Δ.

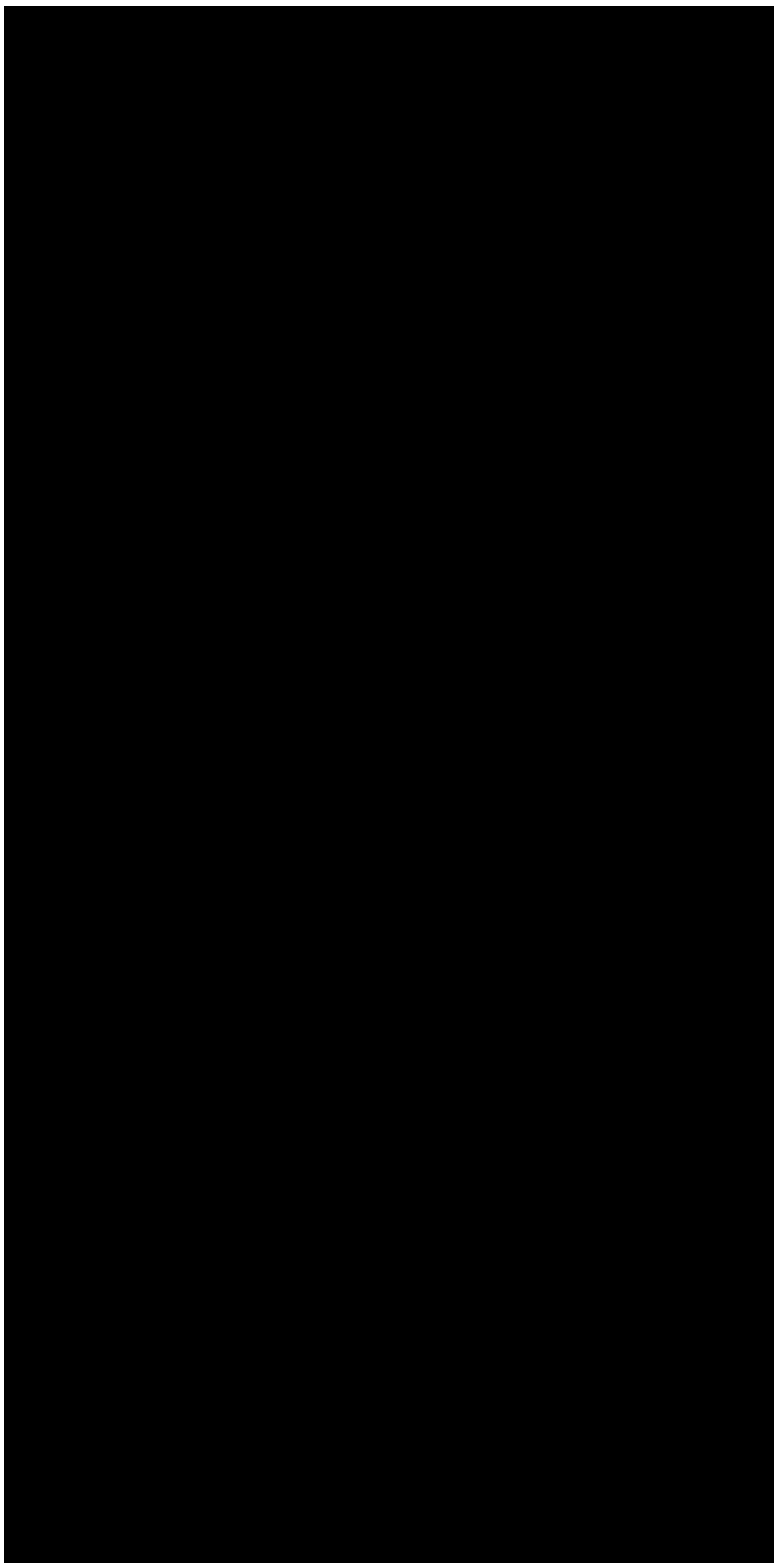


Figure 2.2. Schematic representation of the exercise domains defined by the $\dot{V}O_2$ response to constant work-load exercise. (a) moderate exercise domain (below GET), (b) heavy exercise domain (above the Gas exchange threshold (GET) but below Maximal lactate steady state (MLSS)) and (c) very heavy exercise domain (above MLSS but below $\dot{V}O_2$max).

2.5.1.5. Determination of exercise trial intensity from raw pulmonary gas exchange data

The breath-by-breath data were examined to exclude errant breaths caused by coughing, swallowing, etc. Data from all of the tests were linearly interpolated to provide second-by-second values. Mean values were calculated over consecutive 10 s periods (Astorino, 2009). The GET was determined from a cluster of measurements. Initially, it was determined as the point on the $\dot{V}O_2$ profile at which there was a disproportionate increase in $\dot{V}CO_2$ from visual inspection of individual $\dot{V}CO_2$ and $\dot{V}O_2$ graphs (Beaver et al., 1986b), known as the V-slope method (figure 2.3). Should the V-slope method not prove deterministic, visual inspection of the ventilatory equivalents for an increase in $\dot{V}_E/\dot{V}O_2$ with no increase in $\dot{V}_E/\dot{V}CO_2$, and an increase in end-tidal O_2 tension with no fall in end-tidal CO_2 tension (Wasserman et al., 1999) provided confirmation. The $\dot{V}O_2$max value was determined as the highest 10 s average value. Exercise trial power output corresponding to moderate (80 % GET), heavy (30 % Δ) and very heavy (60 % Δ) exercise intensities were calculated by first determining the power output corresponding to the GET and $\dot{V}O_2$max. Power output corresponding to 80 % of that at GET was designated moderate (80 % GET) intensity exercise. Similarly, power output corresponding to 30 % and 60 % of the difference between that at GET and $\dot{V}O_2$max was designated heavy (30 % Δ) intensity exercise and very heavy (60 % Δ) intensity exercise, respectively. The mean response time of the $\dot{V}O_2$ response to ramp exercise (assumed to approximate 2/3 of the ramp rate, i.e. 20 W) was taken into account in the calculation of these work-loads (Whipp et al., 1981).

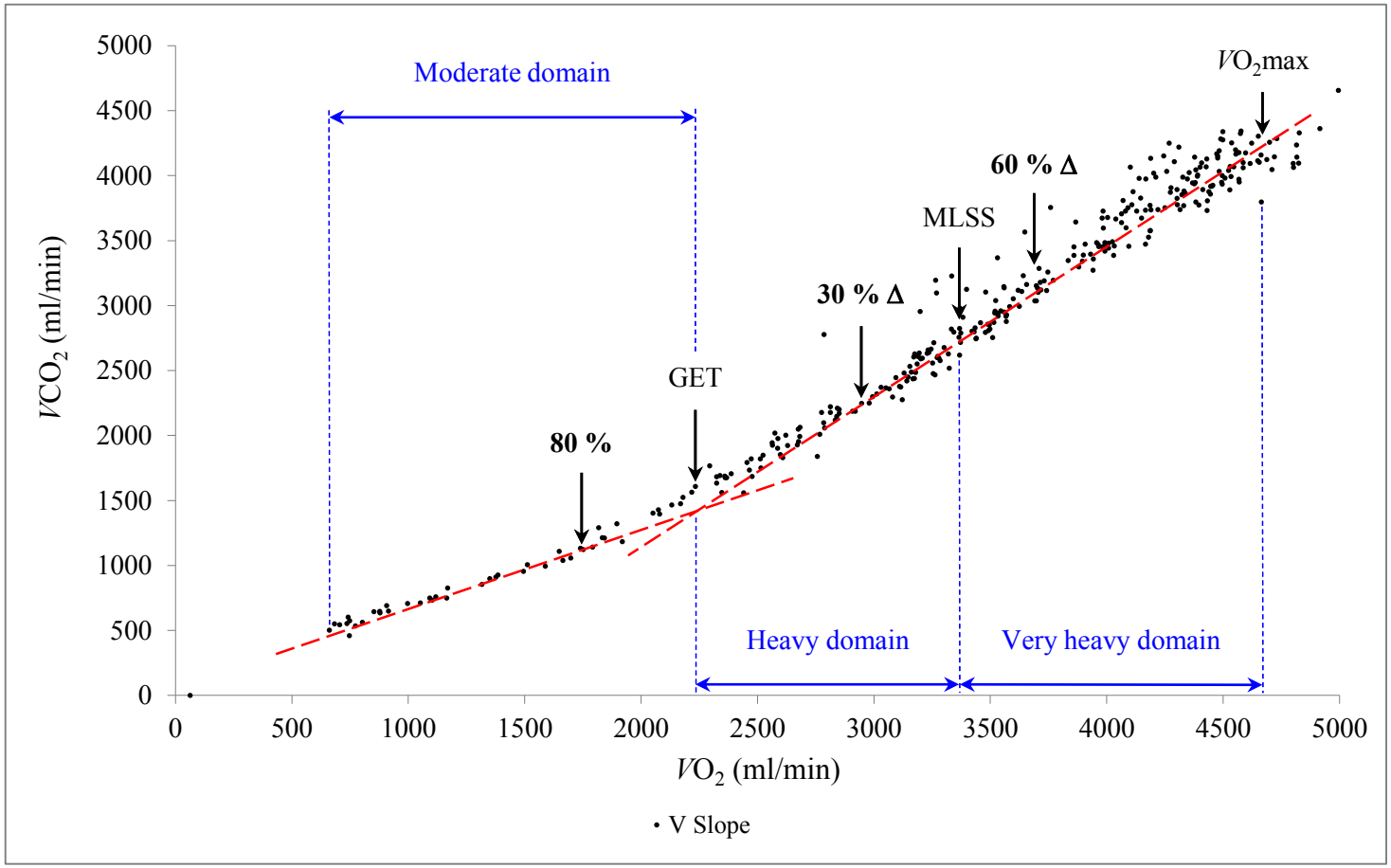


Figure 2.3. The V-slope method. A disproportionate increase in $\dot{V}CO_2$ from visual inspection of individual $\dot{V}CO_2$ and $\dot{V}O_2$ profiles (red dashed lines). The boundaries of the Moderate, Heavy and Very heavy exercise domains are shown (blue dashed lines), as are the threshold events that define them (GET, MLSS and $\dot{V}O_2$max). The exercise trial intensities referred to throughout this thesis are shown in bold (Moderate 80 % GET, Heavy 30 % Δ and Very heavy 60 % Δ).

2.5.1.6. Explanation and justification for exercise trial intensities

Moderate (80 % GET) exercise: exercise at this intensity provides sufficient stimulus for increased physiological responses while falling short of the GET and therefore, avoiding any contribution of energy produced via anaerobic pathways (Carter et al., 2000).

Calculation:

Power output at GET x 0.8 = 80 % GET (moderate) exercise resistance.

Heavy (30 % Δ) exercise: although above the GET and therefore receiving an energy contribution from anaerobic pathways, exercise at this intensity is considerably below Maximal lactate steady state (MLSS) (approximately 54 % Δ (Pringle and Jones, 2002)), which will considerably minimise the effects of the O_2-slow component and increased metabolic acidosis on the measured physiological variables.

Calculation:

((Power output at $\dot{V}O_2$max - Power output at GET) x 0.3) + Power output at GET = 30 % Δ (heavy) exercise resistance.

Very heavy (60 % Δ) exercise: above MLSS, exercise at this intensity produces a rapid increase in the O_2-slow component and in metabolic acidosis.

Calculation:

((Power output at $\dot{V}O_2$max - Power output at GET) x 0.6) + Power output at GET = 60 % Δ (very heavy) exercise resistance.

2.5.1.7. Example of calculations to determine exercise trial duration according to equal work done

Moderate intensity: (e.g. 100 W): 100 W x 30 min (1800 s) = 180000 J total work done.

Heavy intensity: (e.g. 200 W): Total work done at moderate intensity = 180000 J. 180000 J/200 W = 900 s (15 min).

Very heavy intensity: (e.g. 300 W): Total work done at moderate intensity = 180000 J. 180000 J/300 W = 600 s (10 min).

2.5.1.8. Exercise trial protocols

The equipment and procedures identified here have been described in detail previously. Participants were asked to observe the preparation guidelines with regard to fasting, relative hydration and abstinence described previously prior to all exercise trials. Participants were asked politely to ensure they arrived promptly at the appointed time for data collection, and to wear clothing suitable for exercise. Shortly after arriving at the laboratory an indwelling venous cannula was placed. Venous and finger prick blood samples were obtained following 10 min quiet sitting. Prior to the trial the cycle ergometer saddle was correctly positioned according to data recorded during the $\dot{V}O_2$max test. Exercise trials commenced in earnest with the application of a load pre-determined to elicit the physiological response characteristic of the exercise domain (moderate, heavy or very heavy) in which the trial is taking place. Immediately prior to this, resting and exercising baseline $\dot{V}O_2$ data were collected. Heart rate was recorded and participants were asked to breath into a portable cardio-pulmonary testing system during the initial 10 min (including resting and exercising baseline $\dot{V}O_2$ data collection) and the final two min of each exercise trial. Samples of skeletal muscle tissue and adipose tissue were obtained immediately post-exercise (exercise studies 1 and 2 only). Additional blood samples were obtained to establish time-course profiles. Detailed information including an overview schematic relating to the exercise trial protocols employed in each of the interlinking studies follows.

2.5.1.8.1. Overview of exercise studies

A schematic representation of the experimental timelines for the interlinking feasibility and exercise studies performed in this thesis is provided in figure 2.4. Details of the exercise trial protocols used in exercise studies 1, 2, and 3 are provided in tables 2.1., 2.2. and 2.3, respectively.

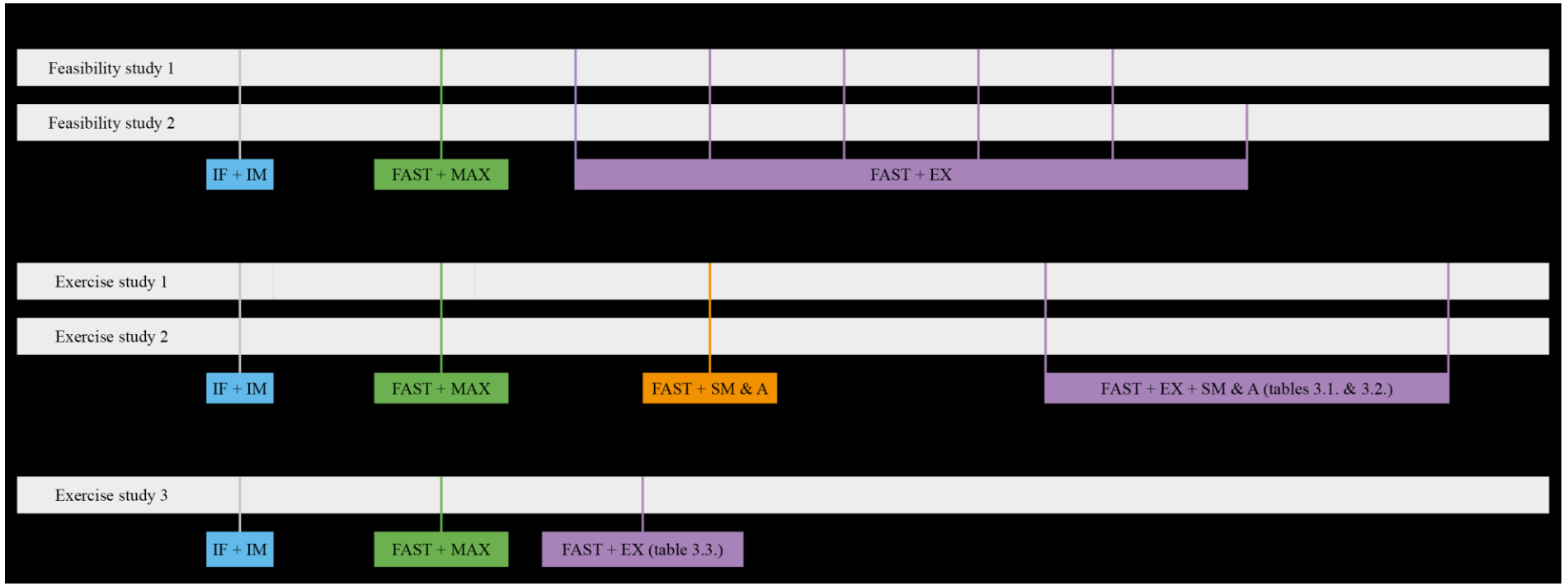


Figure 2.4. Experimental timelines: feasibility studies 1 and 2, and exercise studies 1, 2 and 3. Chronological order represents the minimum duration between subsequent events. IF - participant meeting, informed consent obtained, IM - initial measurements (participant stature and body mass), FAST - 12 hr. overnight fast, MAX - $\dot{V}O_2$ max test, pre- and post-test venous and finger prick (blood glucose and blood lactate) blood samples, SM&A - skeletal muscle and subcutaneous adipose tissue biopsy, EX - randomised exercise trial. Information relating to feasibility studies 1 and 2 is not reported.

Table 2.1. Study 1 exercise trial protocols

Time (min)	**Trial**	
	Moderate (60 min)	**Heavy (equal work: 27:48 ± 02:31)**
0 (baseline)	Blood Exercise trial commenced	
27:48 ± 02:31 (end of exercise) (Heavy (equal work) trial time, mean ± SD)	-	Blood. Muscle and adipose tissue biopsy
30	Blood	-
60 (end of exercise) (Moderate trial time)	Blood. Muscle and adipose tissue biopsy	-
60 (post-exercise)	Blood	
24 hr. (post-exercise)	Blood	

Heavy (equal work) - work done is approximately equal to that done in the moderate (60 min) trial.

Table 2.2. Study 2 exercise trial protocols

Time (min)	**Trial**	
	Heavy (30 min)	**Very heavy (equal work: 22:29 ± 01:31)**
0 (baseline)	Blood Exercise trial commenced	
22:29 ± 01:31 (end of exercise) (Very heavy (equal work) trial time, mean ± SD)	-	Blood. Muscle and adipose tissue biopsy
30 (end of exercise) (Heavy trial time)	Blood. Muscle and adipose tissue biopsy	-
60 (post-exercise)	Blood	
24 hr. (post-exercise)	Blood	

Very heavy (equal work) - work done is approximately equal to that done in the heavy (30 min) trial.

Table 2.3. Study 3 exercise trial protocol

Time (min)	Trial Heavy (30 min)
0 (baseline)	Blood Exercise trial commenced
10	Blood
20	Blood
30 (end of exercise)	Blood.
30 (post-exercise)	Blood
60 (post-exercise)	Blood

2.6. Blood sampling

2.6.1. Venous blood sampling

Samples of whole blood were obtained via venepuncture from veins in close proximity to the cubital fosse of the non-dominant arm pre-exercise, during exercise and post-exercise via indwelling venous cannula (Venflon IV Blue 22G, Beckton Dickinson (BD) Biosciences, Oxford, UK.). Resting baseline samples and 24 hr. post-exercise samples, were collected via needle (NN-2138R, Terumo, Luer 21G, Terumo Europe, Leuven, Belgium) and 10 ml syringe (SS-10ES, Terumo Europe, Leuven, Belgium). Blood samples (3 ml discard, 10 ml collection) were drawn into ice cold S-Monovette® tubes (02.1063.001 Serum Z/9 ml, Sarstedt AG & Co, Germany). To control for the influence of postural changes on body fluid distribution, the pre-exercise resting blood sample was taken 10 min post-cannulation, after the participant had been seated, motionless, for that time. Following blood draw, 10 ml sterile saline was infused to ensure the cannula was kept patent. Following blood clotting on ice, serum was obtained via centrifugation (2000 g, 6 min, 4 °C). The serum was aliquoted into labelled 2 ml Eppendorf® tubes (0030 120.094, Eppendorf AG. Hamburg, Germany) and stored at -80 °C until analysed.

2.6.2. Capillary blood sampling

Samples of whole blood were obtained from the fingertip capillary bed via finger prick pre- and post-exercise to enable measurements of BLa and Blood glucose (BGlu) concentrations.

2.6.2.1. Blood lactate

The concentration of BLa was measured using a YSI 1500 Sport portable BLa analyzer (1500, YSI Inc., Yellow Springs, Ohio, USA). This instrument uses a proprietary electrochemical enzymatic method for detection of lactate in 25 µl samples of whole blood (measurement range: 0 – 30 mmol/l, Precision: Whichever is larger, ± 2 % of reading or 0.1 mmol/l). Calibration checks were performed on this machine daily using a 5 mmol/l standard (2327, YSI Inc., Yellow Springs, Ohio, USA).

Following the relocation of laboratory facilities to central Manchester this instrument was no longer available. Blood lactate concentrations were measured in approximately 5 µl whole blood using a Lactate Pro BLa analyser and specific test strips (Lactate Pro Reagent Strips, Akray KDK, Koyota, Japan). This method was used for all samples collected from participants in the 40 - 50 yr. and 50 - 60 yr. age groups in study 3 only. The measurement principle employed by the instrument is based on the potassium ferricyanide and lactate oxidase method. Independent evaluation confirmed the validity of this device (Pyne et al., 2000) and the relationship of measurements to the YSI 1500 Sport (McLean and Smith, 2004). Equipment and calibration checks were performed on this machine daily using the check strip and a calibration strip associated with the specific test strips being used. Prior to calibration a visual check was performed to confirm serial number agreement between the calibration and test strips.

2.6.2.2. Blood glucose

The concentration of BGlu was measured using a portable BGlu analyser (11418246906, Accutrend® GC, Roche Diagnostics, East Sussex, UK) and test strips for quantification of BGlu (11443054018 BM Accutest, Roche Diagnostics, East Sussex, UK). The measurement principle employed by this instrument is reflectance photometry (optical system: LED 657 nm, measurement range: glucose 3.88 – 7.76 mmol/l). Fasting glucose reference values for healthy adult males are considered to be between 3.4 and 5.5 mmol/l.

Following the relocation of laboratory facilities to central Manchester this instrument also was no longer available. Blood glucose concentrations were measured using a HemoCue® 201+ BGlu analyser (120713, HemoCue® AB, Ängelholm, Sweden) and specific HemoCue® Glucose 201 Microcuvetes (110716, HemoCue® AB, Ängelholm, Sweden). This method was used for all samples collected from participants in the 40 - 50 yr. and 50 - 60 yr. age groups in study 3 only. The measurement principle employed by the instrument is based on a glucose dehydrogenase method. Calibration checks were performed on this machine daily using Level 2 controls - range 6.0 ± 0.9 mmol/l (146.002.002, HemoCue® AB, Ängelholm, Sweden).

2.6.3. Enzyme-linked immunosorbent assay analyses

Serum samples were assayed for specific growth factors and cytokines. Pre-coated, 96-well enzyme-linked immunosorbent assay (ELISA) kits were purchased for determination of specific protein concentrations in human serum. Kits were stored and utilised according to the manufacturers instructions. An automated plate reader (ELx800UV, BIO-TEK Instruments Inc., Winooski, Vermont, USA) was used at relevant wavelengths for all protein determinations. Data were analysed using Microsoft® Excel® 2004 for Mac (version 11.2.5, Microsoft Corporation, Redmond, WA, USA).

The following assays were performed:

- Interleukin-6 (IL-6) (850.035.096, DIACLONE research high sensitivity IL-6, IDS Ltd., Tyne and Wear, UK). Standard curve range 0 – 50 pg/ml, sensitivity 0.8 pg/ml. *†, ††*
- Interleukin-1-alpha (IL-1α) (583301, Cayman Chemical Company, Michigan, USA). Standard curve range 0 – 250 pg/ml, sensitivity 1.5 pg/ml. *††*
- Interleukin-1-beta (IL-1β) (583311, Cayman Chemical Company, Michigan, USA). Standard curve range 0 – 250 pg/ml, sensitivity 1.5 pg/ml. *††*
- Tumour necrosis factor-alpha (TNFα) (589201, Cayman Chemical Company, Michigan, USA). Standard curve range 0 – 250 pg/ml, sensitivity 1.5 pg/ml. *††*
- Cortisol (DX-EIA-1887, DRG Instruments GmbH Corisol ELISA, IDS Ltd., Tyne and Wear, UK). Standard curve range 0 – 800 ng/ml, sensitivity 2.5 ng/ml. *††, ‡, ‡‡, ‡‡‡*
- IL-6 (BMS213INST, Bender MedSystems GmbH, Vienna, Austria). Standard curve range 0 – 200 pg/ml, sensitivity 0.92 pg/ml. *‡, ‡‡, ‡‡‡*
- Growth Hormone (GH) (DX-EIA-3552, DRG Instruments GmbH hGH ELISA, IDS Ltd., Tyne and Wear, UK). Standard curve range 0 – 74 µIU/ml, sensitivity 0.2 µIU/ml. *††, ‡, ‡‡, ‡‡‡*
- Insulin-like growth factor-I (IGF-I) (EL2010, Biocode-Hycel™, IGF-I, Oxford Biosystems Oxford, UK). Standard curve range 0 – 1250 ng/ml, sensitivity 8 ng/ml. *†, ††, ‡, ‡‡, ‡‡‡*
- Leptin (BMS2039INST, Bender MedSystems GmbH, Vienna, Austria). Standard curve range 0 – 4000 pg/ml, sensitivity 20 pg/ml. *‡, ‡‡, ‡‡‡*

- Adiponectin (DEE009, Demeditec Diagnostics GmbH, Kiel, Germany). Standard curve range 0 – 100 ng/ml, sensitivity 0.6 ng/ml. *‡, ‡‡, ‡‡‡*
- Insulin (KAP1251, BioSource Europe S.A., Nivelles, Belgium). Standard curve range 0 – 237 μIU/ml, sensitivity 0.15 μIU/ml. *‡, ‡‡, ‡‡‡*

Feasibility study 1- †, 2 - ††
Exercise study 1 - ‡, 2 - ‡‡, 3 - ‡‡‡

2.7. Skeletal muscle and subcutaneous adipose tissue sampling

Skeletal muscle and subcutaneous adipose tissue samples were obtained post-exercise via conchotome technique (Dietrichson et al., 1980; 1987). The procedure was performed by a suitably qualified, General Medical Council registered, medical doctor experienced in the extraction of skeletal muscle and adipose tissue.

2.7.1. Skeletal muscle and subcutaneous adipose biopsy procedure

The point of incision above the *Vastus Lateralis* was identified following a request to the participant to contract the muscles of the thigh. An area on the skin surface in which an incision would be made was shaved using a disposable prep razor (UN2000, Universal Hospital Supplies, Enfield, UK) and cleaned using alcohol swabs (Sterets pre-injection wipes, 00766691, Seton Healthcare Group plc., Oldham, UK). A sterile field was created and maintained using a sterile procedure pack (Vernaid wound care pack option 2, 28853, Vernon-Carus ltd., Preston, Lancashire, UK). The area around the incision was swabbed with a surgical scrub solution (Betadine Surgical Scrub, Purdue Pharma LP, Stamford, Connecticut, USA). Local anaesthesia of the skin and immediately underlying tissue was achieved using Lidocaine 2 % solution (PL01502/0021R, Lidocaine Hydrochloride, 40 mg in 2 ml, Hamlin Pharmaceuticals Ltd., Gloucester, UK), administered via a 25G hypodermic needle (NN-2516R, Terumo Europe, Leuven, Belgium) and 5 ml syringe (SS-05ES, Terumo Europe, Leuven, Belgium). Sufficient time was allowed for the anaesthetic to act before a 5 mm incision was made in the skin and underlying facia using a sterile disposable scalpel (Size 11, Swann-Morton, General Medical, Aldershot, UK). The sterile biopsy conchotome (Weil-Blakesley Rongeur, 52-030-50, Gebrüder Zepf Medizintechnik, Tüttlingen, Germany) was inserted through the incision, manoeuvred

into position and used to incise tissue from the muscle belly. The instrument was removed and the sample prepared for storage/cell culture.

Immediately after harvesting the skeletal muscle tissue sample, a sample of subcutaneous adipose tissue was obtained from the adipose tissue bed surrounding the site of the incision. Adipose tissue samples were obtained using the conchotome in a similar manner as the skeletal muscle samples. A rest period of at least two weeks was implemented between repeat skeletal muscle and adipose tissue sampling on the same limb. Care was taken to ensure that a distance of at least 2 cm separated the sites of samples taken from the same limb (Malm et al., 2000).

Wound closure was undertaken using sterile adhesive strips (3M Steri-Strip, 3M Healthcare, St. Paul, Minnesota, USA) and overlayed with a sterile dressing (Mepore® Ultra, Mölnlycke Health Care AB, Göteborg, Sweden). Bleeding was minimised by the application of a compressive dressing (3M Coban Self-Adherent Wrap, 1584, 3M Healthcare, St. Paul, Minnesota, USA with Vernaid gauze swab, 28919, Vernon-Carus ltd., Preston, Lancashire, UK). Perfusion of the leg post-biopsy was established by checking the pulse at the ankle. Participants were advised that the compressive dressing should only be removed after a minimum period of 4 hrs. and to keep the wound dry. Participants were further advised to refrain from exercise for 4 days post-procedure, and that they must inform the principal investigator and their general practitioner should the wound become infected.

2.7.2. Skeletal muscle and adipose tissue homogenisation

Cryovials containing the skeletal muscle and adipose tissue samples were removed from liquid nitrogen in small batches before being homogenised in the presence of Trizol (TRI®) Reagent Solution (1 ml) (AM9738 Applied Biosystems, Warrington, UK.) using an T10 basic Ultra-Turrax Homogenizer Workcenter (3519700) fitted with a S10N-5G dispersing tool (3304000, IKA-Werke GmBH & Co. KG, Staufen, Germany). Visual inspection confirmed no visible pieces of tissue remained following the procedure. Cryovials were re-labelled and returned to storage at -80 °C. Between samples the dispersing tool was washed in sterile phosphate buffered saline (BR0014G soluble tablets, OXOID, Basingstoke, UK) followed by 1 ml TRI® Reagent Solution.

2.7.3. Ribonucleic acid isolation

Cryovials containing the homogenised samples were removed from storage at -80 °C in small batches and allowed to thaw completely. Samples were supplemented with 0.2 ml Chloroform (for molecular biology, minimum 99 %, C2432-500ML, Sigma-Aldrich Company Ltd., Poole, UK) per 1 ml of TRI® Reagent Solution and mixed vigorously for 20 s prior to centrifugation at 12000 g for 16 min at 4 ˚C (3K10, SIGMA Laborzentrifugen GmbH, Osterode am Harz, Germany). The aqueous phase was transferred into a 1.5 ml RNase free microcentrifuge tube (AM12400, Applied Biosystems, Warrington, UK). Care was taken not to disturb the interface Deoxyribonucleic acid (DNA) layer. Original cryovials containing the DNA and organic phase were returned to -80 °C for storage. Ribonucleic acid (RNA) was precipitated from the aqueous phase using Isopropanol (2-Propanol for molecular biology, minimum 99 %) (I9516-500 ml, Sigma-Aldrich Company Ltd., Poole, UK) at a ratio of 1:1. Samples were mixed by inversion twice prior to being centrifuged at 12000 g for 10 min at 4 °C. The supernatant was carefully removed and the RNA pellet was washed with 75 % Ethanol (BDH AnalaR 96 % v/v) (10476, VWR International Ltd., Lutterworth, UK) prior to centrifugation at 8500 g for 6 min at 4 ˚C. Ethanol was removed and the RNA pellets were left to air dry before being re-suspended in 100 µl RNA storage solution (AM7001, Applied Biosystems, Warrington, UK). Ribonucleic acid was returned to -80 °C for storage until required for further analyses.

2.7.4. Oligonucleotide primer design and synthesis

The Genome Bioinformatics Group of UC Santa Cruz website genome browser (no date) was used to identify the DNA sequences for the selected genes of investigation. Oligonucleotide primers were designed using Beacon Designer version 5.11 (PREMIER Biosoft International, California, USA). Oligonucleotide primers were designed to yield products spanning exon-intron boundaries to avoid genomic DNA contamination. The inclusion of three or more GC base pairs in the last 5 base pairs at the 3’ end of the primer was avoided in order to minimise nonspecific amplification. All primers had a nucleotide base length of between 18 and 22 base-pair sequences. Beacon Designer version 5.11 was also used to perform searches for secondary structure or inter/intra-molecular interactions such as hairpins, self-dimers and cross-dimers within the primer. Sequence homology searches were performed against the National Center for Biotechnology Information

(NCBI) Genbank® database (NCBI, Maryland, USA) to ensure specific amplification of the associated target gene. Custom oligonucleotide primers (table 2.4) were purchased from Sigma-Genosys (Sigma-Genosys Ltd., Suffolk, UK), or from QIAGEN (QIAGEN, West Sussex, UK).

Table 2.4. Reverse transcription-polymerase chain reaction oligonucleotide primer information

Target Gene (ref. sequence)	Oligonucleotide primer Sequence (5'-3')	Supplier
Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)	^{F} GCAGAACGGACGGAGATG ^{R} TCAACGGCACAGTCAAGG	Sigma-Genosys
IL-6 (NM_000600.3)	^{F} ACAACCTGAACCTTCCAAAG ^{R} TTCCTCACTACTCTCAAATCTG	Sigma-Genosys
TNFα (NM_000594)	^{F} CCAGGGACCTCTCTCTCTAATC ^{R} GCTACAACATGGGCTACAG	Sigma-Genosys
IGF-I (X57025)	^{F} GCTCTTCAGTTCGTGTGTG ^{R} GAAGCAGCACTCATCCAC	Sigma-Genosys
Insulin-like growth factor-I receptor (IGF-IR) (NM_000875)	Not disclosed	QIAGEN
Suppressor of cytokine signalling 3 (SOCS3) (NM_003955.3)	Not disclosed	QIAGEN
Interleukin-15 (IL-15) (NM_000585)	Not disclosed	QIAGEN
Tripartite motif-containing 72 (TRIM72) (NM_001008274)	Not disclosed	QIAGEN
Peroxisome proliferator-activated receptor-γ coactivator 1-alpha (PGC-1α) (NM_013261)	Not disclosed	QIAGEN
Leptin (NM_000230)	Not disclosed	QIAGEN
Adiponectin (NM_004797)	Not disclosed	QIAGEN
Adiponectin receptor 1 (AdipoR1) (NM_015999)	Not disclosed	QIAGEN

F - Forward oligonucleotide primer sequence.

R - Reverse oligonucleotide primer sequence.

2.7.5. Reverse transcription-polymerase chain reaction method and data analysis

Ribonucleaic acid concentration and purity were assessed via ultraviolet spectroscopy from the optical density at 260 and 280 nm using a spectrophotometer (WPA Biowave II, Biochrom Ltd., Cambridge, UK). Seventy ng RNA were used per Reverse transcription-polymerase chain reaction (RT-PCR) (7 µl of RNA at a concentration of 10 ng/µl). Amplification of target genes via RT-PCR was performed using a commercially available Power SYBR Green RNA-to-C_T 1 step kit (4389986, Applied Biosystems, Warrington, UK) and 96-well RT-PCR plates (223-9441, Bio-Rad Laboratories Ltd., Hertfordshire, UK). The custom RT-PCR protocol was controlled using a Chromo4^{TM} DNA engine interfaced with a laptop computer running Opticon Monitor version 3.1.32 (MJ Geneworks Inc, Bio-Rad Laboratories Ltd., Hertfordshire, UK). The following RT-PCR cycles (table 2.5) were performed in order to facilitate gene amplification.

Table 2.5. Reverse transcription-polymerase chain reaction thermal cycle protocol

Description of protocol stage	**Temperature and duration**
1. *cDNA synthesis*	Incubate at 48 °C for 30:00
2. *Transcriptase inactivation*	Incubate at 95 °C for 10:00
3. *Denaturation*	Incubate at 95 °C for 00:15
4. *Annealing/extension*	Incubate at 60 °C for 01:00
5. *Plate read*	-
6. *Goto line 3 for 39 more times*	-
7. *Melting curve analyses*	From 50 to 95 °C read every 1°C, hold 00:00:01
END	-

Opticon Monitor version 3.1.32 constructs a graphical representation of fluorescence yield against RT-PCR cycle. The cycle number at which fluorescence yield crossed a threshold line (C(t)) was obtained by manually positioning a threshold line at a point along the exponential rise in the fluorescence/cycle curve. The data were exported to an Excel® version 12.2.0 spreadsheet (Microsoft® Excel® 2008 for Mac, Microsoft Corporation, Redmond, WA, USA) for analyses. The Delta delta (ΔΔ) C(t) equation (Livak Method) was used to determine normalised gene expression:

> Equation 1: Delta (Δ) C(t) = mean C(t) [gene of interest at exercise condition] – mean C(t) [reference gene at exercise condition].

Where: mean C(t) [gene of interest at exercise condition] = The mean C(t) value (sample and duplicate) of the targeted gene (e.g. IL-6) in the experimental condition (e.g. RNA isolated from skeletal muscle or adipose tissue following moderate, heavy or very heavy intensity cycle ergometer exercise).

Mean C(t) [reference gene at exercise condition] = The mean C(t) value (sample and duplicate) of reference gene (e.g. GAPDH) in the experimental condition (e.g. RNA isolated from skeletal muscle or adipose tissue following moderate, heavy or very heavy intensity cycle ergometer exercise).

> Equation 2: ΔC(t) = mean C(t) [gene of interest at baseline] – mean C(t) [reference gene at baseline].

Where: mean C(t) [gene of interest at baseline] = The mean C(t) value (sample and duplicate) of targeted gene (e.g. IL-6) at baseline (e.g. RNA isolated from skeletal muscle or adipose tissue at rest).

Mean C(t) [reference gene at baseline] = The mean C(t) value (sample and duplicate) of reference gene (e.g. GAPDH) at baseline (e.g. RNA isolated from skeletal muscle or adipose tissue at rest).

> Equation 3: ΔΔC(t) = ΔC(t) of Equation 1 - ΔC(t) of Equation 2.

> Equation 4: 2^-ΔΔC(t) = [normalised expression].

Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the reference gene in all RT-PCR experiments (Barber et al., 2005). Expression (C(t) value) of each gene of interest was normalised against the corresponding GAPDH C(t) value for each sample.

Sterile technique was implemented throughout. RNase contamination from work surfaces and equipment reserved for molecular techniques was minimised prior to use by exposure to RNase Zap. (AM9780, Applied Biosystems, Warrington, UK). Fluid transfer activities were performed using pipettes reserved exclusively for molecular biology techniques. These were used with sterile Aerosol resistant (ART®) pipette tips (Molecular BioProducts Inc., San Diego, CA, USA).

2.8. Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics versions 19 - 21 (IBM Corporation, New York, USA). Checks for parametricity were performed using, where appropriate, Levene's equal variance test and the Shapiro-Wilk normal distribution test and Mauchly's test of sphericity. Data were log (log10) or inverse (1/datum) transformed where applicable to normalise non-normally distributed data. If the assumption of sphericity was violated the degrees of freedom were adjusted accordingly (Greenhouse-Geisser). Non-parametric methods of statistical analyses were employed where a normal distribution could not be reached. Statistical significance was accepted at $P \leq 0.05$. Bonferrini corrections were applied where appropriate. Data are represented as mean ± SD unless otherwise stated.

Chapter 3. Maximal incremental exercise *vs.* acute 'domain-based' constant work-load cycle ergometry: comparison of cardio-respiratory responses

3.1. Abstract

Oxygen uptake dynamics research characterises exercise intensity into 'domains', based upon threshold events in the profiles of the muscle metabolic and pulmonary gas exchange responses. We hypothesised that mean $\dot{V}O_2$, HR and $\dot{V}O_2$/HR data obtained from equal work (varying duration) moderate (80 % GET) (M), heavy (30 % Δ) (H) and very heavy (60 % Δ) (VH) domain cycle ergometer exercise trials would parallel those obtained during a $\dot{V}O_2$max test. Two groups of young recreationally active male participants (Study 1: $n = 6$; age, 28 ± 5 yrs.; body mass, 81.1 ± 14.6 kg; stature 1.79 ± 0.03 m; BMI, 25 ± 4 kg/m^2 - Study 2: $n = 7$; age, 26 ± 7 yrs.; body mass, 82.9 ± 14.3 kg; stature 1.84 ± 0.07 m; BMI, 25 ± 4 kg/m^2) performed a cycle ergometer $\dot{V}O_2$max test (30 W/min ramp, 60 RPM) and equal work (varying duration) M and H (Study 1), and H and VH (Study 2) constant work-load cycle ergometer exercise trials, respectively. Statistical analyses permitted the pooling of data from the H exercise trials. Power Output (PO), $\dot{V}O_2$, HR and $\dot{V}O_2$/HR exercise trial data differed significantly between trials ($P < 0.02$). Analyses comparing M, H and VH trials and $\dot{V}O_2$max text data revieled no significant differences for $\dot{V}O_2$ ($P = 0.16$), HR ($P = 0.5$) and $\dot{V}O_2$/HR ($P = 0.42$) and strong positive correlations ($\dot{V}O_2$: $r = 0.944$, $n = 25$, $P < 0.001$; HR: $r = 0.903$, $n = 24$, $P < 0.001$; and $\dot{V}O_2$/HR: $r = 0.824$, $n = 24$, $P < 0.001$). We concluded that the 'domain-based' constant work-load cycle egometer exercise trials, performed at a power output identified during a incremental exercise test carried out with regard to the principals of oxygen uptake dynamics, yield similar cardio-respiratory characteristics.

3.2. Introduction

The study of the acute responses to physical exercise and the adaptations to regular physical exercise are core to exercise physiology studies. Typically, in such studies participants are required to perform an incremental exercise test until exhaustion, work pioneered by A.V. Hill and colleagues (Hill and Lupton, 1923; Hill et al., 1924). The primary function of this fundamental measure of exercise physiology is to quantify the maximum rate of oxygen uptake ($\dot{V}O_2max$) by an individual during exercise on e.g. a cycle ergometer. To obtain the $\dot{V}O_2max$ value, work-load is progressively increased until the participant is unable to continue due to fatigue; a point termed volitional exhaustion. The rate of oxygen uptake ($\dot{V}O_2$), Heart rate (HR) and Power output (PO), at this point correspond with the maximum aerobic capacity of the participant. The $\dot{V}O_2max$ value has traditionally been used to set constant work-load exercise e.g. a work-load that elicits 60 % $\dot{V}O_2max$. More recently maximal incremental or ramp exercise tests have replaced the discontinuous protocols employed by Hill and colleagues as the preferred method of establishing key parameters of aerobic function in humans, for example work efficiency, the Gas exchange threshold (GET) and $\dot{V}O_2max$ (Whipp et al., 1981).

The accuracy with which exercise trials are assigned to and performed by a group of research study participants is an important methodological consideration, effecting the quality of the data gathered and consequently the research findings. Oxygen uptake dynamics researchers suggest the traditional approach, to assign exercise intensity in relation to $\dot{V}O_2max$, could result in markedly different physiological stress characteristics at what would appear to be identical relative exercise intensities (Whipp et al., 2005) i.e. exercise at 70 % $\dot{V}O_2$ max in a group of participants varying in aerobic fitness might mean one participant is working below his/her Anaerobic threshold (ATh) and another maybe working above his/her ATh. Whipp et al., (2005) suggests that this issue can be overcome by assigning exercise intensity with reference to exercise 'domain-based' threshold events in the profiles of the muscle metabolic and pulmonary gas exchange responses, as described in Chapter 2 'General methods'. Section 2.5.1. 'Methodological considerations: an oxygen uptake dynamics approach'.

We suggest that this approach maybe more appropriate in the study of endurance exercise–induced hormone and cytokine changes than traditional methods given that the

Messenger ribonucleic acid (mRNA) of many metabolically sensitive proteins are expressed in skeletal muscle and/or adipose tissue in response to exercise. Zaldivar et al., (2006) employed this approach by investigating the effect of 30 minute bouts of heavy cycle ergometry exercise on the expression of pro- and anti-inflammatory cytokines and growth factors in leukocytes following exercise. It appears however, that a comparison has not be performed to assses the relationship between physiological data resulting from a cycle ergometer $\dot{V}O_2$max test, performed in a manner capable of distinguishing oxygen uptake dynamics and therefore exercise domains, and constant work-load cycle ergometer exercise trials, conducted at an intensity specifically to elicit cardio-respiratory characteristics reflective of those domains. The objective of this chapter is therefore to compare data from a cycle ergometer $\dot{V}O_2$max test, conducted using principles and techniques associated with oxygen uptake dynamics research, with that from three acute 'domain-based' constant work-load cycle ergometer exercise interventions of varying intensity, but equal total work done. We hypothesise that mean $\dot{V}O_2$, HR and rate of oxygen uptake divided by heart rate ($\dot{V}O_2$/HR) data obtained from moderate, heavy and very heavy domain cycle ergometer exercise trials would parallel those obtained during a $\dot{V}O_2$max test.

3.3. Methods

Methodological information directly pertinent to this chapter is summarised briefly below. Chapter 2. 'General methods', provides a detailed reference resource, documenting the procedures employed in collecting the data presented in this thesis. Relevant sections in Chapter 2 will be referenced in the text by index number, e.g. [2.2.1.] refers to the section 2.2.1. 'Ethical approval'.

3.3.1. Study design

A schematic representation of the interlinking studies presented in this thesis is provided in figure 2.4 [2.5.1.8.1.]. The data presented in this chapter relate to Exercise studies 1 and 2.

3.3.2. Participants [2.2.]

3.3.3. Maximal oxygen uptake determination protocol [2.3.1.]

3.3.4. Determination of exercise trial intensity and duration [2.5.1.5. & 2.5.1.7.]

3.3.5. Exercise trial protocols [2.5.1.8]

3.3.6. Statistical analyses [2.8.]

Participant descriptive data. Independent-samples t-Tests were performed to determine if age, body mass, stature, Body mass index (BMI), $\dot{V}O_2$max, Power output at $\dot{V}O_2$max (POmax), and Heart rate at $\dot{V}O_2$max (HRmax) were significantly different between the participant groups in exercise study 1 and exercise study 2.

Participant cardio-respiratory data. Repeated-measures analysis of variance (ANOVA) with as within factor 'study' and between factor 'work-load' were run to determine whether there were significant differences between the two studies (Study 1 *vs.* Study 2) and three work-loads (moderate (80 % GET), heavy (30 % Delta (Δ)) and very heavy (60 % Δ) intensity exercise) for PO, $\dot{V}O_2$, HR and $\dot{V}O_2$/HR. If significant effects were observed, post-hoc Bonferroni-corrected one-way ANOVA were run to identify the differences.

Exercise trial comparison analyses. Pearson's product-moment correlation coefficients were computed to assess the relationship between $\dot{V}O_2$, HR and $\dot{V}O_2$/HR data for moderate (80 % GET), heavy (30 % Δ) and very heavy (60 % Δ) intensity exercise determined from $\dot{V}O_2$max tests and data measured directly during the exercise trials. Paired samples t-Tests were performed to determine if $\dot{V}O_2$, HR and $\dot{V}O_2$/HR data for moderate (80 % GET), heavy (30 % Δ) and very heavy (60 % Δ) intensity exercise, determined from $\dot{V}O_2$max tests, varied significantly from data measured directly during the exercise trials.

3.4. Results

3.4.1. Participant descriptive data

Studies 1 and 2 were linked by heavy intensity exercise trials to link them, i.e. the studies were designed so participants in both studies 1 and 2 performed heavy intensity exercise of equivalent duration, ensuring equal work was performed within each study and between both trials.

No statistically significant differences were evident between the participant groups in study 1 and 2, and the mean $\dot{V}O_2max$ values suggest excellent aerobic fitness (table 3.1) (Heyward 1998).

Table 3.1. Participant descriptive and maximal exercise cardio-respiratory data

Participant details	**Study 1: Moderate (60 min) and Heavy (equal work) exercise**	**Study 2: Heavy (30 min) and Very Heavy (equal work) exercise**	**Statistical analyses (main effect)**
Gender, *n*	male, 6	male, 7	-
Age (yrs.)	28 ± 5	26 ± 7	$P = 0.57$
Body mass (kg)	81.1 ± 14.6	82.9 ± 14.3	$P = 0.83$
Stature (m)	1.79 ± 0.03	1.84 ± 0.07	$P = 0.15$
BMI (kg/m^2)	25 ± 4	25 ± 4	$P = 0.78$
$\dot{V}O_2max$ (ml/min)	4630 ± 581	4242 ± 874	$P = 0.38$
POmax (W)	334 ± 43	326 ± 71	$P = 0.80$
HRmax (b/min)	180 ± 11	181 ± 14	$P = 0.90$

3.4.2. Participant moderate, heavy and very heavy domain exercise cardio-respiratory data

Participant cardio-respiratory data indicate that there were no statistically significant differences between heavy (30 % Δ) intensity exercise (Study 1 *vs.* Study 2) for the variables analysed (table 3.2). There were no significant differences in total work done. A significant work-load effect was observed, with heavy (30 % Δ) intensity exercise eliciting a greater response than moderate (80 % GET) intensity exercise, and very heavy (60 % Δ) intensity exercise eliciting a greater response than heavy (30 % Δ) intensity exercise for PO (P = 0.01) and $\dot{V}O_2$ (P = 0.02). Similarly, heavy (30 % Δ) intensity exercise elicited a greater response than moderate (80 % GET) intensity exercise for HR ($P < 0.001$) and $\dot{V}O_2$/HR ($P = 0.003$). However, in these cases there were no statistically significant differences between heavy (30 % Δ) and very heavy (60 % Δ) intensity exercise. These data confirm exercise intensity significantly impacts cardio-respiratory responses.

Table 3.2. Participant moderate, heavy and very heavy domain exercise cardio-respiratory data

Exercise variables	Study 1		Study 2		Statistical analyses	
	Moderate (60 min)	Heavy (equal work)	Heavy (30 min)	Very Heavy (equal work)	Study 1 *vs.* Study 2	Work-load
Trial time (min:s)	60:00 ± 00:00	27:48 ± 02:31	30:00 ± 00:00	22:29 ± 01:31	-	-
PO (W)	84 ± 15	182 ± 19	184 ± 51	244 ± 57	NS	$P = 0.01$ M < H < VH
$\dot{V}O_2$ (ml/min)	1541 ± 188	2644 ± 231	2768 ± 670	3467 ± 723	NS	$P = 0.02$ M < H < VH
HR (b/min)	101 ± 10	135 ± 13	134 ± 19	152 ± 12	NS	$P = 0.001$ M < H, VH
$\dot{V}O_2$/HR (ml/b/min)	15 ± 1	20 ± 3	19 ± 4	23 ± 5	NS	$P = 0.003$ M < VH
Total work done (KJ)	303 ± 53	304 ± 53	303 ± 92	331 ± 93	NS	NS

M – moderate, H – heavy, VH – very heavy, NS - Not statistically significant.

3.4.3. Exercise trial comparison analyses

Having ascertained the relevant cardio-respiratory data, we next wished to establish whether a relationship existed between exercise intensity determined from $\dot{V}O_2$max tests and constant work-load exercise trials, for relevant output measures ($\dot{V}O_2$, HR and $\dot{V}O_2$ /HR data). To this end, Pearson's product-moment correlation coefficients indicated significant, positive correlations for $\dot{V}O_2$ ($r = 0.944$, $n = 25$, $P < 0.001$; figure 3.1), HR ($r = 0.903$, $n = 24$, $P < 0.001$; figure 3.2) and $\dot{V}O_2$/HR data ($r = 0.824$, $n = 24$, $P < 0.001$; figure 3.3) between moderate (80 % GET), heavy (30 % Δ) and very heavy (60 % Δ) intensity exercise. No significant differences were revealed between data for $\dot{V}O_2$ ($P = 0.16$), HR ($P = 0.50$) and $\dot{V}O_2$/HR ($P = 0.42$) determined from $\dot{V}O_2$max tests and measured directly during subsequent exercise trials at moderate (80 % GET), heavy (30 % Δ) and very heavy (60 % Δ) intensities.

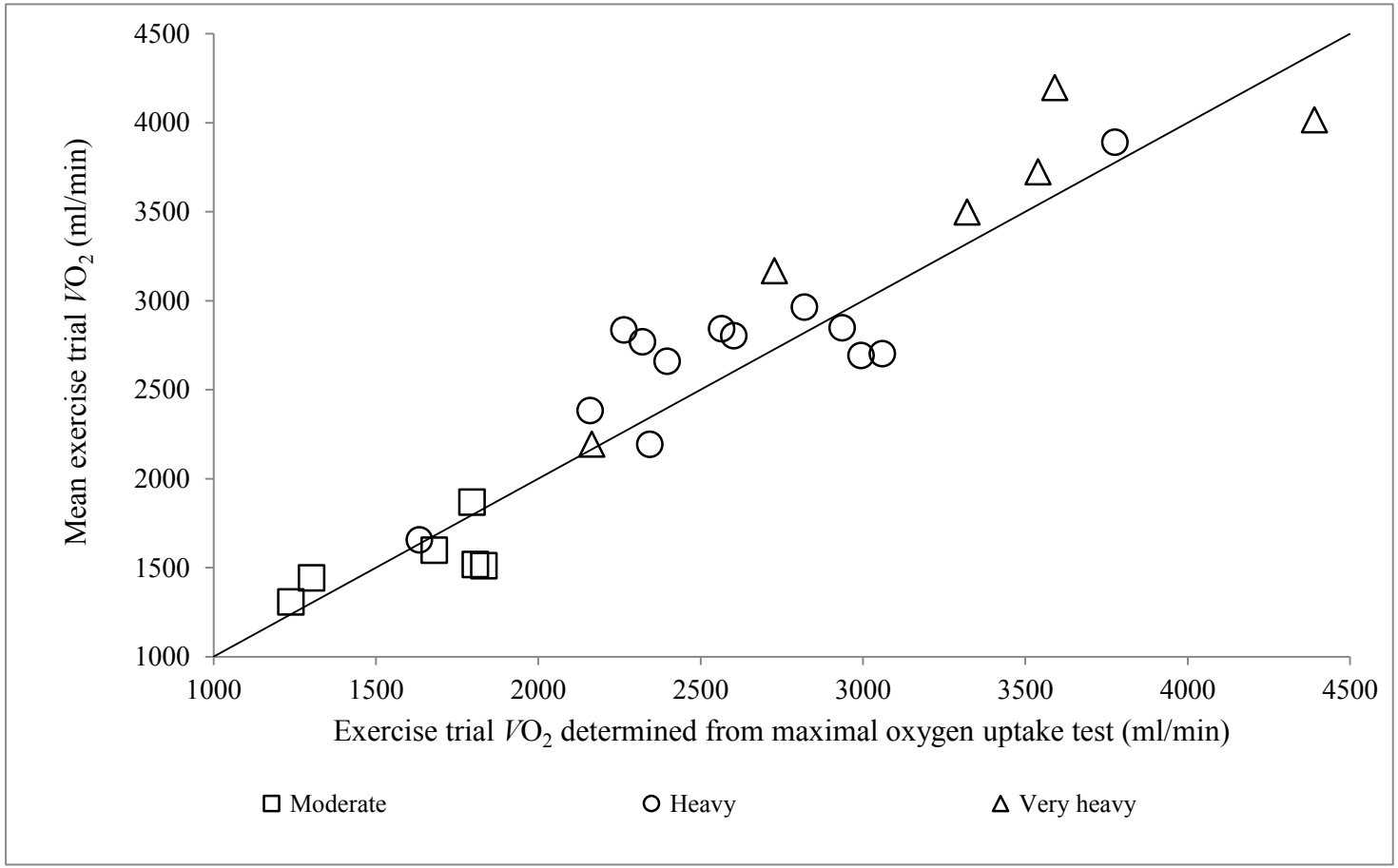


Figure 3.1. Correlation between $\dot{V}O_2$ for moderate (80 % GET), heavy (30 % Δ) and very heavy (60 % Δ) intensity exercise during a $\dot{V}O_2$max test and $\dot{V}O_2$ measured during constant work-load exercise trials at those intensities (r = 0.944, n = 25, P < 0.001).

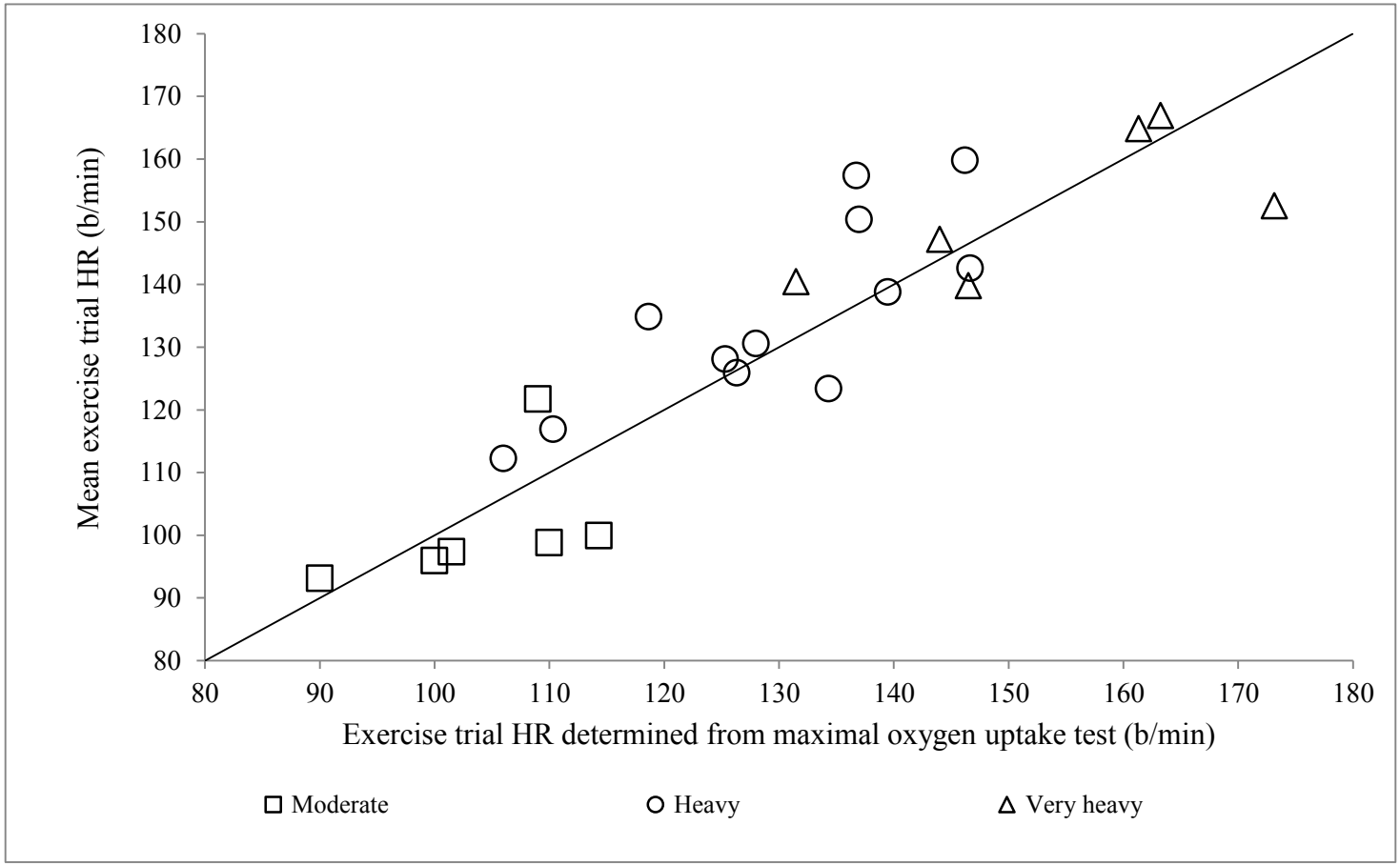


Figure 3.2. Correlation between HR for moderate (80 % GET), heavy (30 % Δ) and very heavy (60 % Δ) intensity exercise during a $\dot{V}O_2$max test and HR measured during constant work-load exercise trials at those intensities (r = 0.903, n = 24, P < 0.001).

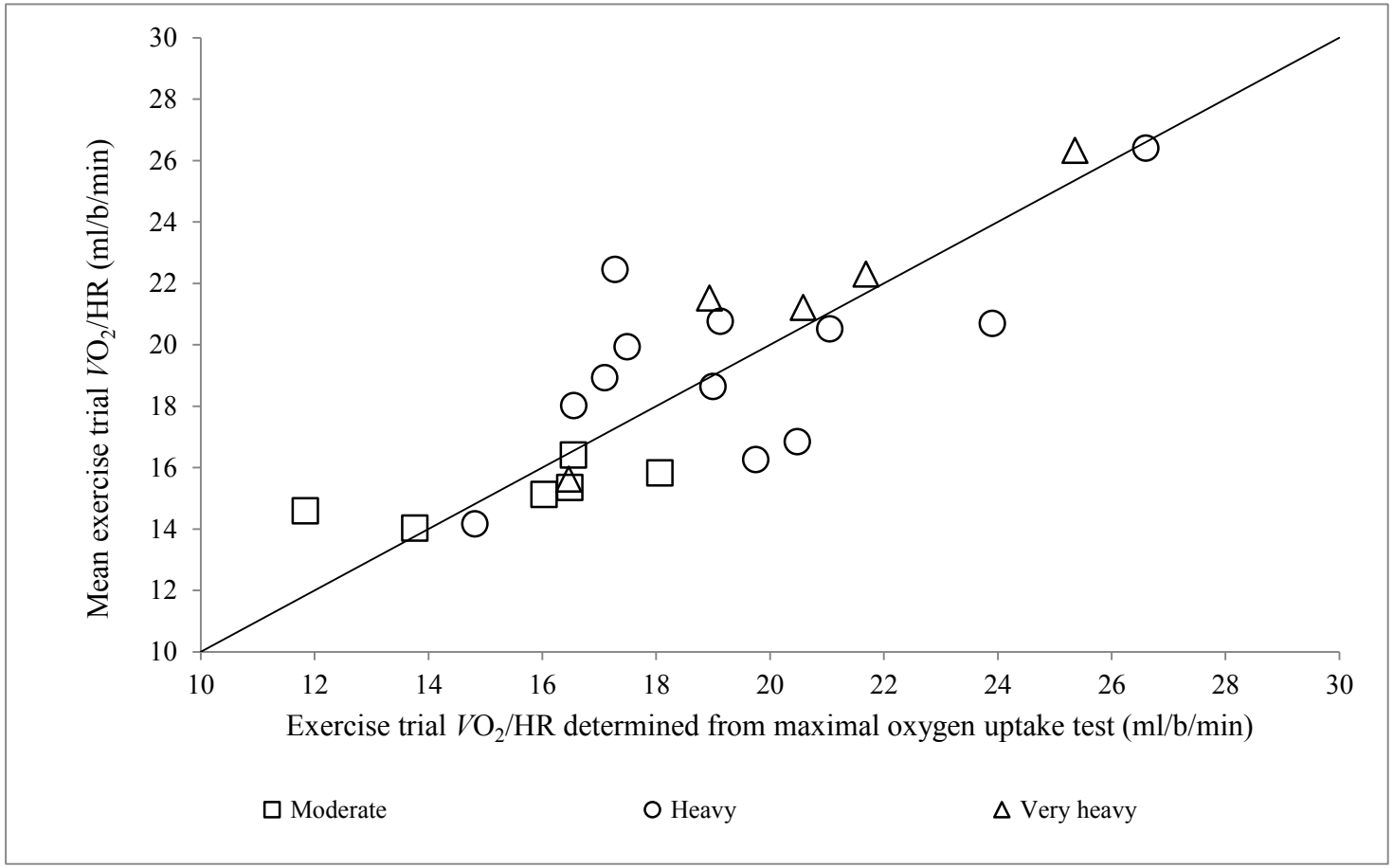


Figure 3.3. Correlation between $\dot{V}O_2$/HR for moderate (80 % GET), heavy (30 % Δ) and very heavy (60 % Δ) intensity exercise during a $\dot{V}O_2$max test and $\dot{V}O_2$/HR measured during constant worload exercise trials at those intensities (r = 0.824, n = 24, P < 0.001).

3.5. Discussion

3.5.1. Study design and participant descriptive data

Two independent studies were designed and linked by the inclusion of heavy domain constant work-load cycle ergometry trials. Within each study, exercise duration was carefully considered to ensure equal total work done between trials and relative commonality between the duration of exercise performed within the heavy domain. Linking two studies in this way enabled them to run in tandem. Successfully recruited participants were allocated to the two studies, thus reducing the required commitment and potentially influencing adherence to and completion of the study protocols in what would otherwise have been a highly demanding protocol for a single study group, while also increasing the number of participants completing heavy domain exercise and avoiding ethical and methodological issues associated with multiple biopsies over a short period of time. Independent-samples t-Tests suggested there were no significant differences between the two participant groups in terms of age (P = 0.57), body mass (P = 0.83), stature (P = 0.15), BMI (P = 0.78), $\dot{V}O_2$max (P = 0.38), POmax (P = 0.80) and HRmax (P = 0.90) (table 3.1). The participants from the heavy domain trial could therefore be considered as a single representative group, indicating that data from these two trials can be pooled.

3.5.2. Methodological considerations

Good practice was followed in that the cycle ergometry performed using a Jaeger Ergoline ER800 electronically-braked cycle ergometer was employed throughout data collection, and participants presented themselves at the laboratory rested, fasted and euhydrated at the same time of day for each session. Detection of the GET, and therefore precise assignment of moderate domain (80 % GET) exercise, was made possible by the use of continuous, breath-by-breath, analyses of expired gas during a fine incremental ramp from the low intensity baseline to the point of volitional exhaustion. This allows the $\dot{V}O_2$ and the rate of carbon dioxide production ($\dot{V}CO_2$) profiles to be recorded in sufficient resolution to accurately establish the GET via the V-slope method. This is in direct contrast to traditional discontinuous protocols utilising Douglas bag expired gas collection methods (Hill and Lupton, 1923; Hill et al., 1924; Astrand, 1967) that at best

provide single mean $\dot{V}O_2$ and $\dot{V}CO_2$ datum values from prolonged collection periods lasting 30 s or more.

The protocol for establishing Maximal lactate steady state (MLSS) typically involves 5 - 6 exercise trials of 20 - 30 min duration conducted at increasing work-loads and with sufficient rest (24 hrs.) to ensure complete recovery. Finger-prick blood samples are also usually collected at 5 minute intervals and analysed for Blood lactate (BLa) to establish the highest constant work-load at which BLa is elevated above that at rest, but maintained at a constant level (Beneke and von Duvillard, 1996; Jones and Doust, 1998). Undertaking this additional work would have greatly prolonged the testing and the participants' involvement, potentially negatively impacting participant recruitment and adherence to and completion of the study protocols. Pringle and Jones (2002) identified MLSS occurred at ~ 54 % Δ during cycle ergometry performed by healthy individuals (n = 8; age, 25 ± 3 yrs.; body mass, 72.1 ± 8.2 kg; stature, 1.76 ± 8.2 m; $\dot{V}O_2$max, 3730 ± 844 ml/min). With reference to this finding, heavy domain exercise trials were conducted at 30 % Δ with very heavy domain exercise conducted at 60 % Δ, and no further sessions were deemed necessary to establish MLSS in each participant.

3.5.3. Criteria for successful determination of $\dot{V}O_2$max

Organisations such as The British Association of Sport and Exercise Sciences (BASES) and American College of Sports Medicine (ACSM) have produced clear guidelines on maximal exercise testing, which include suggested assessment criteria for determining whether or not a participant attained true maximum. The literature is divided between those that do adopt such criteria and those that do not. It appears many studies choose simply to accept the highest $\dot{V}O_2$ value attained before volitional exhaustion as $\dot{V}O_2$max. The most widely accepted criterion is a plateau in the $\dot{V}O_2$ response with increasing work-load, a ceiling effect. The numerous secondary criteria that have been used in the literature appear to have been developed in response to an apparent lack of a plateau in $\dot{V}O_2$ response, a phenomenon brought about in part by the use of discontinuous protocols in which Douglas bags are used to collect expired gas samples, thus providing a low resolution description of the $\dot{V}O_2$ response. Such criteria have received heavy criticism (Howley et al., 1995; Poole et al., 2008; Midgley et al., 2007). The wide natural variability in the measurements themselves is cited as an argument questioning the validity of such

criteria. The result being that by imposing such criteria we may potentially exclude participants that have attained $\dot{V}O_2$max and include those that have not. The use of a verification stage has been suggested as a suitable criterion (Poole et al., 2008; Midgley et al., 2007; Midgley et al., 2009). In performing a verification stage a participant is required to exercise at a work-load in excess of that at which volitional exhaustion occurred. The procedure is typically performed following a short rest, after the $\dot{V}O_2$max test. If $\dot{V}O_2$max was achieved during the $\dot{V}O_2$max test, the $\dot{V}O_2$ value measured after the verification stage will be no higher. If a higher $\dot{V}O_2$ value is measured during the verification stage that value can be accepted as $\dot{V}O_2$max or further verification stages can be performed at increased work-loads until $\dot{V}O_2$max is verified. Verification stages were not employed in the present investigation.

The criteria formulated and adopted in the present investigation are as follows: 1) primary criterion - a plateau in $\dot{V}O_2$ data with increasing work-load, 2) secondary criteria - attainment of age predicted HRmax within 10 b/min, predicted using the equation 205.8 – 0.685 x age (Inbar et al., 1994), 3) attainment of a Respiratory exchange ratio (RER) of 1 or greater, and 4) a Rating of perceived exertion (RPE) of 18 or greater. A re-test was performed if the primary criterion (a plateau in the $\dot{V}O_2$ data with increasing work-load) was not observed, and two out of the three secondary criteria were not met. The $\dot{V}O_2$ plateau is the principle on which the verification stage procedure is founded, and as such is still the criterion by which attainment of $\dot{V}O_2$max can be assessed. The use of modern breath-by-breath expired gas analysis systems allow high resolution $\dot{V}O_2$ and $\dot{V}CO_2$ data to be recorded, facilitating the detection of a $\dot{V}O_2$ plateau. Equations for the prediction of HRmax have been reviewed by Robergs and Landwehr (2002). Robergs and Landwehr (2002) suggested that the widely used equation 220 – age, attributed to Fox and colleagues (Fox et al., 1971), has no scientific merit for use in exercise physiology and related fields as it was not developed from original research, but resulted from observation based on data from 11 references consisting of published research or unpublished scientific compilations. Robergs and Landwehr (2002) also suggested that although at present there is no acceptable equation for the prediction of HRmax, the formula presented by Inbar et al., (1994) provides the most accurate general equation. We have chosen to substitute this equation rather than the traditional 220 – age in our assessment criteria with regard to attainment of HR within 10 b/min of age predicted HRmax. In reviewing $\dot{V}O_2$max test criteria Poole et al., (2008) suggested that in some participants

RER may not increase above 1 (Sidney and Shephard, 1977), whereas in others it may exceed 1.4 (Buchfuhrer et al., 1983). Poole et al., (2008) also suggested that adopting an RER criterion > 1 clearly excludes participants that may have achieved $\dot{V}O_2$max. Achieving an RER of 1 indicates that the exercise intensity was great enough to require some respiratory buffering of lactic acid-derived Hydrogen ions (H^+), and thus suggests that the test was not ended due to lack of participant motivation. The reporting of RPE values (Borg, 1982; 1970) is highly subjective. However, attainment of level 18 on the RPE scale has been used as a criterion for achieving $\dot{V}O_2$max (Tanaka et al., 1997). This criterion provides a method by which the participant can demonstrate that they felt as though they were nearing or applying maximum effort. Participants in the present investigation attained $\dot{V}O_2$max determined by the criteria specified. The $\dot{V}O_2$max test is a fundamental measure of exercise physiology. However, it is clear that a universally acceptable criteria indicating true attainment of $\dot{V}O_2$max has not been reached. Further research is warranted to achieve such a criteria, which should be universally adopted in exercise studies.

3.5.4. Participant moderate, heavy and very heavy domain exercise cardio-respiratory analyses

Data from the two feasibility studies (data not reported) suggested the intensity and duration of all exercise trials would be well tolerated and would stimulate changes in the systemic concentration of a number of hormones and cytokines that could be quantified in our laboratory, via Enzyme-linked immunosorbent assay (ELISA), for further analyses. This was found to be the case. Exercise trials were well tolerated by the participants. The results show significant differences between moderate (80 % GET), heavy (30 % Δ) and very heavy (60 % Δ) exercise, but no significant effect of 'study' on the investigated parameters. These data confirm significant differences exist between exercise trials. Further, no significant differences were observed between overlapping exercise trials conducted in the heavy domain (heavy (equal work), Study 1 and heavy (30 min), study 2), indicating the pooling of data from these two trials is appropriate. However, data suggests that no significant differences are present in HR for exercise trials conducted in the heavy (30 % Δ) and very heavy (60 % Δ) exercise domains ($P = 0.07$), and $\dot{V}O_2$/HR for exercise trials conducted in the moderate (80 % GET) and heavy (30 % Δ) exercise domains ($P = 0.07$), and heavy (30 % Δ) and very heavy (60 % Δ) exercise domains ($P =$

0.19). The lack of statistically significant differences in the post-hoc analyses across trials for variables HR and $\dot{V}O_2$/HR can be accounted for by the variability in HR resulting from the diversity of fitness levels of the participants, compounded by the effect of the $\dot{V}O_2$-slow component (Jones et al., 1999) and the influence of cardio-vascular drift (Coyle and González-Alonso, 2001). Mean values representing the metabolic cost of constant work-load exercise trials were calculated from $\dot{V}O_2$ and HR data during the periods (a) between 2 and 3 min following the onset of the exercise trial work-load, and (b) during the final 60 s. These sample periods were chosen as (a) represents the steady state component, the point at which the $\dot{V}O_2$ response plateaus at the end of the primary component phase, and (b) takes into account any increase in the $\dot{V}O_2$ attributed to the slow component present in exercise in the heavy and very heavy domains (Whipp and Wasserman, 1972; Whipp et al., 1982), thus providing an account of exercise trial oxygen cost regardless of exercise trial duration.

3.5.5. Exercise trial comparison analyses

A measure of the accuracy of assigning constant work-load exercise from a $\dot{V}O_2$max test is provided by the Pearson's product-moment correlation coefficients and paired samples t-Tests, computed to assess the relationship between data calculated from the cycle ergometer $\dot{V}O_2$max test and that measured directly during the constant work-load exercise trials. Strong positive correlation coefficients were obtained for $\dot{V}O_2$, HR and $\dot{V}O_2$/HR (figures 3.1, 3.2 and 3.3) suggesting strong agreement between the two data sets. The results of the paired samples t-Tests suggest that there are no significant differences between the data sets.

3.5.6. Summary and conclusion

In line with the objective of this chapter, to compare data from a cycle ergometer $\dot{V}O_2$ max test, conducted using principles and techniques associated with oxygen uptake dynamics research, with that from three acute 'domain-based' constant work-load cycle ergometer exercise interventions of varying intensity, but equal total work done, we have presented evidence comparing an alternative method with which constant work-load cycle ergometry exercise can be accurately assigned from a $\dot{V}O_2$max test. This represents a departure from the traditional and widely used approach in exercise physiology research

of assigning constant work-load exercise in groups of participants with reference to a percentage of the maximum rate of oxygen uptake (% $\dot{V}O_2$max), a percentage of heart rate at $\dot{V}O_2$max (% HRmax), or a target HR derivative of a % $\dot{V}O_2$max value, as is the case with the Karvonen formula (Karvonen and Vuorimaa, 1988), in favour of an exercise 'domain-based' classification. We hypothesised that mean $\dot{V}O_2$, HR and $\dot{V}O_2$/HR data obtained from equal work (varying duration) moderate (80 % GET) (M), heavy (30 % Δ) (H) and very heavy (60 % Δ) (VH) domain cycle ergometer exercise trials would parallel those obtained during a $\dot{V}O_2$max test. The data support a conclusion that constant work-load cycle egometer exercise trials, performed at a power output identified during a incremental exercise test carried out with regard to the principals and methods of oxygen uptake dynamics research, yield similar cardio-respiratory characteristics. We can therefore continue and test our hypothesis that the constant work-load cycle ergometer exercise trials developed would result in significant differences in the mRNA expression of selected hormones and cytokines despite the measures taken to ensure equal work done.

Chapter 4. Changes in mRNA expression in skeletal muscle and subcutaneous adipose tissue: response to exercise intensity

4.1. Abstract

In the previous chapter we concluded that the equal work (varying duration) moderate (M), heavy (H) and very heavy (VH) constant work-load cycle ergometer exercise trials performed yielded physiological data ($\dot{V}O_2$, HR and $\dot{V}O_2$/HR) parallel to those obtained during a $\dot{V}O_2$max test at the same power output. We were confident that the trials would produce hormone and cytokine responses characteristic of the exercise domain in which they reside in the recreationally active young participant group, and could therefore progress with the analyses of gene expression. We hypothesised that the constant work-load cycle ergometer exercise trials developed in the previous chapter would result in significant differences in the mRNA expression of selected hormones and cytokines despite the measures taken to ensure equal work done. We analysed the expression of hormone and cytokine mRNA in skeletal muscle and subcutaneous adipose tissue bioptic material collected at baseline (B) and following the M, H and VH exercise trials. Absolute expression at B of PGC-1α ($P = 0.02$), leptin ($P < 0.001$) and adiponectin ($P < 0.001$) were found to be greater in subcutaneous adipose tissue than skeletal muscle. Conversely, expression of TNFα ($P = 0.003$), TRIM72 ($P < 0.001$), SOCS3 ($P = 0.003$) and housekeeping gene GAPDH ($P < 0.001$) were found to be significantly greater in skeletal muscle than subcutaneous adipose tissue. Post exercise, increased expression (GAPDH normalised) of IL-6: B *vs.* M ($P = 0.006$), H ($P < 0.001$) and VH ($P < 0.001$) and M *vs.* VH ($P = 0.02$); TNFα: B *vs.* VH ($P = 0.04$) and SOCS3: B *vs.* M ($P = 0.02$), and VH ($P = 0.04$) was observed. Exercise was without effect on gene expression in subcutaneous adipose tissue. We concluded that our data confirm the importance of skeletal muscle as a source of endurance exercise responsive hormones and cytokines, and highlight exercise intensity and duration as a mechanisms regulating the expression of IL-6, TNFα and SOCS3, proteins with metabolic, inflammatory and cytokine suppressive roles respectively, in the tissue.

4.2. Introduction

Pedersen (2009) suggested the beneficial effects of endurance exercise are due to the anti-inflammatory effects of regular exercise, through a reduction in adipose tissue mass and/or induction of an anti-inflammatory environment with each acute bout of endurance exercise. Myokines, cytokines and other peptides released by skeletal muscle fibres as a result of contractile activity (Pedersen et al., 2003), are thought to play a role in mediating the beneficial effects of exercise (Pedersen, 2009; Pedersen and Febbraio, 2012). Adipokines, secreted from adipose tissue regulate energy metabolism (Yu and Ginsberg, 2005; Ronti et al., 2006) and have been implicated in related pathological conditions such as chronic low-grade systemic inflammation (Tilg and Moschen, 2006; Arai et al., 2011). Cross-talk between these two tissues, 'the adipose-muscular axis', may function to satisfy metabolic demand during and post-exercise. The mechanisms however require clarification. The objective of this chapter is therefore to establish the changes in the Messenger ribonucleic acid (mRNA) expression of selected hormones and cytokines that occur locally in skeletal muscle and subcutaneous adipose tissue as a function of our acute 'domain-based' constant work-load cycle ergometer exercise interventions in recreationally active young males. We hypothesise that the constant work-load cycle ergometer exercise trials developed in the previous chapter will result in significant differences in the mRNA expression of selected hormones and cytokines despite the measures taken to ensure equal work done.

4.3. Methods

Methodological information directly pertinent to this chapter is summarised briefly below. Chapter 2. 'General methods', provides a detailed reference resource, documenting the procedures employed in collecting the data presented in this thesis. Relevant sections in Chapter 2 will be referenced in the text by index number, e.g. [2.2.1.] refers to the section 2.2.1. 'Ethical approval'.

4.3.1. Study design

A schematic representation of the interlinking studies presented in this thesis is provided in figure 2.4 [2.5.1.8.1.]. The data presented in this chapter relate to Exercise studies 1 and 2.

4.3.2. Participants [2.2.]

Participant descriptive and exercise cardio-respiratory data are provided in Chapter 3, Tables 3.1. and 3.2.

4.3.3. Determination of exercise trial intensity and duration [2.5.1.5. & 2.5.1.7.]

4.3.4. Exercise trial protocols [2.5.1.8.]

4.3.5. Skeletal muscle and subcutaneous adipose tissue sampling [2.7.]

Skeletal muscle and subcutaneous adipose tissue samples were obtained post-exercise via conchotome technique (Dietrichson et al., 1980; 1987). The procedure was performed by a suitably qualified, General Medical Council registered, medical doctor experienced in the extraction of skeletal muscle and adipose tissue. Skeletal muscle samples were obtained from the *Vastus Lateralis* through a small incision following preparation of the skin surface and local anaesthesia of the skin and immediate underlying tissue. Immediately after harvesting the skeletal muscle tissue sample, a sample of subcutaneous adipose tissue was obtained from the adipose tissue bed surrounding the site of the incision. Wound closure was undertaken using sterile adhesive strips and overlayed with a sterile compressive dressing. Participants were advised when to remove the dressing and how to care for the wound, to refrain from exercise for at least 4 days and to inform the principal investigator and their general practitioner should the wound become infected.

Skeletal muscle tissue and subcutaneous adipose tissue samples were transferred to a Class II Biological Safety Cabinet, washed in ice-cold phosphate buffered saline to remove blood and then divided for tissue culture studies and for detection of mRNA expression. Samples were transferred to 1.2 ml cryovials rapidly frozen and stored in liquid nitrogen to await further analyses.

4.3.6. Skeletal muscle and adipose tissue homogenisation [2.7.2.] and ribonucleic acid isolation [2.7.3.]

Cryovials containing the skeletal muscle and adipose tissue samples were removed from liquid nitrogen and homogenised in the presence of Trizol (TRI®) Reagent Solution before being returned to storage at -80 °C for one freeze thaw cycle before Ribonucleic acid (RNA) isolation. Samples were supplemented with 0.2 ml Chloroform and mixed vigorously for 20 s prior to centrifugation at 12000 g for 16 min at 4 ˚C. The aqueous phase was transferred into a 1.5 ml RNase-free microcentrifuge tube. Care was taken not to disturb the interface Deoxyribonucleic acid (DNA) layer. Ribonucleic acid was precipitated from the aqueous phase using Isopropanol. Samples were mixed by inversion twice prior to being centrifuged at 12000 g for 10 min at 4 °C. The supernatant was carefully removed and the RNA pellet was washed with 75 % ethanol prior to centrifugation at 8500 g for 6 min at 4 ˚C. Ethanol was removed and RNA pellets were left to air dry before being re-suspended in 100 µl RNA storage solution. Ribonucleic acid was returned to -80 °C for storage until further analyses.

4.3.7. Oligonucleotide primer design and synthesis [2.7.4.]

The Genome Bioinformatics Group of UC Santa Cruz website genome browser (no date) was used to identify the DNA sequences for the selected genes of investigation. Oligonucleotide primers were designed using Beacon Designer version 5.11. Oligonucleotide primers were designed to yield products spanning exon-intron boundaries to avoid genomic DNA contamination. The inclusion of three or more GC base pairs in the last 5 base pairs at the 3' end of the primer was avoided in order to minimise nonspecific amplification. All primers had a nucleotide base length of between 18 and 22 base-pair sequences. Beacon Designer version 5.11 was also used to perform searches for secondary structure or inter/intra-molecular interactions such as hairpins, self-dimers and cross-dimers within the primer. Sequence homology searches were performed against the National Center for Biotechnology Information (NCBI) Genbank® database to ensure specific amplification of the associated target gene. Custom oligonucleotide primers were purchased from Sigma-Genosys or from QIAGEN.

4.3.8. Reverse transcription-polymerase chain reaction method and data analysis [2.7.5.]

Ribonucleic acid concentration and purity were assessed via ultraviolet spectroscopy from the optical density at 260 and 280 nm using a spectrophotometer (WPA Biowave II, Biochrom Ltd., Cambridge, UK). Seventy ng RNA were used per Reverse transcription-polymerase chain reaction (RT-PCR) (7 µl of RNA at a concentration of 10 ng/µl). Amplification of target genes via RT-PCR was performed using a commercially available Power SYBR Green RNA-to-C_T 1 step kit and 96-well RT-PCR plates. The custom RT-PCR protocol was controlled using a Chromo4^{TM} DNA engine interfaced with a laptop computer running Opticon Monitor version 3.1.32. Opticon Monitor version 3.1.32 constructs a graphical representation of fluorescence yield against RT-PCR cycle. The cycle number at which fluorescence yield crossed a threshold line (C(t)) was obtained by manually positioning a threshold line at a point along the exponential rise in the fluorescence/cycle curve. The data were exported to an Excel® version 12.2.0 spreadsheet (Microsoft® Excel® 2008 for Mac, Microsoft Corporation, Redmond, WA, USA) for analyses. The Delta delta (ΔΔ) C(t) equation (Livak Method) was used to determine normalised gene expression.

Sterile technique was implemented throughout. RNase contamination from work surfaces and equipment reserved for molecular techniques was minimised prior to use by exposure to RNase Zap. Fluid transfer activities were performed using pipettes reserved exclusively for molecular biology techniques. These were used with sterile Aerosol Resistant (ART®) pipette tips.

4.3.9. Statistical analyses [2.8.]

Absolute Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) expression in skeletal muscle and adipose tissue comparison. Paired samples t-Tests were performed to assess whether there was a significant difference between absolute expression of the house-keeping gene GAPDH in skeletal muscle compared with adipose tissue.

Heavy exercise-induced local mRNA expression trial comparisons. Independent samples t-Tests were performed to assess whether there were significant differences in local mRNA expression induced by heavy exercise performed in study 1 and study 2. Where data were non-normally distributed, and could not be successfully log or inversely transformed, non-parametric Mann-Whitney U statistical analyses were performed. A repeated-measures Analysis of variance (ANOVA) was subsequently performed,

incorporating baseline and post-heavy exercise Suppressor of cytokine signaling 3 (SOCS3) data with as within factor 'time' and between factor 'study' to check for a 'time x study interaction'.

Baseline absolute mRNA expression in skeletal muscle and adipose tissue. Paired samples t-Tests were performed to assess whether there were significant differences between baseline absolute mRNA expression in skeletal muscle compared with adipose tissue.

Moderate, heavy and very heavy exercise-induced local mRNA abundance. Repeated-measures ANOVA with as within factor 'time' and between factor 'work-load' were run to determine whether there were significant differences between local mRNA expression induced by moderate, heavy and very heavy exercise. If significant effects were observed, post-hoc Bonferroni-corrected one-way ANOVA were run to identify the difference. Where data were non-normally distributed, and could not be successfully log or inversely transformed, non-parametric Kruskal Wallis statistical analyses were run to determine whether there were significant differences. If significant effects were observed, post-hoc Mann-Whitney U tests for individual data set pairs were performed to locate the difference. Bonferroni-corrections were applied to identify true significance.

4.4. Results

4.4.1. Variation in the reference gene GAPDH and the effect of 'study'

The expression of GAPDH in skeletal muscle tissue was higher than that in adipose tissue ($P < 0.001$; figure 4.1). Furthermore, absolute expression of GAPDH was unchanged as a consequence of the exercise interventions in both skeletal muscle and adipose tissue (data not shown).

Gene expression induced by heavy (30 % Δ) exercise performed in study 1 and study 2 was analysed for an effect of 'study'. There were no significant differences for most of the investigated proteins. SOCS3 was the exception and mRNA levels were lower in study 1 than study 2 (mean ± SD, Study 1 1.03 ± 0.06, Study 2 1.10 ± 0.04; $P = 0.02$). However, heavy exercise induced a similar increase in SOCS3 gene expression in study 1 and 2, as reflected by an absence of a 'time x study interaction' ($P = 0.24$). The mRNA

data from heavy exercise trials performed in study 1 and study 2 were therefore grouped and analysed as one trial.

4.4.2. Baseline and exercise-induced changes in local mRNA expression in skeletal muscle and adipose tissue

Baseline absolute gene expression of Peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) ($P = 0.02$), leptin ($P < 0.001$) and adiponectin ($P < 0.001$) were found to be greater in adipose tissue than in skeletal muscle. Conversely, expression of Tumour necrosis factor-alpha (TNFα) ($P = 0.003$), Tripartite motif-72 (TRIM72) ($P < 0.001$), and SOCS3 ($P = 0.003$) were found to be significantly greater in skeletal muscle than adipose tissue (figure 4.1).

Significant exercise-induced increases were evident in skeletal muscle following GAPDH normalisation for Interleukin-6 (IL-6) ($P < 0.001$), TNFα ($P = 0.02$), and SOCS3 ($P = 0.006$; figure 4.2). Post-hoc Bonferroni corrected comparisons identified significant increases in IL-6 expression between baseline and moderate ($P = 0.006$), heavy ($P < 0.001$) and very heavy ($P < 0.001$) exercise, also moderate and very heavy ($P = 0.02$) exercise. A significant increase in TNFα expression was revealed between baseline and very heavy exercise ($P = 0.04$). A significant increase in SOCS3 expression was revealed between baseline and moderate ($P = 0.02$), and very heavy ($P = 0.04$) exercise. No significant exercise-induced changes in gene expression were evident in adipose tissue (figure 4.3).

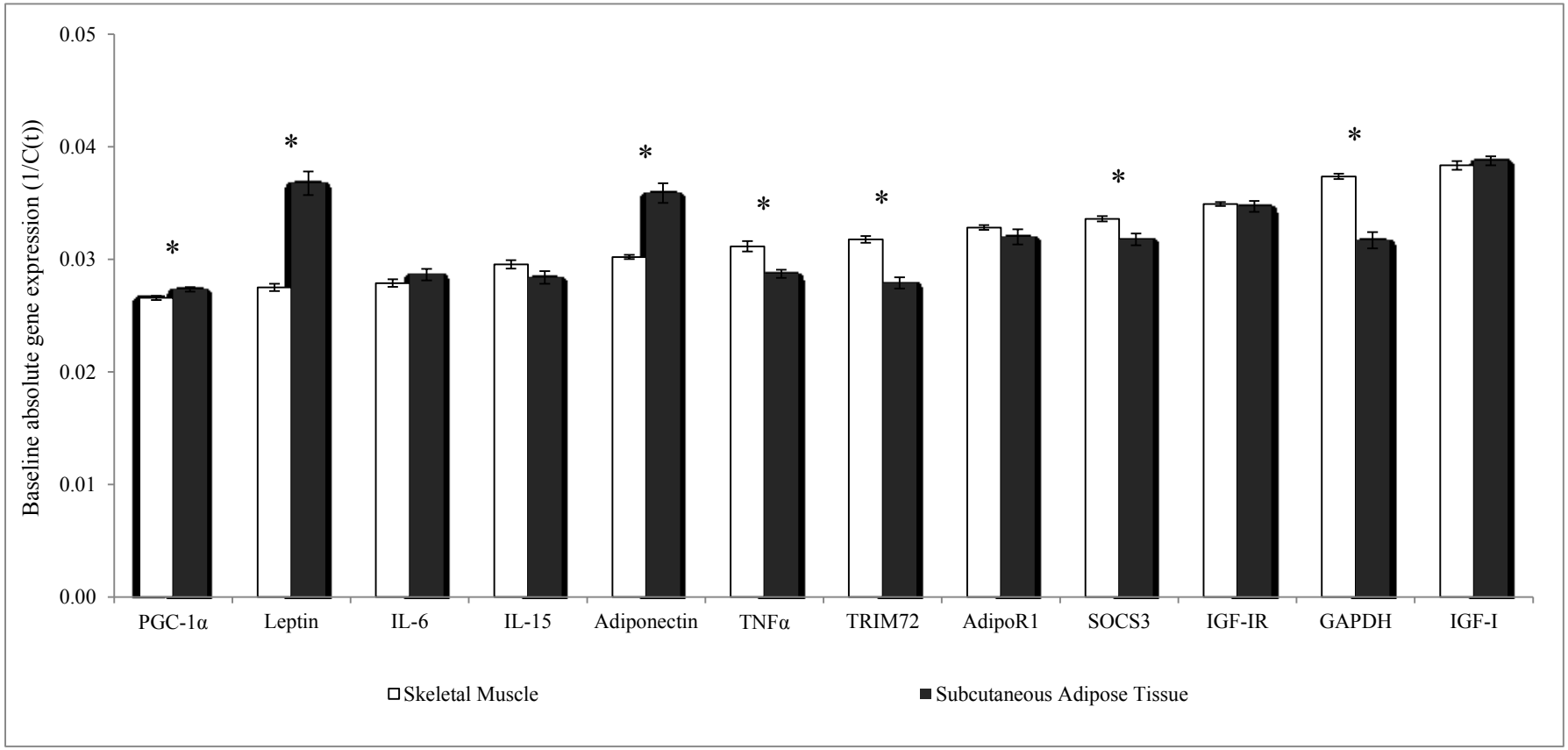


Figure 4.1. Baseline absolute gene expression in skeletal muscle tissue and subcutaneous adipose tissue. * Significantly different ($P < 0.05$). Data are (mean ± SEM).

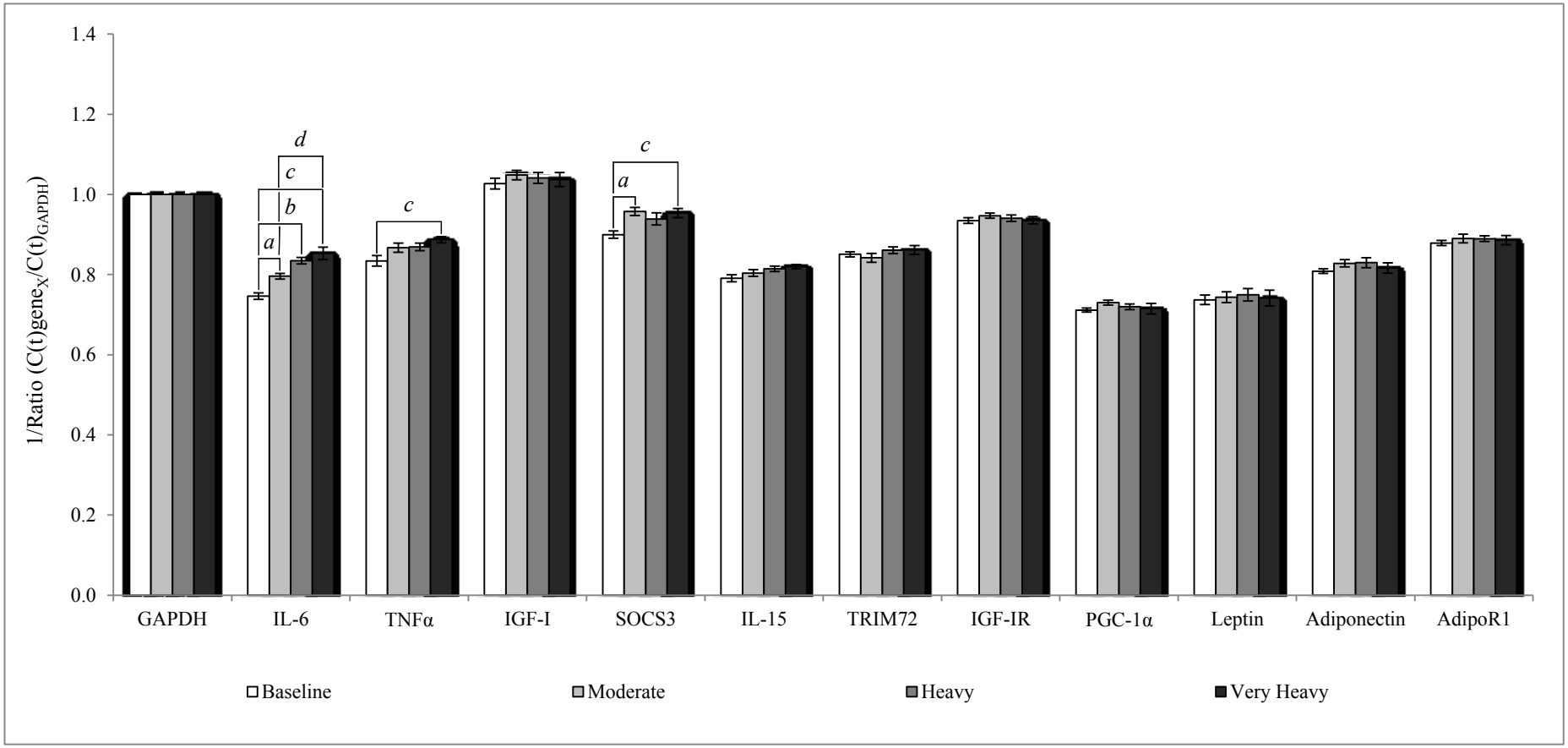


Figure 4.2. Normalised gene expression in skeletal muscle tissue at baseline and in response to moderate (60 min), heavy (27:48 ± 2:31 min) and very heavy (22:29 ± 1:31 min) domain cycle ergometer exercise. * - Significant effect of 'trial': IL-6 ($P < 0.001$); Post-hoc analyses, baseline *vs.* moderate ($P = 0.006$; *a*), heavy ($P < 0.001$; *b*) and very heavy ($P < 0.001$; *c*). Moderate *vs.* very heavy ($P = 0.02$; *d*). TNFα ($P = 0.02$); baseline *vs.* very heavy ($P = 0.04$; *c*). SOCS3 ($P = 0.006$); Post-hoc analyses, baseline *vs.* moderate ($P = 0.02$; *a*) and very heavy ($P = 0.04$; *d*). Data are (mean ± SEM).

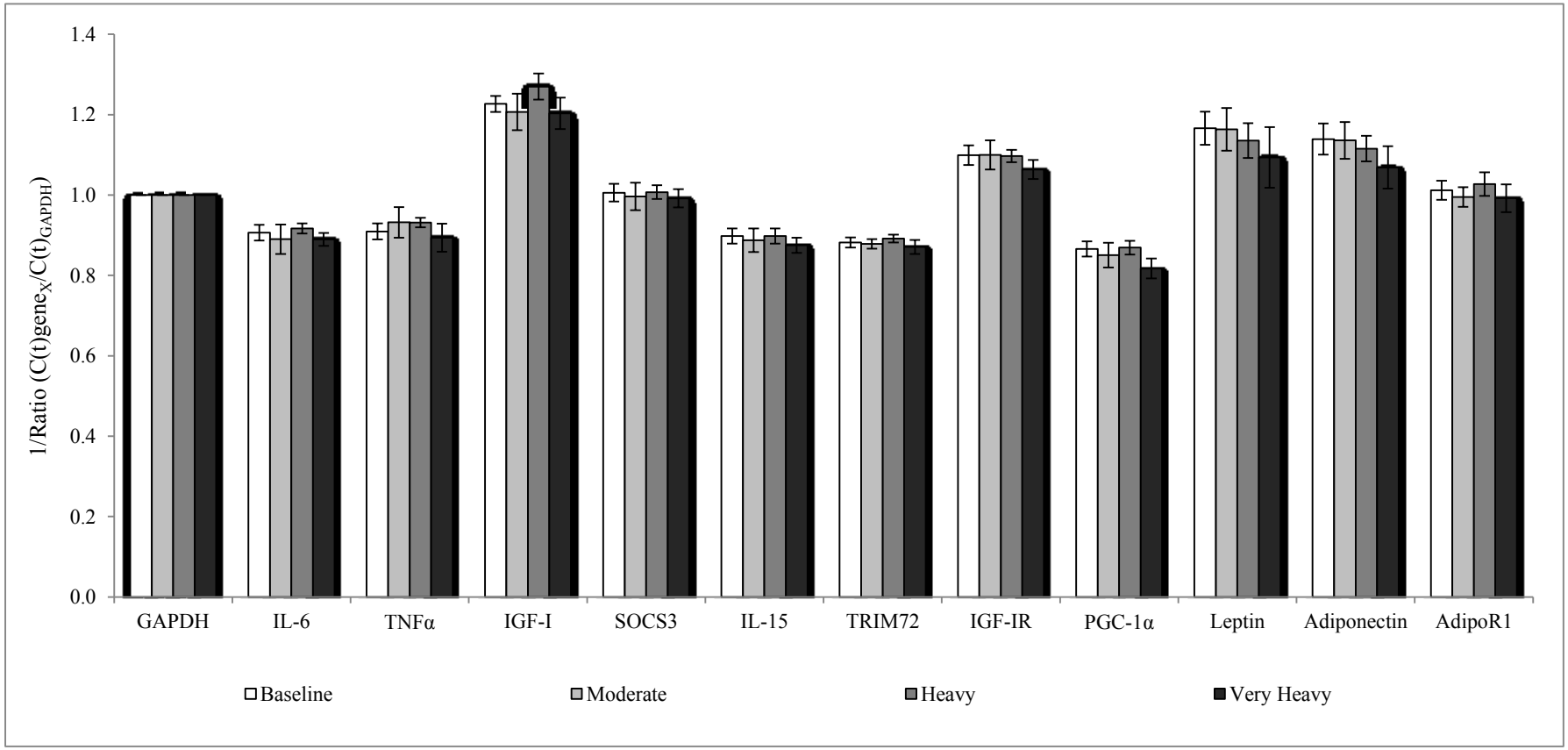


Figure 4.3. Normlised gene expression in subcutaneous adipose tissue at baseline and in response to moderate (60 min), heavy (27:48 ± 2:31 min) and very heavy (22:29 ± 1:31 min) domain cycle ergometer exercise. No significant effect of 'trial'. Data are (mean ± SEM).

4.5. Discussion

4.5.1. Baseline absolute expression

The absolute C(t) values of 30 or greater for PGC-1α, IL-6, Interleukin-15 (IL-15), TNFα, TRIM72 and Adiponectin receptor 1 (AdipoR1) suggest that their mRNA was expressed at low levels in both tissues. Of these, PGC-1α was more abundant in adipose tissue while TNFα and TRIM72 were more abundant in skeletal muscle. The absolute C(t) values of 30 or less for Leptin, adiponectin, SOCS3, Insulin-like growth factor-I receptor (IGF-IR), GAPDH, and Insulin-like growth factor-I (IGF-I) suggest that their mRNA was expressed at higher levels. Of these, leptin and adiponectin were more abundant in adipose tissue, while SOCS3 and GAPDH were more abundant in skeletal muscle. Insulin-like growth factor-I and IGF-IR were expressed at a similar level in both tissues. The expression of SOCS3 at this level suggests the importance of regulatory signalling in skeletal muscle in response to exercise (Babon and Nicola, 2012; Yoshimura et al., 2012; Carow and Rottenberg, 2014). The relatively high expression of the reference gene GAPDH in skeletal muscle compared to adipose tissue supports the findings of Barber et al., (2005), in which expression in skeletal muscle and adipose tissue were ranked 1st and 58th respectively, in order of GAPDH mRNA abundance, from 72 human tissues analysed. The relatively high and uniform tissue expression of IGF-I and IGF-IR highlights the importance of the roles that these proteins play, e.g. survival, cell growth and differentiation in both tissue types (Stewart and Rotwein, 1996; Kurmasheva and Houghton, 2006; Puche and Castilla-Cortázar, 2012).

4.5.2. Local gene expression adaptations

No significant exercise-induced changes in gene expression were found in adipose tissue. In skeletal muscle, exercise induced a significant increase in the expression of IL-6 following all trials. Only after very heavy exercise was TNFα elevated. The expression of SOCS3 in skeletal muscle increased in response to moderate and very heavy exercise. We suggest that the changes in IL-6 reflect the metabolic environment within working skeletal muscle, and in association with TNFα, potentially activation of repair mechanisms. Up-regulation of SOCS3 serves to inhibit and therefore guard against inflammatory/catabolic influence of excessive IL-6 and TNFα.

4.5.2.1. Exercise-induced adaptations normalised to GAPDH

In addition to myocytes, skeletal muscle contains smooth muscle cells, fibroblasts, endothelial cells, and macrophages. These cells have all been shown to produce IL-6 (Podor et al., 1989; Cicco et al., 1990; Klouche et al., 1999; De Rossi et al., 2000). It is possible that the changes in gene expression observed in the present study may have been due to increased mRNA expression in cells other than myocytes in the skeletal muscle biopsy samples obtained. In the present study we measured changes in mRNA expression and not the rate of gene transcription. It is possible that data reflect an increase in transcription and/or increases in mRNA stability, or a combination of both. However, Keller et al., (2001) demonstrated that the rate of IL-6 gene transcription closely matches IL-6 mRNA abundance during exercise. We are therefore confident that our results reflect the rate of IL-6 gene transcription. In line with previous studies, we found that acute endurance exercise promoted an increase in IL-6 mRNA expression within human skeletal muscle (Ostrowski et al., 1998a; Keller et al., 2001; Starkie et al., 2001) and that acute very heavy domain exercise resulted in significantly greater IL-6 mRNA expression, compared to acute moderate domain exercise. It is recognised that the magnitude of the IL-6 response is related to the intensity and duration of exercise (Fischer, 2006). Evidence suggests that Calcium (Ca^{2+}) release during Excitation-contraction coupling (ECC) (Calderón et al., 2014) is related to skeletal muscle IL-6 transcriptional activity. Stimulation of human primary skeletal myotubes with the Ca^{2+} ionophore ionomycin increased IL-6 mRNA time- and dose-dependently, an effect that was blunted by ~ 75 % in the presence of Cyclosporin A (CSA), an inhibitor of the eukaryotic Ca^{2+} and calmodulin-dependent serine/threonine protein phosphatase calcineurin (Keller et al., 2002; Keller et al., 2006). Similar findings have been observed in animal models (Holmes et al., 2004; Allen et al., 2010). Whitham et al., (2012) established further support for Ca^{2+}-dependent regulation of IL-6, and identified c-Jun NH_2-terminal kinase (JNK)/Activator protein-1 (AP-1) as a novel transcription pathway in skeletal muscle. To mimic exercise conditions Whitham et al., (2012) mechanically stimulated murine C2C12 myotubes via Electrical pulse stimulation (EPS) and compared the responses with those from stimulation with the pharmacological Ca^{2+} carrier calcimycin (A23187) and the classical IL-6 IKappa-B kinase kinase complex (IKK)/Nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) inflammatory response (De Waal Malefyt et al., 1991; Kreutz et al., 1997) elicited by Hydrogen peroxide (H_2O_2). Neither calcimycin- nor EPS-induced IL-6 mRNA

expression was found to be under the transcriptional control of NFκB. Electrical pulse stimulation increased the phosphorylation of JNK and the reporter activity of the downstream transcription factor AP-1. In addition, JNK inhibition abolished the EPS-induced increase in IL-6 mRNA and protein expression. Importantly, an exercise-induced increase in both JNK phosphorylation and IL-6 mRNA expression in the dissected whole mixed hind limb muscles of mice was observed following 30 min of treadmill running, whereas no increase was observed in skeletal muscle specific JNK-deficient mice (Whitham et al., 2012). Therefore, although we do not have a precise mechanism for the regulators of increased IL-6 mRNA in skeletal muscle in response to exercise, there maybe a role for calcium, which warrants further investigation.

During endurance exercise the contribution to energy provision from the various metabolic pathways is determined by the relative intensity of the exercise bout. At low exercise intensities, glucose derived from hepatic glycogenolysis or oral ingestion, and free fatty acids from adipose tissue lipolysis are the primary fuel sources supplying skeletal muscle. With increasing exercise intensity, muscle utilisation of systemic free fatty acids declines, whereas utilisation of circulating glucose and intramuscular glycogen progressively increases. Glucose derived from skeletal muscle glycogenolysis is the predominant carbohydrate fuel source during intense exercise (Coyle, 1995; Houten and Wanders, 2010; Egan and Zierath, 2013). Calculation of carbohydrate oxidation as a percentage of total substrate oxidation (Péronnet and Massicotte, 1991) during moderate (mean ± SEM: 30 ± 9 %), heavy (69 ± 10 %) and very heavy (92 ± 3 %) exercise trials support these statements. The design of the present study was such that the exercise intensity and duration were manipulated to ensure equal work done in all trials. Expression of IL-6 mRNA in skeletal muscle was greater following an acute bout of very heavy exercise compared to an acute bout of moderate exercise. The lack of a significant difference between heavy and very heavy exercise maybe due to the trade off between intensity and duration in accomplishing equal total work done, or an increasing dependence on intramuscular glycogen stores that do not require Glucose transporter type 4 (GLUT4) translocation. This is in agreement with the suggestion that the primary role of IL-6 during exercise is to support glucose uptake with increasing rates of carbohydrate oxidation (Pal et al., 2014).

It has also been suggested that IL-6 functions as a key stimulator of lipid mobilisation and metabolism, to spare muscle glycogen (Lyngso et al., 2002; van Hall et al., 2003;

Petersen et al., 2005). Recent evidence suggests IL-6 may even have a role in modulating the choice of substrate in skeletal muscle during exercise by altering Pyruvate dehydrogenase (PDH) complex via Adenosine monophosphate-activated protein kinase (AMPK) (Biensø et al., 2014). It is therefore somewhat surprising that we found no increase in IL-6 mRNA in adipose tissue. The reason for this maybe the relatively short exercise duration in the present study, and the lack of repeated tissue sampling post-exercise. Keller et al., (2003a) identified different IL-6 mRNA induction kinetics in skeletal muscle and adipose tissue; a significant increase in skeletal muscle IL-6 mRNA was observed immediately post- and 1.5 hrs. post-cycle ergometer exercise of 3 hrs. duration at 60 % of the maximum rate of oxygen uptake ($\dot{V}O_2max$), whereas adipose tissue IL-6 mRNA was significantly increased at 1.5 hrs. post-exercise only. Further, subcutaneous adipose tissue lipolysis in the lower limbs has been shown to contribute less to whole body lipolytic rate than that of abdominal subcutaneous adipose tissue (Arner et al., 1990; Horowitz and Klein, 2000; Horowitz, 2003).

Data from the present study show increased SOCS3 mRNA expression in human skeletal muscle in response to acute endurance exercise. Increased SOCS3 mRNA has previously been observed in the skeletal muscle of Sprague-Dawley rats following treadmill endurance training (Steinberg et al., 2004; Spangenburg et al., 2006) and in human skeletal muscle following resistance exercise (Trenerry et al., 2007; 2008; 2011; Toth et al., 2011). The expression of SOCS3 mRNA in skeletal muscle was increased after moderate and very heavy exercise. The SOCS3 is a potent inhibitor of IL-6 signalling by exerting negative feedback control on the Janus kinase (JAK)/Signal transducer and activator of transcription (STAT) pathway. Classical IL-6 signalling, via the phosphorylated tyrosine residue Tyr759 of its signal transducer GP130, results in activation of the JAK/STAT signalling pathway and the rapid induction of SOCS3 (Naka et al., 1997; Starr et al., 1997; Nicola and Greenhalgh, 2000; Fischer et al., 2004). Additionally, IL-6 is targeted for proteasomal degradation through the E3 ubiquitin-ligase complex bound to the 'SOCS box' motif (Starr et al., 1997; Hilton et al., 1998). Paradoxically, the greatest increase in SOCS3 mRNA expression in skeletal muscle was induced by moderate exercise, whereas the greatest increase in IL-6 mRNA expression was induced by heavy and very heavy exercise. However, evidence indicates that elevated systemic concentrations of IL-6 result in increased expression of SOCS3 protein in skeletal muscle (Rieusset et al., 2004; Carey et al., 2006; Holmes et al., 2008). We have shown that, under conditions of equal total work done, the greatest increase in systemic

IL-6 concentration occurred in response to moderate exercise (data presented in Chapter 5). Interleukin-6 release from skeletal muscle has been shown to account for the exercise-induced increase in systemic concentrations (Steensberg et al., 2000). Therefore, we suggest that the increase in skeletal muscle SOCS3 mRNA expression in response to moderate exercise in the present study maybe due, at least in part, to systemic accumulation of IL-6 over the relatively greater duration of the moderate exercise bout.

Given the suggested importance of IL-6 activation of AMPK in metabolism and insulin sensitivity, inhibition of IL-6 signalling by SOCS3 appears a negative consequence of exercise. Sarvas et al., (2013) argues that context determines the negative consequences of SOCS3 inhibition. The transient nature of SOCS3 induction in response to exercise has a limited effect on the beneficial metabolic and insulin sensitising effects promoted by exercise-induced IL-6. Chronically elevated IL-6, such as in obesity and Type 2 diabetes mellitus (T2DM), leads to increased and sustained expression of SOCS3 proteins in skeletal muscle, adipose tissue and in the liver, and inhibitory effects on insulin signalling and glucose metabolism (Rui et al., 2002; Ueki et al., 2004; 2005; Shi et al., 2006), as well as leptin adipostat function (Bjørbaek et al., 1999; Lubis et al., 2008; Fuentes et al., 2010). Further, Sarvas et al., propose the concept of 'IL-6 resistance'. Similar to leptin resistance, in which SOCS3 is induced by and binds to the leptin receptor thereby limiting leptin action, chronically elevated IL-6 results in sustained SOCS3 activity, diminished AMPK activity and increased insulin resistance (Sarvas et al., 2013).

Data from the present study show an increase in TNFα mRNA expression in human skeletal muscle relative to baseline, only in response to very heavy exercise. In comparison to the prototypical myokine IL-6, relatively little is known about exercise-induced TNFα mRNA expression in skeletal muscle. However, TNFα is generally considered an inflammatory cytokine generated by the immune system in response to infection or inflammation. Traditionally, TNFα has been associated with muscle pathology, originally designated 'cachectin' in recognition of its catabolic action. In humans skeletal muscle catabolism has been attributed to TNFα in various diseases that showed muscle loss in combination with chronically elevated levels of TNFα, most notably cancer (Argilés and Lopez-Soriano, 1999; Tisdale, 1999). However, evidence from a study by Petersen et al., (2009) suggested TNFα *per se* does not increase skeletal muscle protein loss *in vivo* in humans, but that skeletal muscle loss might be attributed to

high TNFα concentrations mediating catabolism via an enhanced effect on the levels of other cytokines and catabolic hormones.

Skeletal muscle TNFα mRNA is increased in the elderly (Greiwe et al., 2001), in obesity (Ferrier et al., 2004) and in patients with T2DM (Saghizadeh et al., 1996). In contrast to IL-6, the exercise-induced increase in skeletal muscle TNFα mRNA is very small and results in little if any measurable increase in systemic concentration indicating a local role in skeletal muscle (Steensberg et al., 2002; Febbraio et al., 2003). A complete explanation of the mechanisms involved remains elusive, but a direct effect of TNFα on insulin sensitivity in skeletal muscle has been demonstrated *in vitro* (Hotamisligil et al., 1996), *in vivo* in animals (Miles et al., 1997) and *in vivo* in humans (Plomgaard et al., 2005). Tumour necrosis factor-alpha inhibits insulin signalling through key regulatory proteins Insulin receptor substrate-1 (IRS1) and Protein kinase B (PKB) substrate 160 in human skeletal muscle *in vitro* (Bouzakri and Zierath, 2007) and *in vivo* (Plomgaard et al., 2005). These data show that elevated TNFα plays a direct pathogenic role in glucose metabolism and the pathological conditions associated with insulin resistance. In addition, TNFα has been shown to increase lipolysis without enhancing skeletal muscle fat metabolism *in vivo* in humans, highlighting a role in dyslipidemia and the development of lipid-induced insulin resistance (Plomgaard et al., 2008). The mechanism is suggested to be direct inhibition of AMPK signalling through Protein phosphatase 2C (PP2C) activation (Steinberg et al., 2006; 2009).

Systemically, exercise is suggested to promote an anti-inflammatory environment in which TNFα concentrations generally do not increase (Pedersen and Hoffman-Goetz, 2000; Brandt and Pedersen, 2010) due, in part, to the inhibitory effects of IL-6 on TNFα and Interleukin-1 (IL-1) through IL-6 induction of Soluble tumour necrosis factor receptors (sTNFrs), Interleukin-1 receptor antagonist (IL-1ra) and Interleukin-10 (IL-10) (Ostrowski et al., 1999; Starkie et al., 2003; Steensberg et al., 2003). However, skeletal muscle TNFα mRNA increases in response to 3 hrs. of treadmill running and cycle-ergometer exercise at ~ 70 and 75 % $\dot{V}O_2$max, respectively (Nieman et al., 2003, 2005) and 30 min of treadmill running at 75 % $\dot{V}O_2$max (Louis et al., 2007). Our data supports the findings of these studies and further suggests that high intensity endurance exercise promotes an increase in skeletal muscle TNFα mRNA expression, regardless of duration. In the latter study IL-6 and TNFα mRNA expression relative to GAPDH followed a similar profile in the 24 hrs. post-exercise. Specifically, a significant increase

immediately post-exercise followed by a decline and peak 8 hrs. post-exercise. At 24 hrs. post-exercise IL-6 and TNFα mRNA concentrations remained elevated at a concentration similar to that observed immediately post-exercise (Louis et al., 2007). Despite similar post-exercise induction profiles, Keller et al., (2006) demonstrated differential IL-6 and TNFα mRNA expression in a primary human skeletal muscle cell model in response to the calcium ionophore ionomycin, mimicking contraction-mediated Ca^{2+} release from the sarcoplasmic reticulum. Interleukin-6 mRNA increased in a time- and dose-dependent fashion with ionomycin stimulation. The effect was blunted by ~ 75 % by calcineurin phosphatase inhibitor CSA. In contrast, TNFα mRNA expression decreased by ~ 70 % in response to ionomycin, but increased in response to the addition of CSA. As previously stated, the calcineurin pathway appears to play a major role in the Ca^{2+}-induced IL-6 response. However, IL-6 and TNFα appear to be regulated differently in skeletal muscle cells in response to a Ca^{2+} stimulus. Further, as low glycogen levels have been associated with decreased re-uptake of Ca^{2+} into the sarcoplasmic reticulum (Booth et al., 1997), these data may provide an explanation for increased exercise-induced IL-6 mRNA expression when skeletal muscle glycogen concentrations are low (Keller et al., 2001; Steensberg et al., 2001; Hiscock et al., 2004). These data fail, however, to provide an explanation for the significant increase in skeletal muscle TNFα mRNA expression following acute very heavy exercise in the present study, in what the data from Keller et al., (2006) suggested should be an environment inhibiting TNFα mRNA expression.

4.5.3. Summary and conclusion

In line with the objective of this chapter, to establish the changes in the mRNA expression of selected hormones and cytokines that occur locally in skeletal muscle and subcutaneous adipose tissue as a function of our acute 'domain-based' constant work-load cycle ergometer exercise interventions in recreationally active young males, we analysed the expression of hormone and cytokine mRNA in skeletal muscle and subcutaneous adipose tissue bioptic material collected at baseline and following the moderate, heavy and very heavy domain exercise trials. We hypothesised that the constant work-load cycle ergometer exercise trials would result in significant differences in mRNA expression despite the measures taken to ensure equal work done in each. We found significant increases in the mRNA expression (normalised to GAPDH) in skeletal muscle of IL-6 following all trials immediately post-exercise, whereas increases in SOCS3 mRNA were observed following moderate and very heavy domain exercise only.

TNFα mRNA increased immediately post-very heavy domain exercise only. No effects on gene expression in subcutaneous adipose tissue were observed immediately post-exercise. We conclude that our data confirm the importance of skeletal muscle as a source of exercise responsive hormones and cytokines, and highlight exercise intensity and duration as a mechanisms regulating the expression of IL-6, TNFα and SOCS3, proteins with acknowledged metabolic, inflammatory and cytokine suppressive roles respectively, in the tissue. Using this approach we can proceed with testing our hypothesis that acute 'domain-based' constant work-load cycle ergometer exercise interventions will result in significant changes in the systemic abundance of the selected hormones and cytokines under investigation.

Chapter 5. Changes in systemic hormone and cytokine abundance: response to exercise intensity

5.1. Abstract

In the previous chapter we concluded that exercise intensity and exercise duration, rather than equal work done, were potent drivers behind IL-6 in particular, but also TNFα and SOCS3 mRNA expression in skeletal muscle, confirming the importance of this tissue as a source of exercise responsive hormones and cytokines. Again, confident that the trials would produce hormone and cytokine responses characteristic of the exercise domain in which they reside, we wished to supplement these local data by investigating systemic responses to equal work (varying duration) moderate (M) heavy (H) and very heavy (VH) exercise in the recreationally active, young participant group. We hypothesised that the constant work-load cycle ergometer exercise trials developed in chapter 3 would result in significant systemic changes despite the relative differences in trial duration, manipulated to ensure equal work done. A workload comparison (baseline (B) *vs.* End of Exercise) indicated only IL-6 increased in response to M (1.08 – 5.05 pg/ml, $P = 0.001$). In response to M, H and VH, IL-6 increased and remained elevated for 24 hrs. ($P = 0.03$). IGF-I and cortisol concentrations declined by 60 min post-exercise ($P = 0.001$ and $P = 0.04$, respectively). GH increased to peak at the end of exercise ($P < 0.001$) before declining sharply. We concluded that endurance exercise promotes significant changes in the systemic abundance of hormones and cytokines. The changes strongly suggest activation of mechanisms of metabolic rather than anabolic control in our young participant group, specifically, the mobilisation of energy from fat stores.

5.2. Introduction

Pedersen (2009) suggested the beneficial effects of endurance exercise are due to the anti-inflammatory effects of regular exercise, through a reduction in adipose tissue mass and/or induction of an anti-inflammatory environment with each acute bout of endurance exercise. Analysis of systemic changes in the abundance of selected hormones and cytokines (myokines and adipokines) with roles thought to contribute to the regulation of adipose tissue accumulation and inflammation may provide clarification of the mechanisms conferring the beneficial effects of endurance exercise. The objective of this chapter is therefore to establish the changes that occur systemically in the abundance of selected hormones and cytokines, therefore supplementing data gathered previously in skeletal muscle and adipose tissue, as a function of our acute 'domain-based' constant work-load cycle ergometer exercise interventions in recreationally active young males. We hypothesise that the constant work-load cycle ergometer exercise trials developed in Chapter 3 will result in significant systemic changes despite the relative differences in trial duration, manipulated to ensure equal work done.

5.3. Methods

Methodological information directly pertinent to this chapter is summarised briefly below. Chapter 2. 'General methods', provides a detailed reference resource, documenting the procedures employed in collecting the data presented in this thesis. Relevant sections in Chapter 2 will be referenced in the text by index number, e.g. [2.2.1.] refers to the section 2.2.1. 'Ethical approval'.

5.3.1. Study design

A schematic representation of the interlinking studies presented in this thesis is provided in figure 2.4 [2.5.1.8.1.]. The data presented in this chapter relate to Exercise studies 1 and 2.

5.3.2. Participants [2.2.]

Participant descriptive and exercise cardio-respiratory data are provided in Chapter 3, Tables 3.1. and 3.2.

5.3.3. Determination of exercise trial intensity and duration [2.5.1.5. & 2.5.1.7.]

5.3.4. Exercise trial protocols [2.5.1.8.]

5.3.5. Blood sampling [2.6.]

Samples were obtained pre-, during and post-exercise via indwelling venous cannula. A needle and syringe were used to collect blood samples 24 hrs. post-exercise. Samples were transferred to ice cold S-Monovette® tubes and then centrifuged. The serum was aliquoted into 2 ml Eppendorf® tubes and frozen at -80 °C until analysed. Serum samples were assayed for specific proteins via Enzyme-linked immunosorbent assay (ELISA). Pre-coated, 96-well ELISA kits were purchased for determination of specific protein concentrations in human serum.

5.3.6. Statistical analyses [2.8.]

Maximal exercise-induced hormonal adaptations. Paired t-Tests were performed to determine if systemic Growth Hormone (GH), Insulin-like growth factor-I (IGF-I), cortisol and Interleukin-6 (IL-6) concentrations measured post-cycle ergometry maximum rate of oxygen uptake ($\dot{V}O_2max$) test were significantly different to concentrations measured at baseline.

Heavy exercise-induced hormonal adaptations, trial comparisons. Repeated-measures Analysis of variance (ANOVA) with as within factor 'time' (4 levels; Baseline, End of exercise, Post-exercise (60 min) and Post-exercise (24 hrs.)) and between factor 'study' (2 levels; Study 1 and Study 2) were run to determine whether there were significant differences between systemic protein abundance induced by heavy exercise performed in Study 1 and Study 2. If significant effects were observed, post-hoc Bonferroni-corrected one-way ANOVA were run to identify the difference.

Work-load comparison, Baseline vs. End of exercise. Repeated-measures ANOVA with as within factor 'time' (2 levels; Baseline and End of exercise) and between factor 'work-load' (3 levels; moderate, heavy and very heavy) were run to determine whether there were significant differences between systemic protein abundance induced by moderate, heavy and very heavy exercise, immediately post-exercise. If a significant 'time' x 'work-

load' interaction was observed, the data file was split on between factor 'work-load' and independent t-tests were performed to locate the difference. Post-hoc Bonferroni-corrections were applied.

Moderate, heavy and very heavy exercise-induced hormonal adaptations. Repeated-measures ANOVA with as within factor 'time' (4 levels; Baseline, End of exercise, Post-exercise (60 min) and Post-exercise (24 hrs.)) and between factor 'work-load' (3 levels) were run to determine whether there were significant differences between systemic protein abundance induced by moderate, heavy and very heavy exercise. If significant effects were observed, post-hoc Bonferroni-corrected one-way ANOVA were run to identify the difference.

5.4. Results

5.4.1. Exercise-induced hormonal adaptations

Maximal exercise induced a significant increase in the systemic concentrations of IL-6, IGF-I and GH, and a decrease in the concentration of cortisol, immediately post-exercise (figure 5.1).

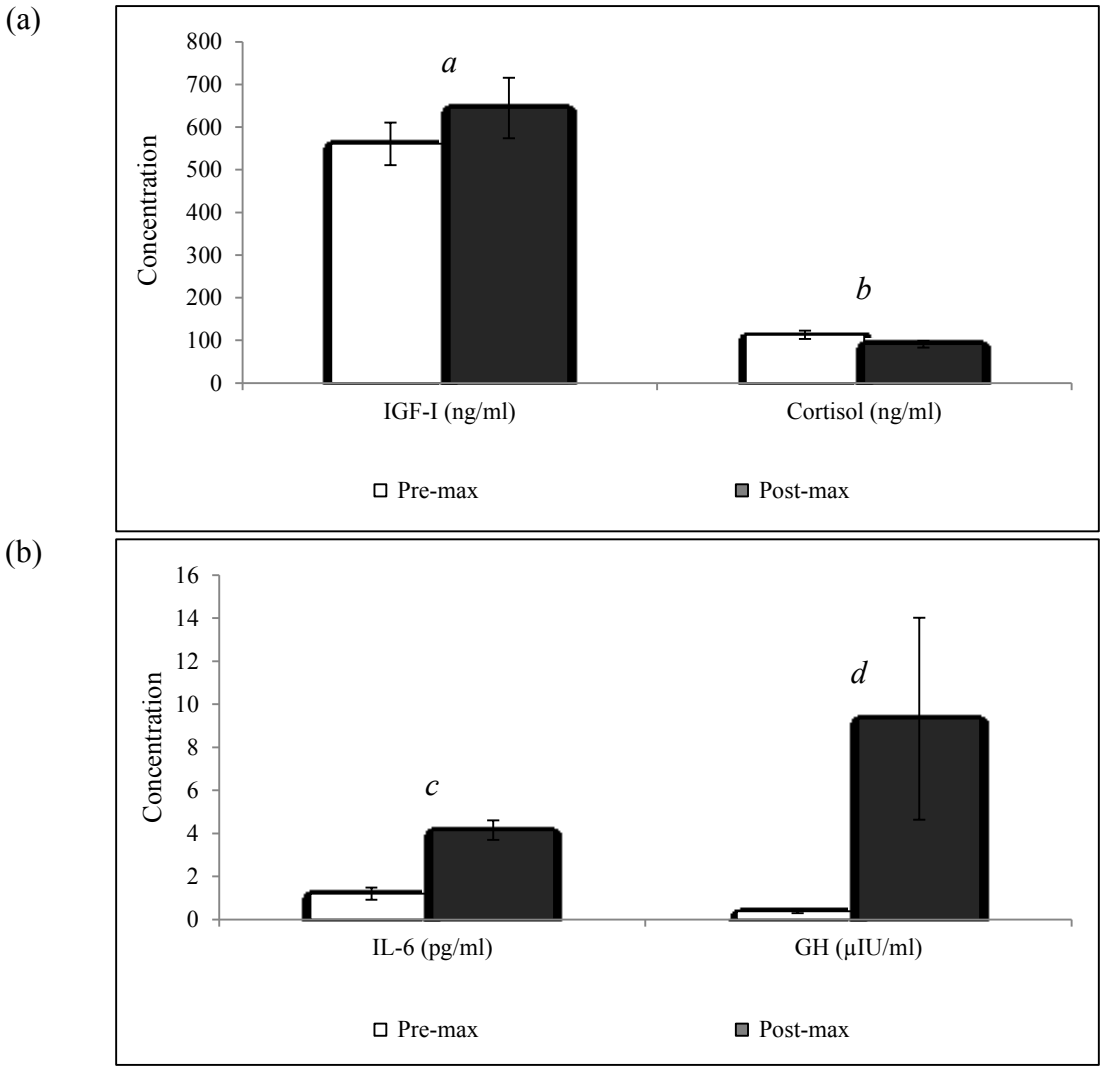


Figure 5.1. Maximal exercise-induced hormonal adaptation. Significant effect of 'time'. (a) Pre-max *vs.* Post-max IGF-I ($P = 0.01$; *a*) and Cortisol ($P = 0.02$; *b*), and (b) IL-6 ($P = 0.005$; *c*) and GH ($P = 0.008$; *d*). Data are (mean ± SEM).

No significant differences were found between the systemic protein concentrations induced by heavy (30 % Δ) exercise performed in study 1 and study 2 (effect of 'study') for cortisol ($P = 0.37$), IL-6 ($P = 0.15$), GH ($P = 0.86$) or IGF-I ($P = 0.27$). The data from the heavy exercise interventions were therefore pooled and analysed as a single heavy exercise trial.

The results of the comparison to determine the effect of work-load on exercise-induced hormonal adaptations pre-post-exercise (Baseline *vs*. End of exercise) indicate moderate exercise promoted a significant increase in IL-6 ($P = 0.001$; figure 5.2.). Heavy and very heavy exercise-induced responses were not significantly different. No effect of work-load was observed on IGF-I, GH or cortisol.

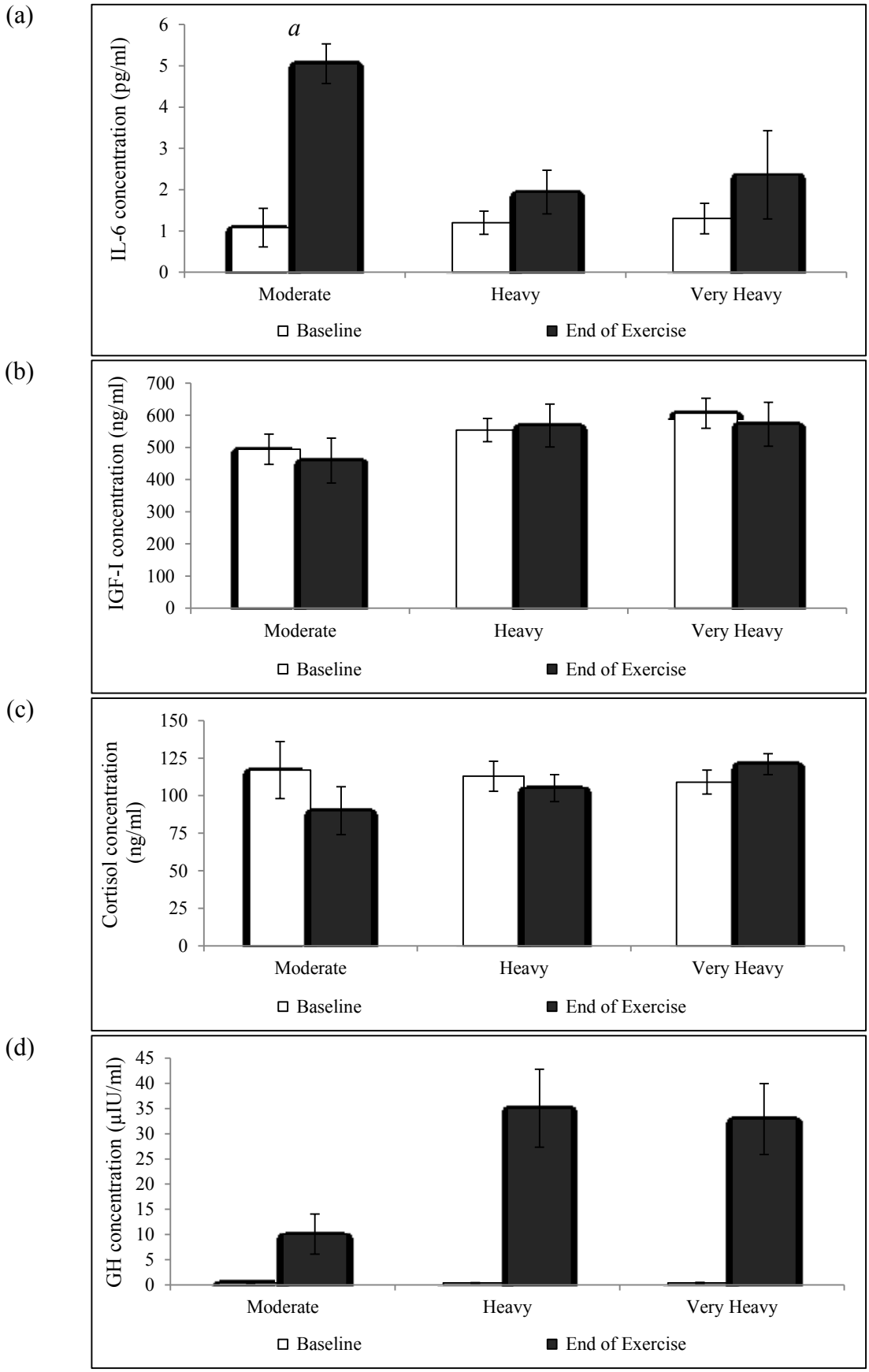


Figure 5.2. Work-load comparison, Baseline *vs*. End of Exercise. IL-6 ($P = 0.001$; *a*) (a), IGF-I (b), Cortisol (c) and GH (d). Data are (mean ± SEM).

5.4.1.1. Interleukin-6

Systemic IL-6 concentrations increased significantly above that at baseline in response to all exercise trials (significant effect of 'time' $P < 0.001$; figure 5.3). Post-hoc analyses indicated the IL-6 concentrations remained elevated above baseline for approximately 24 hrs. Analyses conducted to establish the effect of work-load (Baseline *vs.* End of exercise) indicated that moderate exercise promoted the greatest increase in IL-6 concentration at the end of exercise ($P = 0.001$).

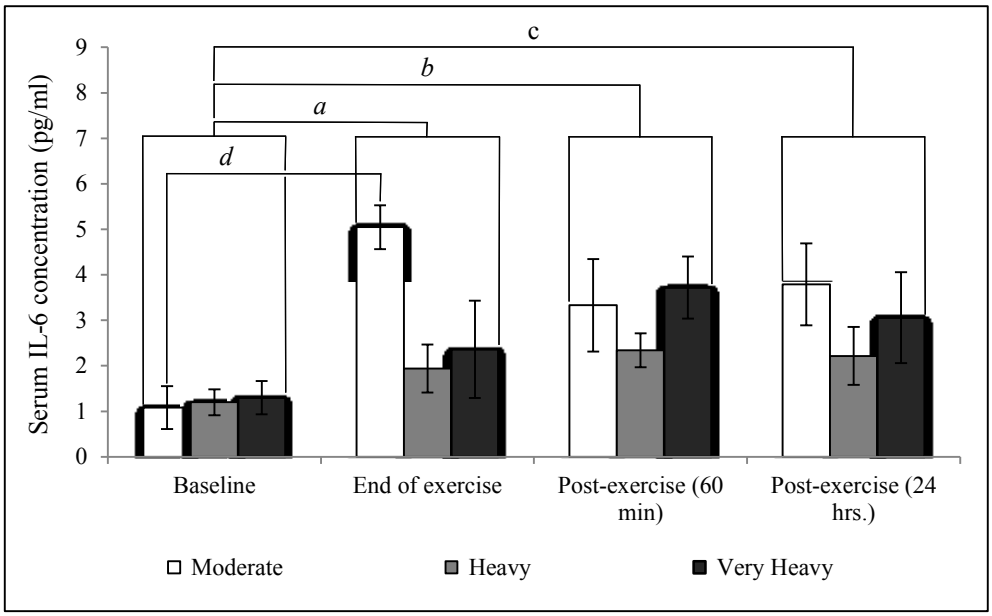


Figure 5.3. Systemic IL-6 response to moderate (60 min), heavy (27:48 ± 2:31 min) and very heavy (22:29 ± 1:31 min) domain cycle ergometer exercise. Significant effect of 'time' ($P < 0.001$). Post-hoc analyses, Baseline *vs.* End of exercise ($P = 0.003$; *a*); Post-exercise (60 min) ($P = 0.002$; *b*); Post-exercise (24 hrs.) ($P = 0.03$; *c*). Baseline *vs*. End of exercise: moderate significantly different to heavy and very heavy ($P = 0.001$; *d*). Data are (mean ± SEM).

5.4.1.2. Insulin-like growth factor-I

When monitoring the impact of exercise intensity and duration on IGF-I profiles, it was evident that a temporal adaptation occurred. Indeed, statistical analyses identified a significant effect of 'time' on IGF-I ($P < 0.001$; figure 5.4). Post-hoc analyses indicate the variation in the systemic concentration of IGF-I became significant following the cessation of exercise, during which time the concentration declined after all trials. No effect of 'work-load' ($P = 0.6$) or 'work-load' x 'time' interaction ($P = 0.7$) was observed indicating the lack of variation in systemic IGF-I abundance in response to the exercise trials, further highlighted by overlapping Standard error of the mean (SEM) error bars at all time points.

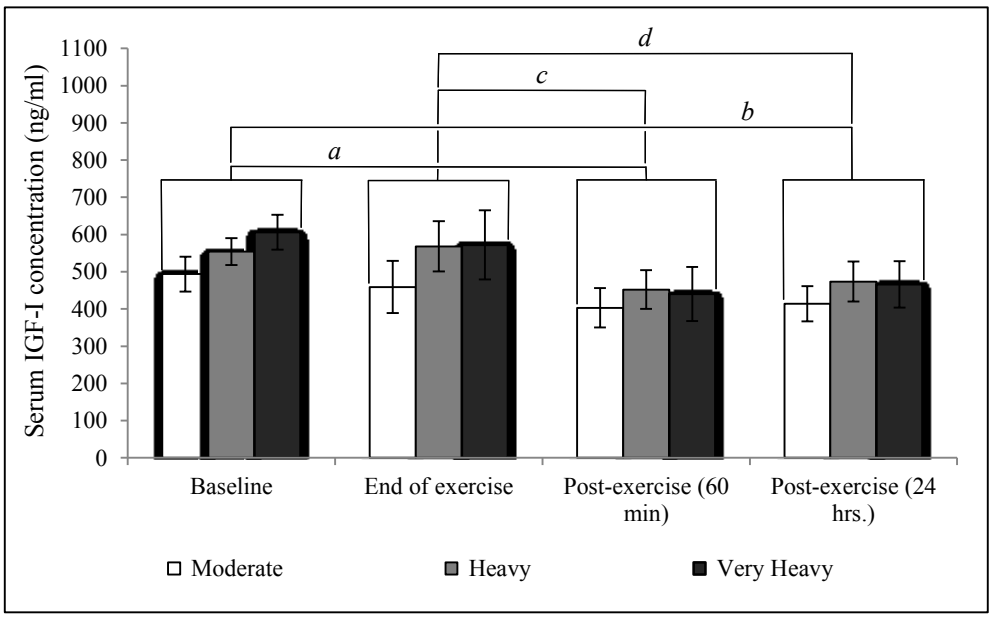
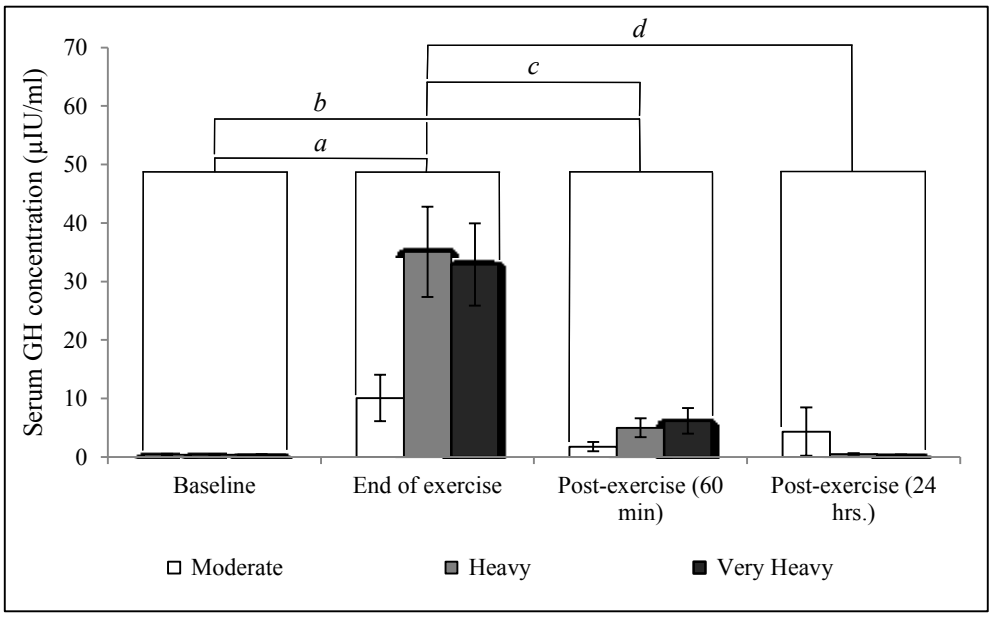


Figure 5.4. Systemic IGF-I response to moderate (60 min), heavy (27:48 ± 2:31 min) and very heavy (22:29 ± 1:31 min) domain cycle ergometer exercise. Significant effect of 'time' ($P < 0.001$). Post-hoc analyses, Baseline *vs.* Post-exercise (60 min) ($P = 0.001$; *a*) and Post-exercise (24 hrs.) ($P = 0.009$; *b*), End of exercise *vs.* Post-exercise (60 min) ($P < 0.001$; *c*) and Post-exercise (24 hrs.) ($P < 0.001$; *d*). Data are (mean ± SEM).

5.4.1.3. Growth hormone

Statistical analyses identified a significant effect of 'time' on GH ($P < 0.001$; figure 5.5 Post-hoc analyses indicated systemic GH concentrations increased significantly in response to all exercise trials above baseline to peak at the end of exercise. Growth hormone concentrations decreased significantly following the end of exercise, but remained significantly elevated above that at baseline 60 min post-exercise. Data presented as mean ± SEM belie the variability in the GH response observed in the present study. Despite a ~ 4-fold greater increase in systemic concentrations of GH at the end of heavy and very heavy exercise relative to that following at the end of moderate exercise, significance was not achieved due to the variation in participant response.

Figure 5.5. Systemic GH response to moderate (60 min), heavy (27:48 ± 2:31 min) and very heavy (22:29 ± 1:31 min) domain cycle ergometer exercise. Significant effect of 'time' ($P < 0.001$). Post-hoc analyses, Baseline *vs.* End of exercise ($P < 0.001$; *a*) and Post-exercise (60 min) ($P = 0.008$; *b*), End of exercise *vs.* Post-exercise (60 min) ($P < 0.001$; *c*) and Post-exercise (24 hrs.) ($P < 0.001$; *d*).



5.4.1.4. Cortisol

Once again, there was a significant effect of 'time' on the systemic concentration of cortisol ($P = 0.04$; figure 5.6). Post-hoc analyses indicated cortisol concentrations were lower at 60 min post-exercise than at baseline and at the end of exercise.

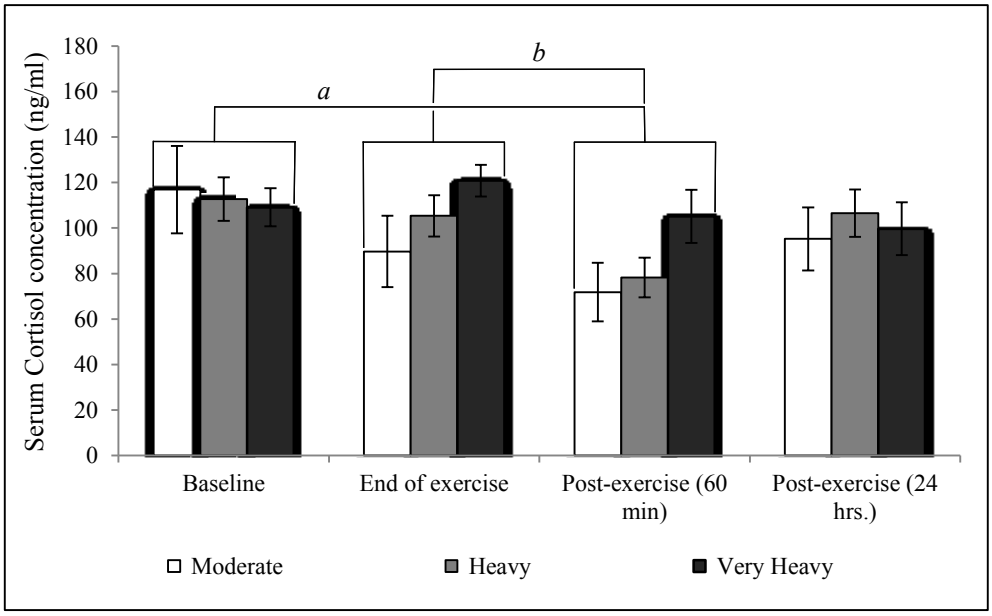


Figure 5.6. Systemic cortisol response to moderate (60 min), heavy (27:48 ± 2:31 min) and very heavy (22:29 ± 1:31 min) domain cycle ergometer exercise. Significant effect of 'time' ($P = 0.04$). Post-hoc analyses, Baseline *vs.* Post-exercise (60 min) ($P = 0.04$; *a*), End of exercise *vs.* Post-exercise (60 min) ($P = 0.01$; *b*).

5.4.1.5. The Growth hormone/Cortisol ratio

All trials induced a significant increase in the GH to cortisol ratio (significant effect of 'time', $P < 0.001$; figure 5.7), which peaked at the end of exercise and remained elevated above that at baseline at 60 min post-exercise.

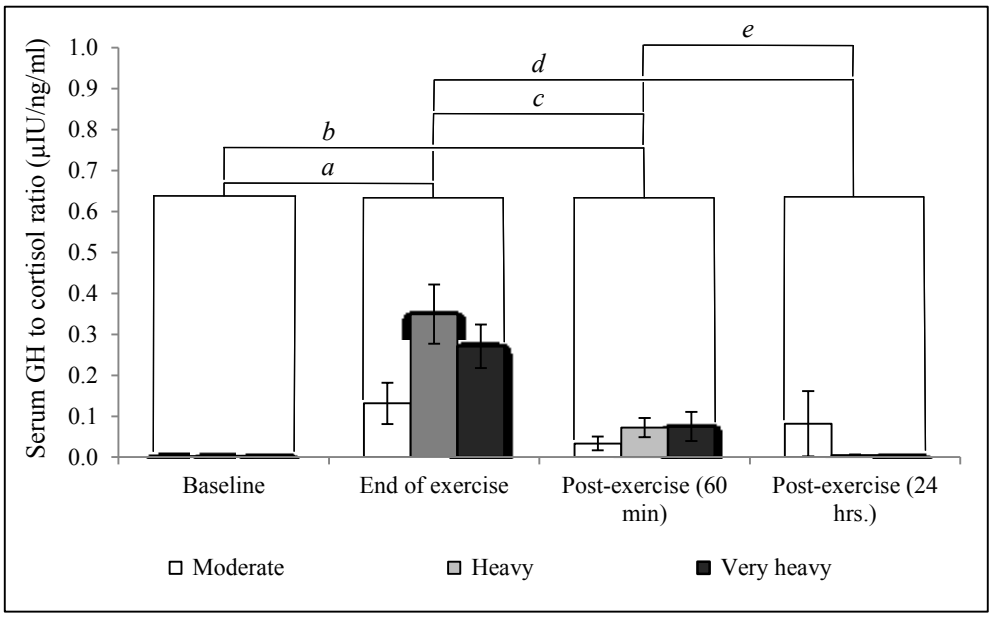


Figure 5.7. Systemic GH to cortisol ratio response to moderate (60 min), heavy (27:48 ± 2:31 min) and very heavy (22:29 ± 1:31 min) domain cycle ergometer exercise. Significant effect of 'time' ($P < 0.001$). Post-hoc analyses, Baseline *vs.* End of exercise ($P < 0.001$; *a*) and Post-exercise (60 min) ($P < 0.001$; *b*), End of exercise *vs.* Post-exercise (60 min) ($P = 0.002$; *c*) and Post-exercise (24 hrs.) ($P < 0.001$; *d*), Post-exercise (60 min) *vs.* Post-exercise (24 hrs.) ($P < 0.001$; *e*). Data are (mean ± SEM).

5.5. Discussion

5.5.1. Endocrine adaptations to exercise interventions

It is acknowledged that high intensity exercise can elicit damage (Córdova Martínez et al., 2014) with associated systemic increases in inflammatory cytokines (Zaldivar et al., 2006). We found no changes in circulating Tumour necrosis factor-alpha (TNFα), Interleukin-1-beta (IL-1β) and Interleukin-1-alpha (IL-1α); indeed the levels remained below the detection threshold of the ELISA kits (data not shown), as observed by others (Ullum et al., 1994; Drenth et al., 1995; Brüünsgaard et al., 1997). The systemic concentration of IL-6 in resting, healthy individuals is ~ 1 pg/ml or lower (Brüünsgaard et al., 1997; Ostrowski et al., 1998a; Knudsen et al., 2008). The findings from the present study support this. Acute exercise resulted in a significant increase in the systemic concentration of IL-6 above baseline, which remained elevated up to 24 hrs. post-exercise. It is well established that systemic concentrations of IL-6 are increased in response to exercise (Northoff and Berg, 1991; Fischer, 2006). Data from 74 exercise trials involving ~ 800 participants (Fischer, 2006) suggested that the variation in the IL-6 increase is especially dependent on duration, and that exercise of 60 min duration typically yields < 10-fold increase in the systemic concentration of IL-6, regardless of exercise mode. Further, IL-6 has been suggested to be a key modulator of lipid homeostasis and metabolism, connected with the substrate availability and glycogen sparing (van Hall et al., 2003; Wolsk et al., 2010). Compared to heavy or very heavy domain exercise, where oxidative phosphorylation alone is unable to satisfy energy demands and where blood flow to adipose tissue depots maybe restricted, long duration and low intensity moderate domain exercise performed in the present study had the greatest potential to benefit metabolism of free fatty acids derived from adipose tissue (Fischer, 2006; Frayn, 2010). Although the greatest increase in systemic IL-6 concentrations in response to moderate (~ 5-fold) compared to heavy and very heavy exercise bouts (~ 2-fold increase) supports this notion, the absence of a difference between heavy and very heavy exercise, together with a large increase following maximal exercise, challenges the prime importance of duration. It suggests that besides duration, intensity also impacts systemic concentrations of IL-6, at least in cycling. Interestingly, the IL-6 levels remained ~ 3-fold higher for up to 24 hrs. after exercise, irrespective of intensity or duration. Given the reported emphasis of exercise duration and intensity on

IL-6 levels, it is important to note that the conclusions which are drawn maybe influenced by the timing of sampling following exercise cessation.

Baseline GH concentrations in the present study are within the range of those reported by Drobny et al., (1983). Roth et al., (1963) first described the marked increase in GH in response to exercise, which has subsequently been well documented in endurance, sprint and resistance exercise (Godfrey et al., 2003; Stokes, 2003; Gibney et al., 2007; Gilbert et al., 2008) and can occur as early as 15 min after the onset of exercise (Lassarre et al., 1974; Raynaud et al., 1981). Studies suggest that endurance exercise above the Anaerobic threshold (ATh) elicits the most marked increase in GH (Felsing et al., 1992; Pritzlaff et al., 1999; Wahl et al., 2010; 2013) which positively correlates with exercise duration performed at 70 % peak rate of oxygen uptake ($\dot{V}O_2$peak) (Wideman et al., 2006). In line with this, we observed that systemic GH concentrations increased more in response to heavy and very heavy than moderate exercise, though this did not reach significance. Growth hormone concentrations peak at the end of exercise and return to baseline after approximately 60 min. (Lassarre et al., 1974; Raynaud et al., 1981; Viru et al., 1992; Zaldivar et al., 2006). The findings from the present study support this also.

In line with previous studies (Kanaley et al., 2005; Eliakim et al., 2006; Zaldivar et al., 2006; Nindl et al., 2009; 2010; Wahl et al., 2010) we found no change in IGF-I at end of exercise. However, a significant decline in IGF-I was observed at 60 min and up to 24 hrs. post-exercise. These observations occurred irrespective of exercise intensity and duration. Data from short-term training studies identified that systemic IGF-I concentrations decline with training (Eliakim et al., 1996; 1998; Nishida et al., 2010). In the study by Nishida et al., (2010), the decline in IGF-I was accompanied by a decrease in basal glucose and insulin concentrations and an increase in Insulin-like growth factor binding protein-1 (IGFBP-1), without a change in Insulin-like growth factor binding protein-3 (IGFBP-3). The authors suggested the decline in IGF-I maybe an adaptive response to prevent hypoglycemia following insulin-sensitising training. Thus, changes in systemic IGF-I concentrations in response to an acute bout of exercise may reflect regulatory behaviour priming glucose homeostasis.

The systemic concentrations of IGF-I increased significantly in response to the $\dot{V}O_2$max test. These data support the findings of others (De Palo et al., 2008; Zebrowska et al., 2009). Although it appears sub-maximal exercise does not elicit an elevation in IGF-I

levels, the increase following maximal exercise would imply an environment more conducive to anabolic adaptation. The relationship between local and systemic IGF-I in mediating the outcomes of exercise are still not fully understood and further investigation is required to confirm whether local production and release from skeletal muscle augments systemic concentrations following maximal exercise. Given the widely acknowledged regulatory links between GH and IGF-I, it is interesting to note that the peak in systemic GH concentrations induced by the three exercise-trials is not reflected in the IGF-I response, thus representing an uncoupling of the Growth hormone/Insulin-like growth factor-I (GH/IGF-I) axis. Zaldivar et al., (2006) observed similar results in which no change in systemic IGF-I was observed in response to 30 min of heavy domain cycle ergometer exercise, despite a peak in systemic GH. Frystyk (2010) suggested the uncoupling maybe due to the transient nature of the exercise-induced systemic GH response as administration with recombinant growth hormone (rhGH), eliciting a similar peak but longer-lasting increase in systemic GH concentration, has been shown to elevate the systemic concentrations of IGF-I within 4 hrs. post-administration (Lee et al., 1997; Stokes et al., 2005).

Baseline systemic cortisol concentrations in the present study appear lower than those observed by others (Silverman and Mazzeo, 1996; Del Corral et al., 1998; Hill et al., 2008; Wahl et al., 2010; Rosa et al., 2012). However, several studies reported a decline in cortisol prior to exercise (Del Corral et al., 1998; Hill et al., 2008; Wahl et al., 2010). These lower concentrations more closely resemble baseline values in the present study. It has been shown that exercise above 60 % $\dot{V}O_2$max induces an increase in systemic cortisol concentrations, whilst exercise below this intensity resulted in a decline in cortisol (Davies and Few, 1973; Bloom et al., 1976). The duration of exercise at a specific intensity then determines the final cortisol concentrations (Hill et al., 2008). The findings of the present study do not support this. Maximal exercise induced a reduction in systemic cortisol concentrations, which suggests either a lack of Hypothalamic-pituitary-adrenal (HPA) axis stimulation or a rate of cortisol uptake into target tissues outweighing that of release from the adrenal gland. Systemic cortisol concentrations remained unchanged as a result of the three exercise trials, but had declined significantly at 60 min post-exercise. The cortisol responses in our study were too small to have an impact on the GH to cortisol ratio. The small cortisol responses were independent of exercise intensity and duration.

5.5.2. Summary and conclusion

In line with the objective of this chapter, to establish the changes that occur systemically in the abundance of selected hormones and cytokines as a function of our acute 'domain-based' constant work-load cycle ergometer exercise interventions in recreationally active young males, we analysed the systemic abundance of IL-6, IGF-I, cortisol and GH at baseline, end of exercise and at 60 minutes and 24 hrs. post-moderate, heavy and very heavy domain exercise trials. We hypothesised that the constant work-load cycle ergometer exercise trials would result in significant systemic changes despite the relative differences in trial duration, manipulated to ensure equal work done. We observed an increase in IL-6 during moderate exercise only, but significant increases post-heavy and very heavy exercise that remained elevated for 24 hrs. We observed an uncoupling of GH and IGF-I with GH increasing to peak at the end of exercise before declining sharply. No such increase was observed in IGF-I, which declined post-exercise and remained significantly below baseline concentrations for 24 hrs. Cortisol displayed a similar profile of decline. We conclude that endurance exercise promotes significant changes in the systemic abundance of hormones and cytokines. The changes strongly suggest activation of mechanisms of metabolic rather than anabolic control, specifically, the mobilisation of energy from fat stores. We feel confident in our 'domain-based' approach and will therefore proceed with testing our hypothesis that ageing will be reflected in systemic hormone and cytokine temporal and/or abundance variations in older participant groups.

Chapter 6. Age-related changes in systemic protein abundance: response to heavy domain cycle ergometry

6.1. Abstract

In the previous chapters we established the impact of equal work (varying duration) moderate, heavy and very heavy domain constant work-load cycle ergometer exercise on local (mRNA expression in skeletal muscle and subcutaneous adipose tissue) and systemic (protein abundance) responses of selected hormones and cytokines in recreationally active young male participants. We wish to augment the data from the heavy domain (30 % Δ) (H) exercise trial with those from a wider age range (20 - 60 yrs.). We hypothesised that ageing would be reflected in systemic hormone and cytokine temporal and/or abundance variations in the older participant groups. Four groups of recreationally active male participants (Study 3: 20 - 30 yrs., n = 8; body mass, 79.5 ± 10.1 kg; stature 1.78 ± 0.04 m; BMI, 25 ± 2 kg/m^2. 30 - 40 yrs., n = 10; body mass, 79.0 ± 13.1 kg; stature 1.78 ± 0.07 m; BMI, 25 ± 3 kg/m^2. 40 - 50 yrs., n = 8; body mass, 75.6 ± 18.9 kg; stature 1.74 ± 0.10 m; BMI, 23 ± 5 kg/m^2. 50 - 60 yrs., n = 8; body mass, 80 ± 13 kg; stature 1.74 ± 0.10 m; BMI, 26 ± 3 kg/m^2) performed 30 min of heavy domain (30 % Δ) (H) constant work-load cycle ergometer exercise. In all groups IL-6 displayed a similar bi-phasic profile, with an increase during exercise at 10 min and subsequently at 60 min post-exercise ($P < 0.001$). Insulin and leptin declined in all groups during exercise ($P < 0.001$ and $P = 0.002$, respectively). Exercise was without effect on adiponectin. GH increased similarly in all groups to peak again at the end of exercise ($P < 0.001$), although the peak appears depressed in the 40 - 60 yr. age range. IGF-I was unchanged, but concentrations were consistently higher in the 20 – 30 yr. age group at all time points ($P = 0.001$). Cortisol concentrations declined similarly in all groups post-exercise ($P < 0.001$). We concluded that systemic IGF-I concentrations clearly decline between the 3rd and 6th decades of life, and that age (20 - 60 yrs.) is not reflected by temporal and/or abundance variations in systemic insulin, leptin, adiponectin, IL-6, GH, IGF-I or cortisol in response to an acute 30 min bout of heavy domain cycle ergometer exercise.

6.2. Introduction

Evidence suggests endurance exercise may serve a function as an exercise component in therapeautic interventions targeted at ageing individuals, to help address age-related skeletal muscle functional decline by reducing visceral and ectopic (in skeletal muscle and organs) adipose tissue accumulation, and therefore, potentially, systemic and chronic inflammation. Although not the focus of the present work, it is also envisaged that incorporating endurance exercise into such an intervention may provide additional benefits such as improvements in cardio-respiratory fitness and minor improvements in or maintenance of skeletal muscle strength and power, potentially promoting functional improvements in the performance of activities of daily living. Our data suggest cycle ergometer exercise in the heavy domain, specifically, exercise above the Gas exchange threshold (GET) but below Maximal lactate steady state (MLSS), might present such an endurance exercise component. The mechanisms however require clarification. The objective of this chapter is therefore to establish the impact of age (20 - 60 yrs.) on the systemic hormone and cytokine response to an acute, 30 min, heavy domain constant work-load cycle ergometer exercise intervention in recreationally active males. We hypothesise that, even in this relatively young and recreationally active cohort, that ageing will be reflected in systemic hormone and cytokine temporal and/or abundance variations in the older participant groups.

6.3. Methods

Methodological information directly pertinent to this chapter is summarised briefly below. Chapter 2. 'General methods', provides a detailed reference resource, documenting the procedures employed in collecting the data presented in this thesis. Relevant sections in Chapter 2 will be referenced in the text by index number, e.g. [2.2.1.] refers to the section 2.2.1. 'Ethical approval'.

6.3.1. Study design

A schematic representation of the interlinking studies presented in this thesis is provided in figure 2.4 [2.5.1.8.1.]. The data presented in this chapter relate to Exercise study 3.

6.3.2. Participants [2.2.]

Participant descriptive and exercise cardio-respiratory data are provided in Table 6.1. and 6.3.

6.3.3. Determination of exercise trial intensity and duration [2.5.1.5. & 2.5.1.7.]

6.3.4. Exercise trial protocols [2.5.1.8.]

6.3.5. Blood sampling [2.6.]

Samples of whole blood were obtained from the fingertip capillary bed via finger prick pre- and post-exercise to enable measurements of glucose and lactate concentrations. Samples of whole blood were collected via venepuncture from veins in proximity to the cubital fosse of the non-dominant arm. Samples were obtained pre-, during and post-exercise via indwelling venous cannula. Samples were transferred to ice cold S-Monovette® tubes and centrifuged. The serum was aliquoted into 2 ml Eppendorf® tubes and stored at -80 °C until analysed. Serum samples were assayed for specific proteins via Enzyme-linked immunosorbent assay (ELISA). Pre-coated, 96-well ELISA kits were purchased for determination of specific protein concentrations in human serum.

6.3.6. Statistical analyses [2.8.]

Participant descriptive and maximal exercise cardio-respiratory data. One-way Analysis of variance (ANOVA) were run to determine if age, body mass, stature, Body mass index (BMI), maximum rate of oxygen uptake ($\dot{V}O_2$max), Power output at $\dot{V}O_2$max (POmax) and Heart rate at $\dot{V}O_2$max (HRmax) were significantly different between participant groups 20 - 30 yrs., 30 - 40 yrs., 40 - 50 yrs. and 50 - 60 yrs. Where data did not satisfy the assumptions of parametricity, the Kruskal-Wallis test was performed.

Participant heavy domain exercise cardio-respiratory data. One-way ANOVA were run to determine if Power output (PO), rate of oxygen uptake ($\dot{V}O_2$), Heart rate (HR), rate of oxygen uptake divided by heart rate ($\dot{V}O_2$/HR), and Total work done were significantly different for heavy domain exercise trials between participant groups 20 - 30 yrs., 30 - 40 yrs., 40 - 50 yrs. and 50 - 60 yrs. Data were log transformed where applicable.

Heavy domain exercise trial comparison analyses. Pearson's product-moment correlation coefficients were computed to assess the relationship between $\dot{V}O_2$, HR and $\dot{V}O_2$/HR data for heavy domain exercise determined from $\dot{V}O_2$max tests and data measured directly during subsequent heavy domain exercise trials. Paired samples t-Tests were performed to determine if $\dot{V}O_2$, HR and $\dot{V}O_2$/HR data for heavy domain exercise, determined from $\dot{V}O_2$max tests, varied significantly from data measured directly during subsequent heavy domain exercise trials.

Blood lactate and blood glucose analyses. Repeated-measures ANOVA with as within factor 'time' and between factor 'group' were run to determine whether there were significant time and age effects. If significant effects were observed post-hoc Bonferroni-corrected one-way ANOVA were run to identify the difference.

Maximal and heavy domain exercise-induced hormonal adaptation analyses. Repeated-measures ANOVA with as within factor 'time' and between factor 'group' were run to determine whether there were significant time and age effects. If significant effects were observed post-hoc Bonferroni-corrected one-way ANOVA were run to identify the difference.

Delta analyses. In the case of a significant 'time' x 'group' interaction, a post-hoc ANOVA was run on the absolute delta values to identify the different responses between age groups.

6.4. Results

6.4.1. Participant descriptive and maximal exercise cardio-respiratory data

No statistically significant differences were evident between the participant groups for body mass, stature, BMI, $\dot{V}O_2$max or POmax. HRmax, not unexpectedly, was higher in the 20 - 30 yr. olds compared to the other age groups (table 6.1).

6.4.2. Participant heavy domain exercise cardio-respiratory data

There were no significant differences between the participant groups for PO, $\dot{V}O_2$, $\dot{V}O_2$ /HR, or Total work done during the heavy domain exercise. The HR was, however, significantly higher in the 20 - 30 *vs*. the 30 - 40 yr. old participants (table 6.2).

Table 6.1. Participant descriptive and maximal exercise cardio-respiratory data

Participant details	**20 - 30 yrs.**	**30 - 40 yrs.**	**40 - 50 yrs.**	**50 - 60 yrs.**	**Statistical analyses (main effects)**
Gender, *n*	male, 8	male, 10	male, 8	male, 8	-
Age (yrs.)	22 ± 2	34 ± 3	45 ± 3	57 ± 4	$P < 0.001$ *
Body mass (kg)	79.5 ± 10.1	79.0 ± 13.1	75.6 ± 18.9	80 ± 13	$P = 0.91$
Stature (m)	1.78 ± 0.04	1.78 ± 0.07	1.79 ± 0.07	1.74 ± 0.10	$P = 0.51$
BMI (kg/m^2)	25 ± 2	25 ± 3	23 ± 5	26 ± 3	$P = 0.33$
$\dot{V}O_2max$ (ml/min)	3741 ± 837	3723 ± 807	3456 ± 639	3348 ± 821	$P = 0.58$
POmax (W)	272 ± 62	289 ± 53	236 ± 50	247 ± 58	$P = 0.17$
HRmax (b/min)	187 ± 14	170 ± 8	169 ± 3	171 ± 14	$P = 0.03$ **

Post-hoc analyses. Age (*) all groups ($P < 0.001$), HRmax (**) 20 - 30 yrs. *vs*. 30 - 40 yrs. ($P = 0.03$) and 20 - 30 yrs. *vs*. 40 - 50 yrs. ($P = 0.02$).

Table 6.2. Participant heavy domain exercise cardio-respiratory data

Exercise variables	20 - 30 yrs.	30 - 40 yrs.	40 - 50 yrs.	50 - 60 yrs.	Statistical analyses
Trial time (min:s)	30:00 ± 00:00	30:00 ± 00:00	30:00 ± 00:00	30:00 ± 00:00	-
PO (W)	138 ± 35	146 ± 27	121 ± 19	129 ± 24	$P = 0.28$
$\dot{V}O_2$(ml/min)	2076 ± 535	2148 ± 310	2099 ± 372	2148 ± 372	$P = 0.93$
HR (b/min)	141 ± 14	123 ± 16	125 ± 8	123 ± 10	$P = 0.04$ ***
$\dot{V}O_2$/HR (ml/b/min)	15 ± 4	18 ± 3	17 ± 2	18 ± 3	$P = 0.44$
Total work done (KJ)	248 ± 72	262 ± 49	218 ± 34	233 ± 43	$P = 0.28$

Post-hoc analyses. HR (***) 20 - 30 yrs. *vs*. 30 - 40 yrs. ($P = 0.05$).

6.4.3. Heavy domain exercise trial comparison analyses

Having examined the relationship between relevant output measures ($\dot{V}O_2$, HR and $\dot{V}O_2$ /HR data) determined from the $\dot{V}O_2$max tests and measured directly during subsequent exercise trials for moderate, heavy and very heavy domain exercise (Chapter 3. Section 3.4.3. 'Exercise trial comparison analyses'), we wished to build on the findings and examine the relationship between these output measures established from participants in the present study. To this end, Pearson's product-moment correlation coefficients indicated significant positive correlations for $\dot{V}O_2$ ($r = 0.828$, $n = 34$, $P < 0.001$; figure 6.1), HR ($r = 0.729$, $n = 30$, $P < 0.001$; figure 6.2) and $\dot{V}O_2$/HR ($r = 0.877$, $n = 30$, $P = 0.001$; figure 6.3) in participants between the 20 - 60 yrs. of age, performing heavy domain exercise. Comparison of $\dot{V}O_2$, HR and $\dot{V}O_2$/HR data determined from $\dot{V}O_2$max tests with that measured during heavy domain exercise revealed no significant differences for HR ($P = 0.06$) and $\dot{V}O_2$/HR ($P = 0.48$). A significant difference was observed for $\dot{V}O_2$ ($P = 0.02$), reflecting the higher $\dot{V}O_2$ values measured in response to the heavy domain exercise trial.

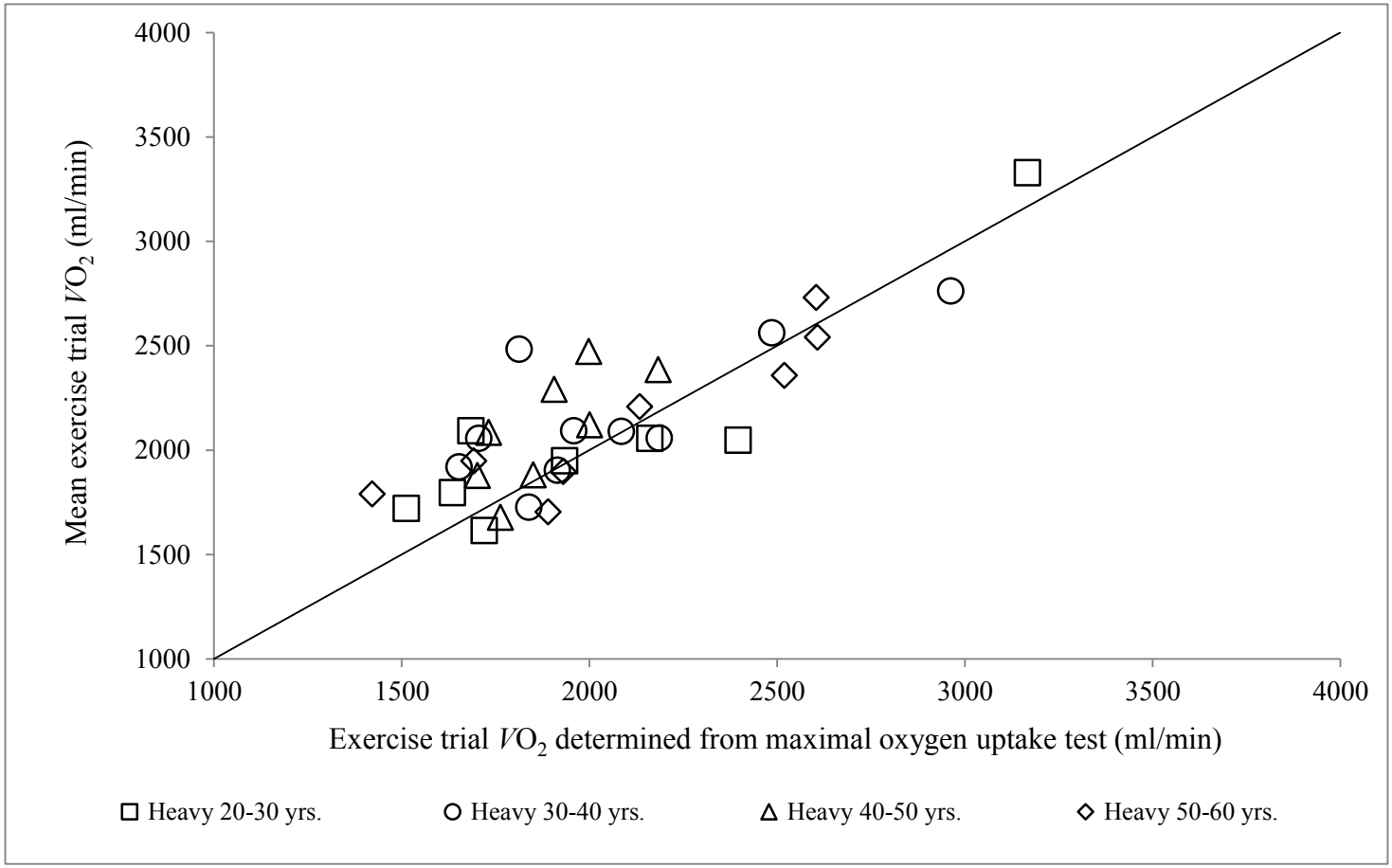


Figure 6.1. Correlation between $\dot{V}O_2$ measured during $\dot{V}O_2$max tests and heavy (30 % Δ) intensity exercise trials in 20 - 30, 30 - 40, 40 - 50 and 50 - 60 yr. old males ($r = 0.828$, $n = 34$, $P < 0.001$).

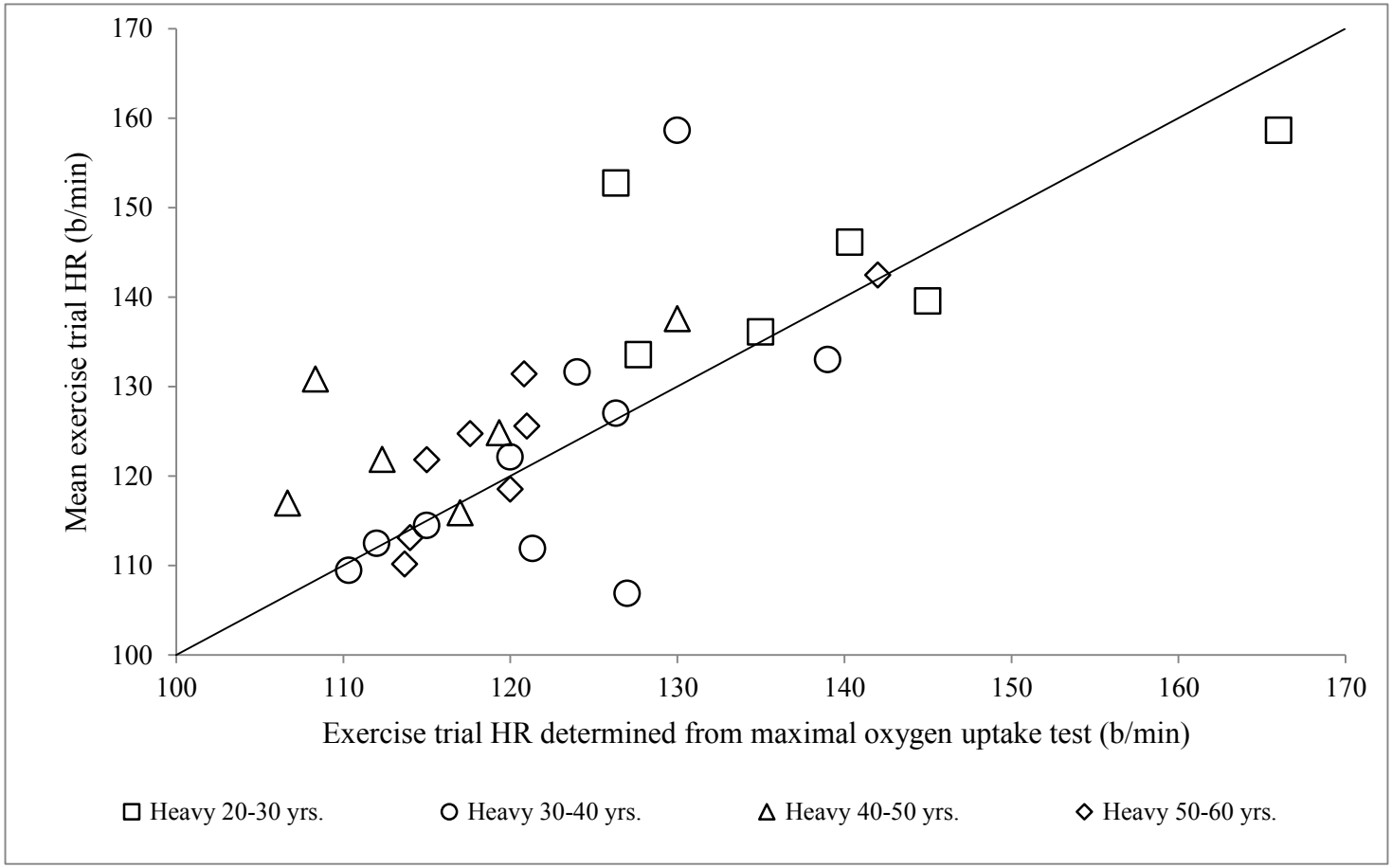


Figure 6.2. Correlation between HR measured during $\dot{V}O_2$max tests and heavy (30 % Δ) intensity exercise trials in 20 - 30, 30 - 40, 40 - 50 and 50 - 60 yr. old males (r = 0.729, n = 30, P < 0.001).

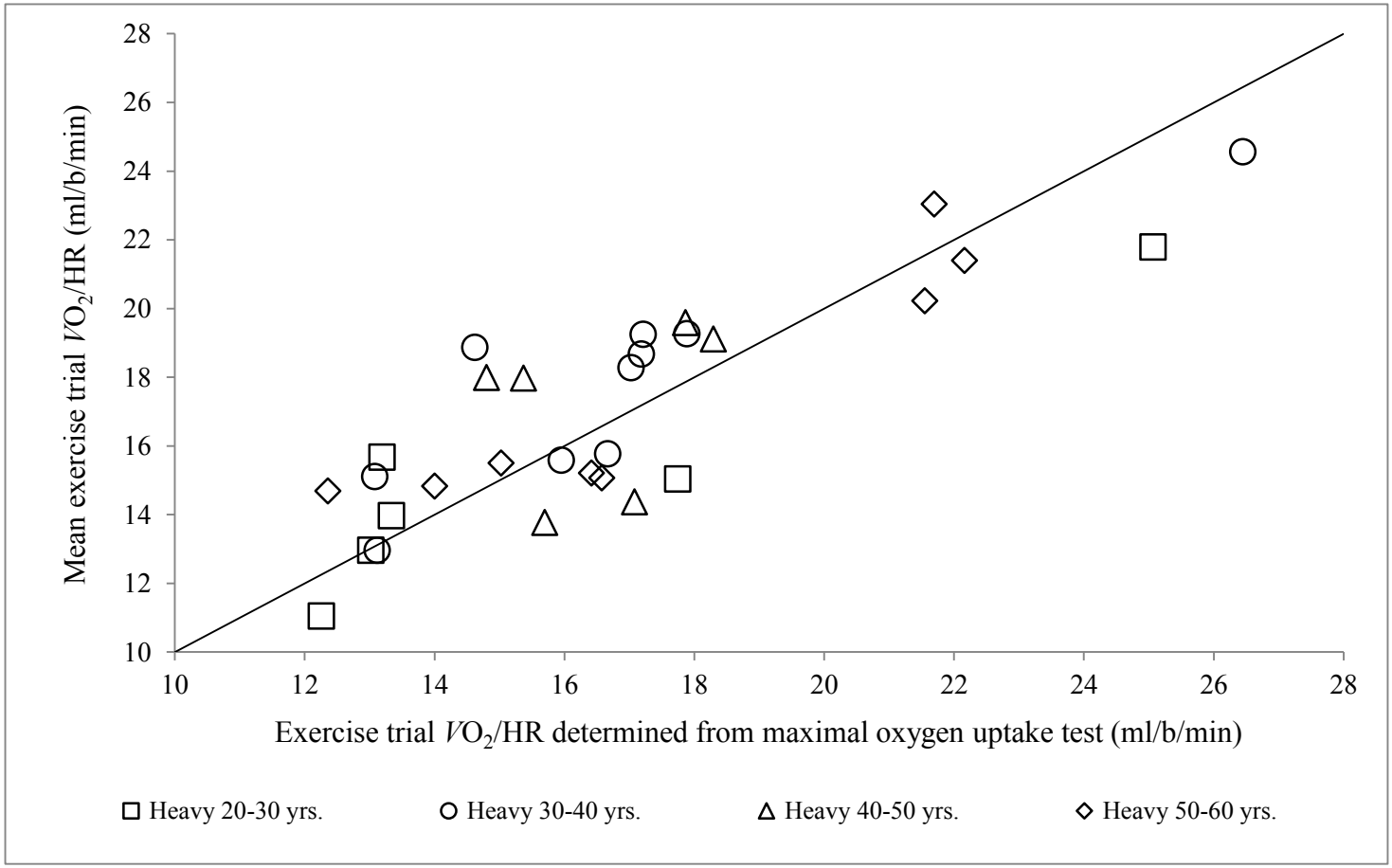


Figure 6.3. Correlation between $\dot{V}O_2$/HR measured during $\dot{V}O_2$max tests and heavy (30 % Δ) intensity exercise trials in 20 - 30, 30 - 40, 40 - 50 and 50 - 60 yr. old males ($r = 0.877$, $n = 30$, $P = 0.001$).

6.4.4. Blood lactate and blood glucose analyses

Blood lactate (BLa) increased significantly during both maximal and heavy domain exercise ($P < 0.001$) (figure 6.4), with 3.5 - 4-fold greater increases in BLa in response to maximal exercise. The significant effect of 'group' observed in response to both maximal ($P < 0.001$) and heavy domain ($P = 0.008$) exercise highlights the greater post-exercise BLa concentrations in the older individuals. A significant 'group' x 'time' interaction ($P = 0.02$) highlights the greater increase in BLa in 40 - 50 *vs.* 20 - 30 and 30 - 40 yr. old participants, during maximal exercise, which is supported by post-hoc comparison data ($P = 0.001$). Post-hoc comparison data from heavy domain exercise support a similar finding, with lower BLa values in the 30 - 40 *vs.* 40 - 50 ($P = 0.04$) and 50 - 60 ($P = 0.02$) yr. olds. A significant increase in Blood glucose (BGlu) was observed only during maximal exercise ($P < 0.001$). Post-hoc analyses were unable to locate the difference despite a significant effect of 'group' ($P = 0.04$). However, the greatest and similar increases were observed in the 20 - 30 and 30 - 40 yr. old participants. No significant effects were observed with regard to heavy domain exercise.

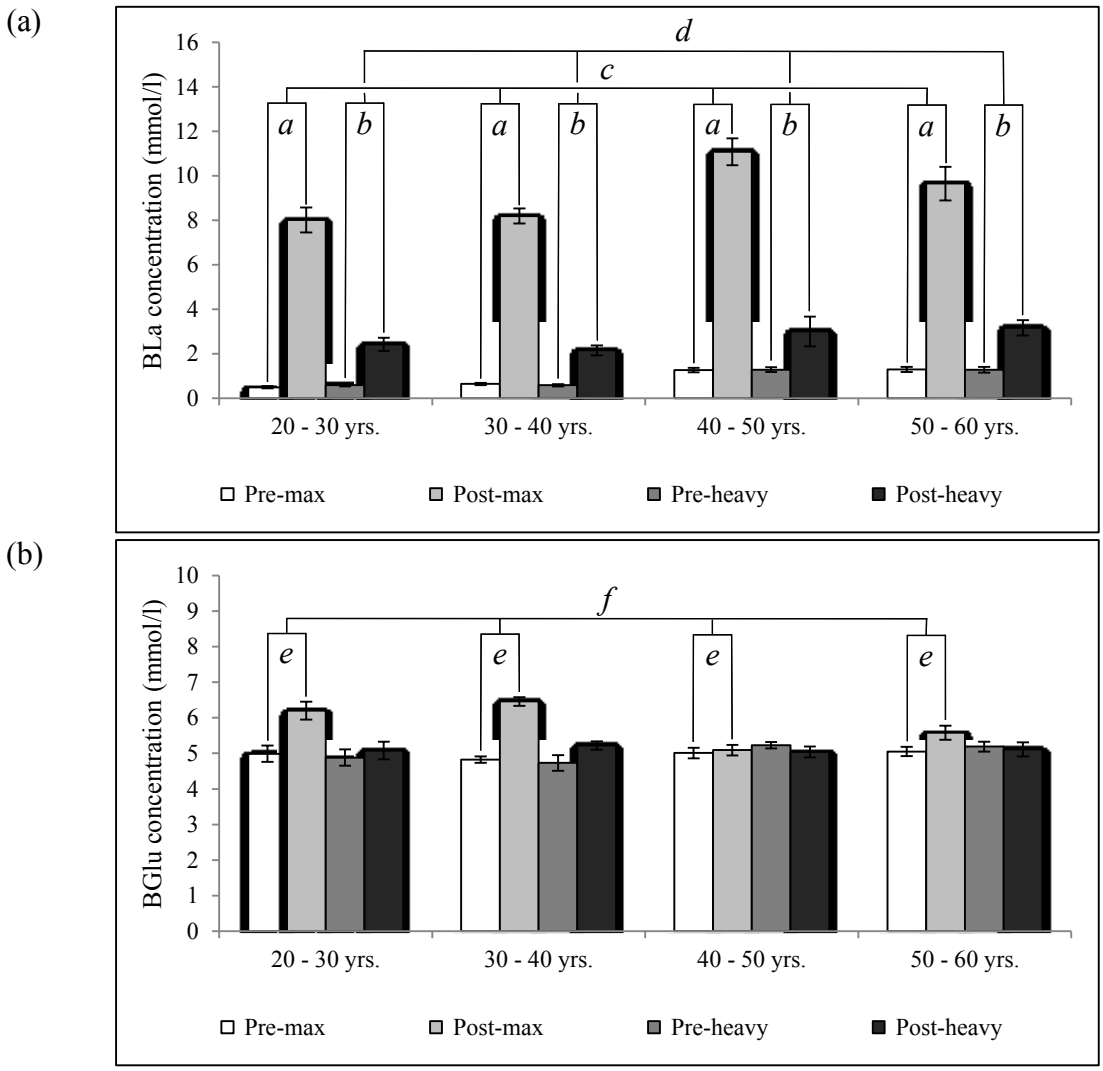


Figure 6.4. Maximal and heavy domain cycle ergometer exercise adaptation. Blood lactate (Bla) (a) and blood glucose (BGlu) (b). (a) Significant effect of 'time'. Pre-max *vs.* Post-max ($P < 0.001$; *a*), Pre-heavy vs. Post-heavy ($P < 0.001$; *b*). Significant effect of 'group'. Pre-max *vs.* Post-max ($P < 0.001$; *c*), Pre-heavy vs. Post-heavy ($P < 0.001$; *d*). (b) Significant effect of 'time'. Pre-max *vs.* Post-max ($P < 0.001$; *e*). Significant effect of 'group'. Pre-max *vs.* Post-max ($P = 0.04$; *f*). Data are (mean ± SEM).

6.4.5. Maximal exercise-induced hormonal adaptation

Insulin concentration increased during maximal exercise (figure 6.5 and 6.5, *continued*). A 'group' x 'time' interaction ($P = 0.03$) highlights a somewhat attenuated response in the 40 - 50 and 50 - 60 yr. old participants. Growth hormone (GH) increased in all age groups in response to maximal exercise. Baseline Insulin-like growth factor-I (IGF-I) levels were highest in the youngest age group compared with all other groups, which did not differ from each other. Maximal exercise resulted in an increase in IGF-I concentration, which was attenuated progressively with respect to increasing age ('group' x 'time' interaction, $P = 0.002$). No differences in cortisol concentration were apparent. The Interleukin-6 (IL-6) concentration increased in all age groups in response to maximal exercise.

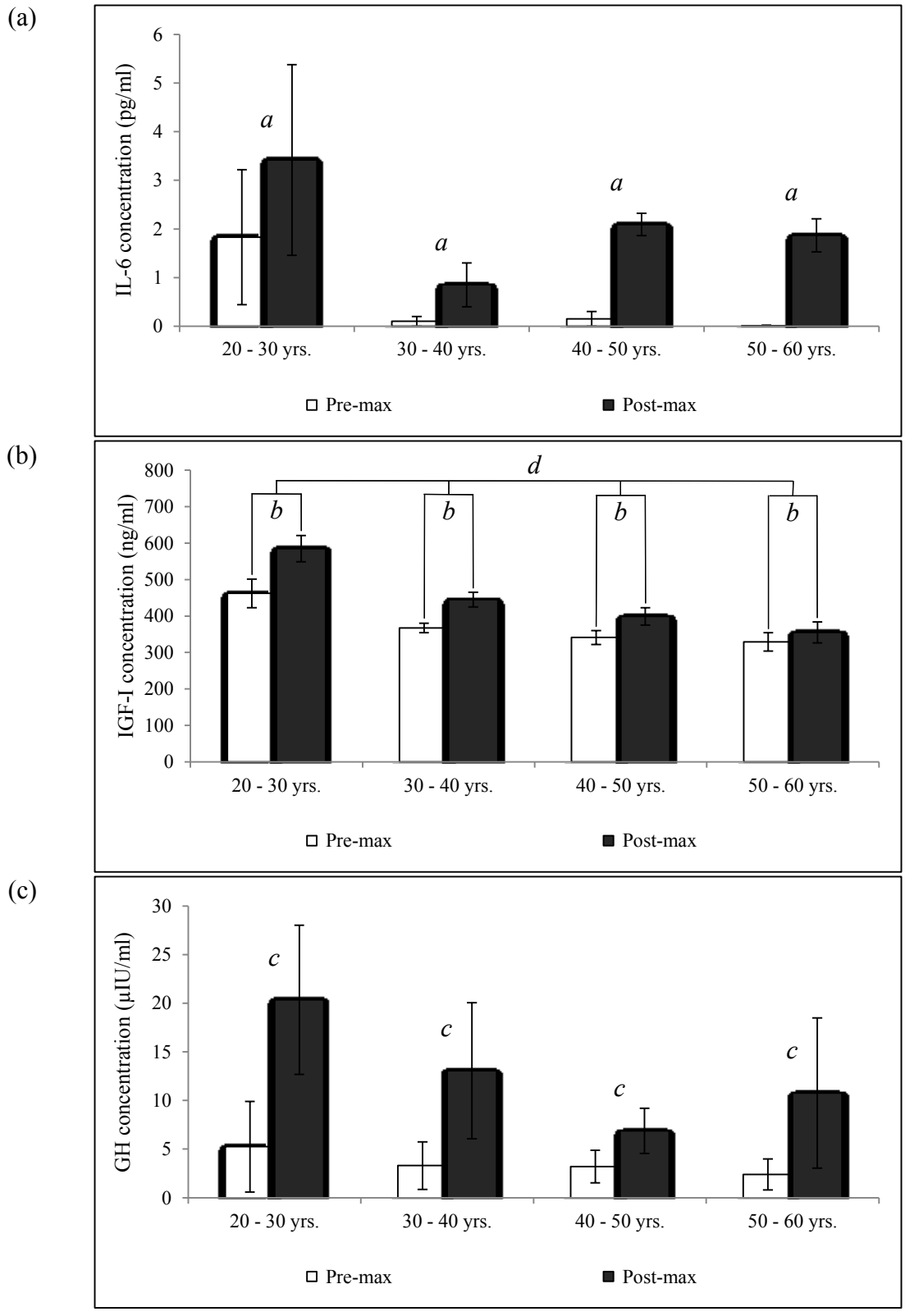
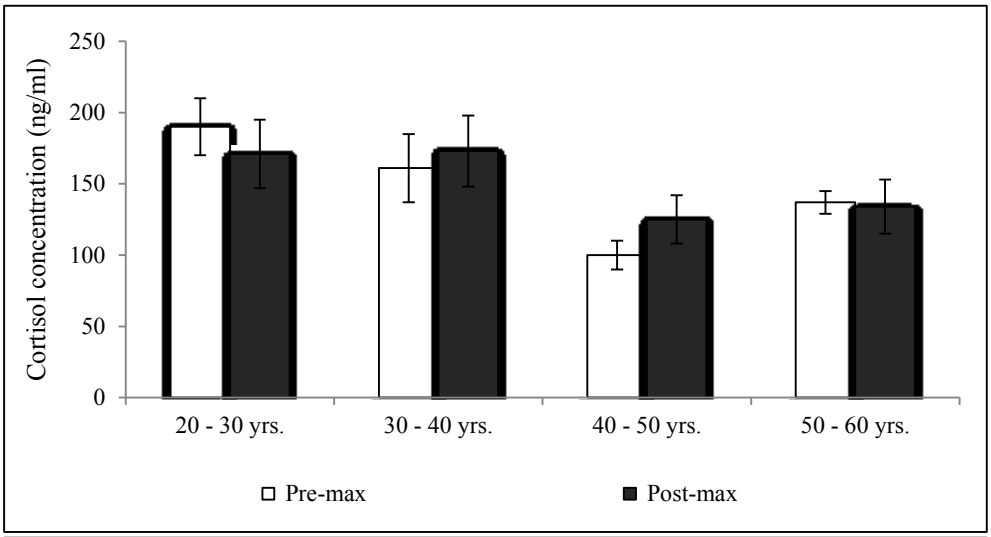


Figure 6.5. Maximal exercise-induced hormonal adaptation. IL-6 (a), IGF-I (b) and GH (c). Significant effect of 'time'. Pre-max *vs.* Post-max (a) ($P < 0.001$; *a*), (b) ($P < 0.001$; *b*), (c) ($P = 0.003$; *c*). Significant effect of 'group'. Pre-max *vs.* Post-max (b) ($P < 0.001$; *d*). Data are (mean ± SEM).

(d)



(e)

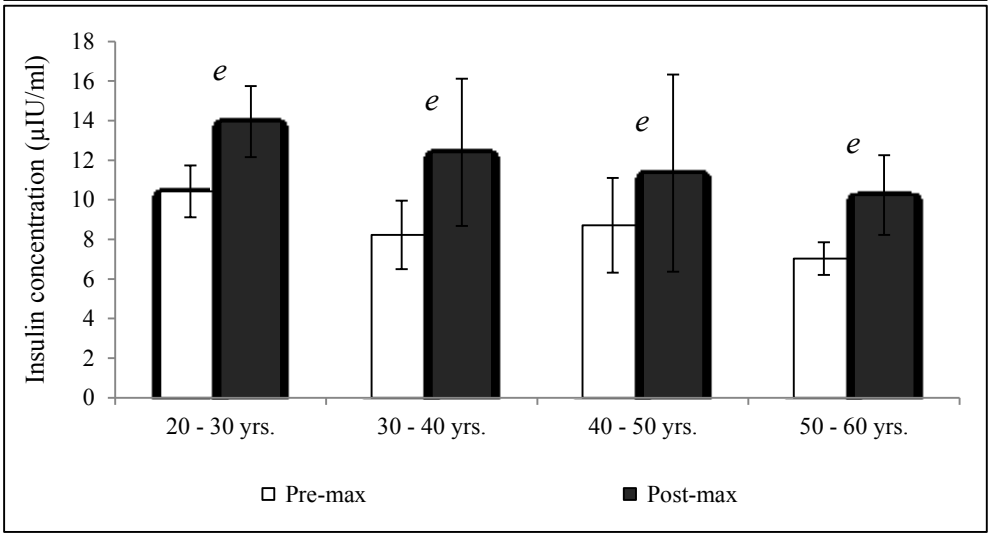


Figure 6.5, *continued.* Maximal exercise-induced hormonal adaptation. Cortisol (d) and Insulin (e). Significant effect of 'time'. Pre-max *vs.* Post-max (e) ($P = 0.02$; *e*). Data are (mean ± SEM).

6.4.6. Heavy domain exercise-induced hormonal adaptation

6.4.6.1. Insulin, leptin and adiponectin adaptations

Heavy domain exercise resulted in a decrease in systemic insulin concentration in all age groups (figure 6.6). A significant decrease in systemic leptin concentration post-exercise was observed, which was similar in all age groups (figure 6.7). No significant differences were observed in systemic adiponectin concentration (figure 6.8).

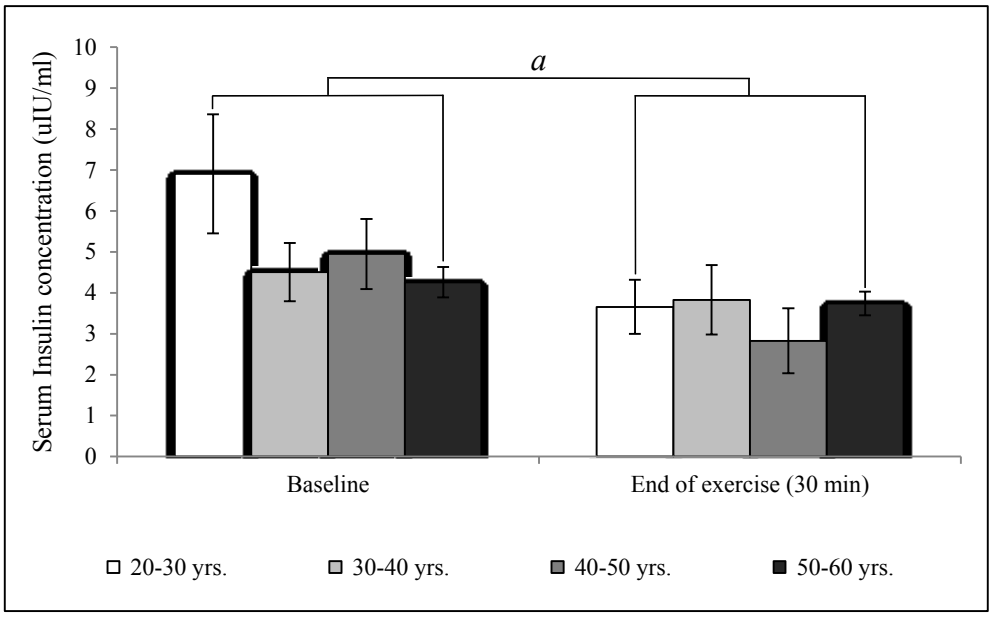


Figure 6.6. Systemic insulin response to heavy domain cycle ergometer exercise in 20 - 30, 30 - 40, 40 - 50 and 50 - 60 yr. old males. Significant effect of 'time' ($P < 0.001$; *a*). Data are (mean ± SEM).

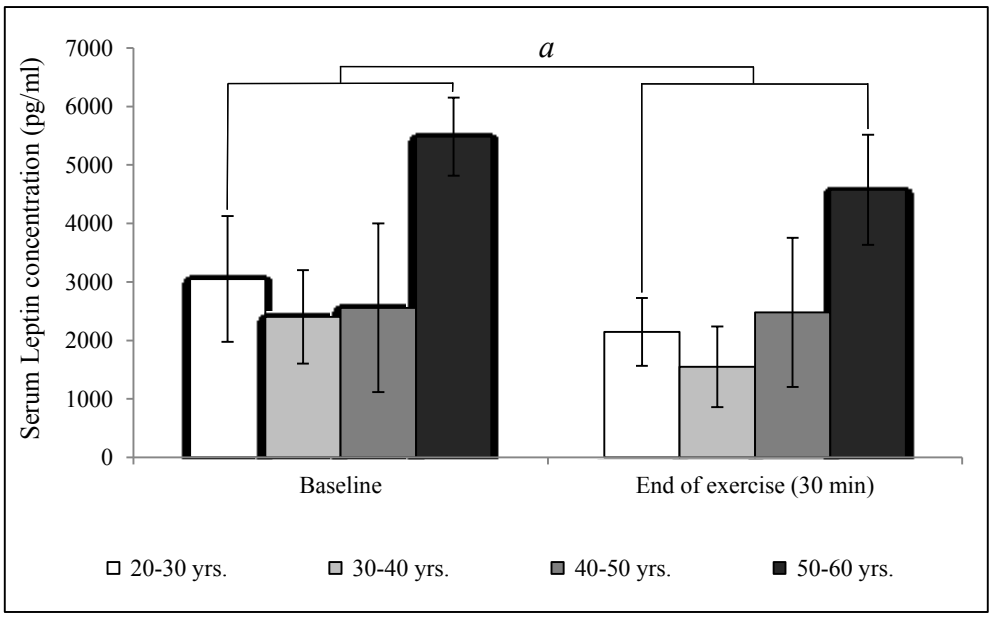


Figure 6.7. Systemic leptin response to heavy domain cycle ergometer exercise in 20 - 30, 30 - 40, 40 - 50 and 50 - 60 yr. old males. Significant effect of 'time' ($P = 0.002$; *a*). Data are (mean ± SEM).

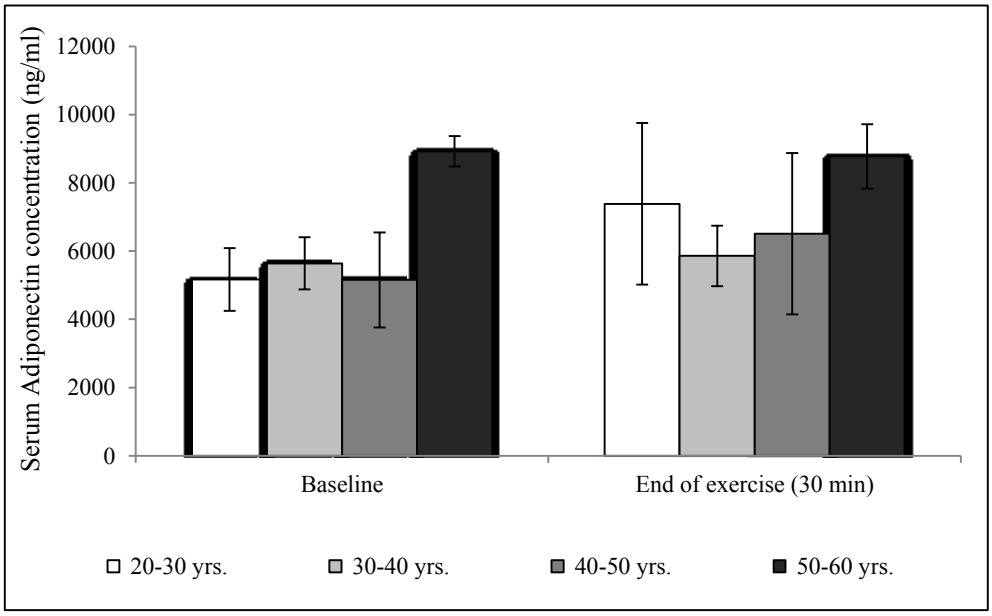


Figure 6.8. Systemic adiponectin response to heavy domain cycle ergometer exercise in 20 - 30, 30 - 40, 40 - 50 and 50 - 60 yr. old males. Data are (mean ± SEM).

6.4.6.2. Anabolic adaptations

During heavy domain exercise GH increased significantly in all age groups (figure 6.9). Growth hormone returned to baseline levels 60 min after cessation of exercise. The most significant variation occurred between GH concentrations at the end of exercise compared with those at baseline and 60 min post-exercise. Although post-hoc comparisons revealed no significant group differences, the GH response in the older age groups appears reduced in comparison with that of the younger age groups. During heavy domain exercise IGF-I concentrations did not increase, but were consistently higher in the youngest age group (figure 6.10).

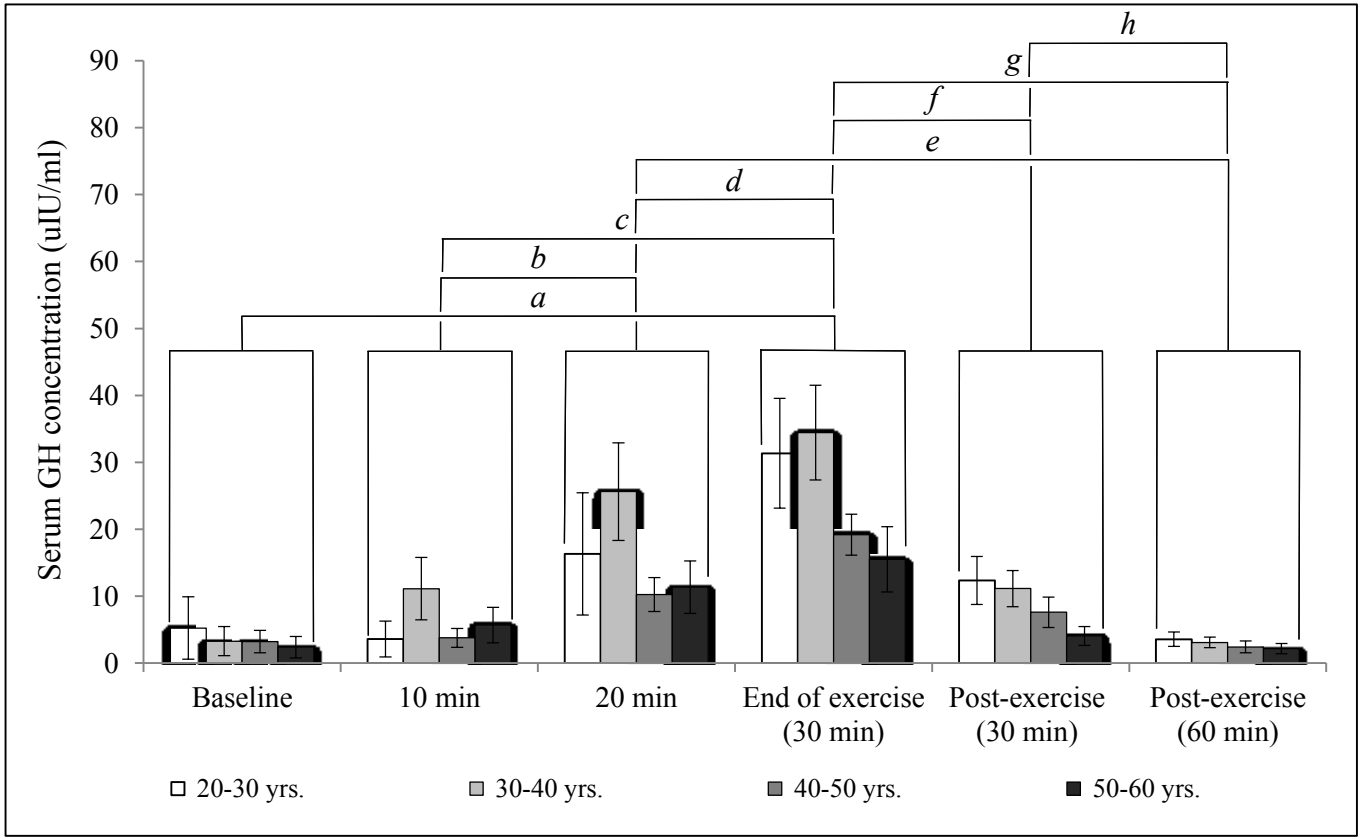


Figure 6.9. Systemic GH response to heavy domain cycle ergometer exercise in 20 - 30, 30 - 40, 40 - 50 and 50 - 60 yr. old males. Significant effect of 'time' ($P < 0.001$). Post-hoc analyses, Baseline *vs.* End of exercise ($P < 0.001$; *a*), 10 min *vs.* 20 min ($P = 0.006$; *b*) and End of exercise ($P < 0.001$; *c*), 20 min *vs.* End of exercise ($P = 0.02$; *d*) and Post-exercise (60 min) ($P = 0.01$; *e*), End of exercise *vs.* Post-exercise (30 min) ($P < 0.001$; *f*) and Post-exercise (60 min) ($P < 0.001$; *g*), Post-exercise (30 min) *vs.* Post-exercise (60 min) ($P < 0.001$; *h*). Data are (mean ± SEM).

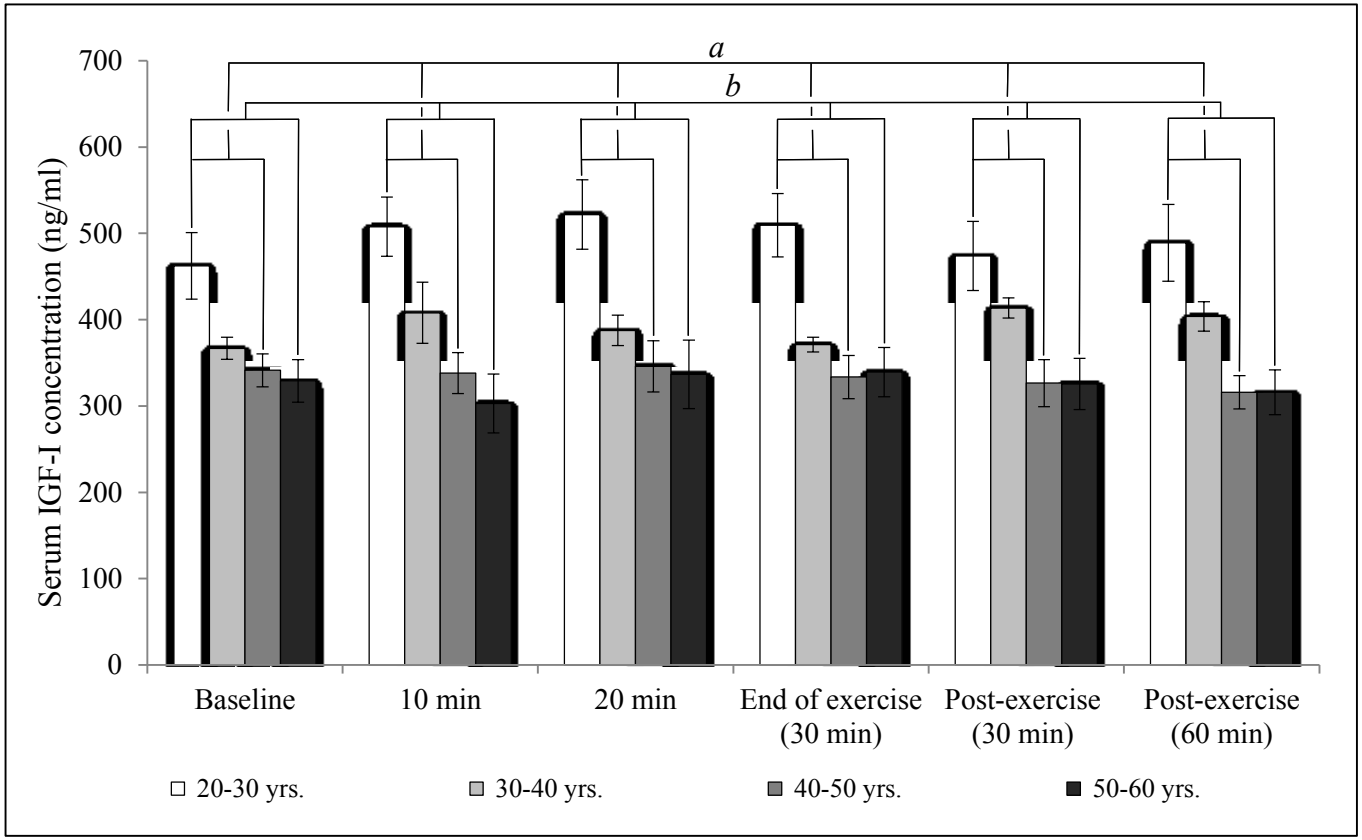


Figure 6.10. Systemic IGF-I response to heavy domain cycle ergometer exercise in 20 - 30, 30 - 40, 40 - 50 and 50 - 60 yr. old males. Significant effect of 'group' ($P = 0.001$). Post-hoc analyses, 20 - 30 *vs.* 40 - 50 ($P = 0.004$; *a*) and 50 - 60 ($P = 0.002$; *b*) yrs. Data are (mean ± SEM).

6.4.6.3. Catabolic and metabolic/inflammatory adaptations

During heavy domain exercise cortisol concentrations decreased (figure 6.11). Post-hoc analyses indicated the decline in cortisol became significant in the post-exercise period. The absence of an effect of 'group' and 'group' x 'time' interaction indicated that the decrease in systemic cortisol was similar for all age groups. Heavy domain exercise resulted in an increase in IL-6 abundance in the first 10 min of exercise and again post-exercise (figure 6.12). Post-hoc analyses revealed that all time points differed significantly, except at 20 min, where data were similar to baseline. A significant 'group' x 'time' interaction ($P < 0.001$) was reflected by a greater post-exercise increase in IL-6 in the 40 - 50 and 50 - 60 yr. age groups. Post-hoc 'Delta' analyses indicated a significant impact of 'time' 60 min post-exercise ($P = 0.001$). Post-hoc multiple comparisons on 'group' indicate the 30 - 40 yr. old participants differed significantly from those in the 40 - 50 and 50 - 60 yr. age groups ($P = 0.002$). The response in the 20 - 30 yr. old participants did not differ from any other group.

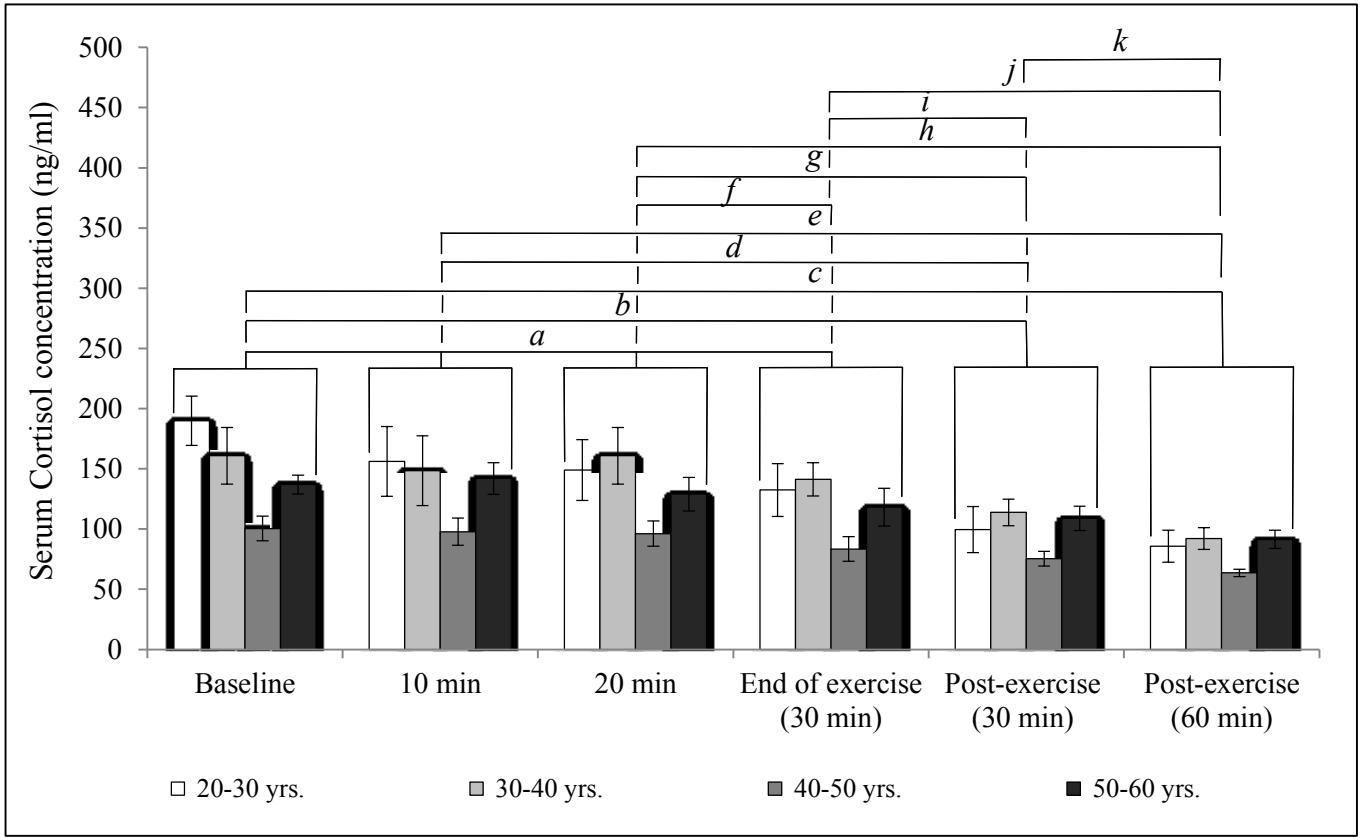


Figure 6.11. Systemic cortisol response to heavy domain cycle ergometer exercise in 20 - 30, 30 - 40, 40 - 50 and 50 - 60 yr. old males. Significant effect of 'time' ($P < 0.001$). Post-hoc analyses, Baseline *vs.* End of exercise ($P = 0.009$; *a*), Post-exercise (30 min) ($P < 0.001$; *b*) and Post-exercise (60 min) ($P < 0.001$; *c*), 10 min *vs.* Post-exercise (30 min) ($P = 0.007$; *d*) and Post-exercise (60 min) ($P = 0.001$; *e*), 20 min *vs.* End of exercise ($P = 0.03$; *f*), Post-exercise (30 min) ($P = 0.001$; *g*) and Post-exercise (60 min) ($P < 0.001$; *h*), End of exercise *vs.* Post-exercise (30 min) ($P < 0.001$; *i*) and Post-exercise (60 min) ($P < 0.001$; *j*), Post-exercise (30 min) *vs.* Post-exercise (60 min) ($P = 0.001$; *k*). Data are (mean ± SEM).

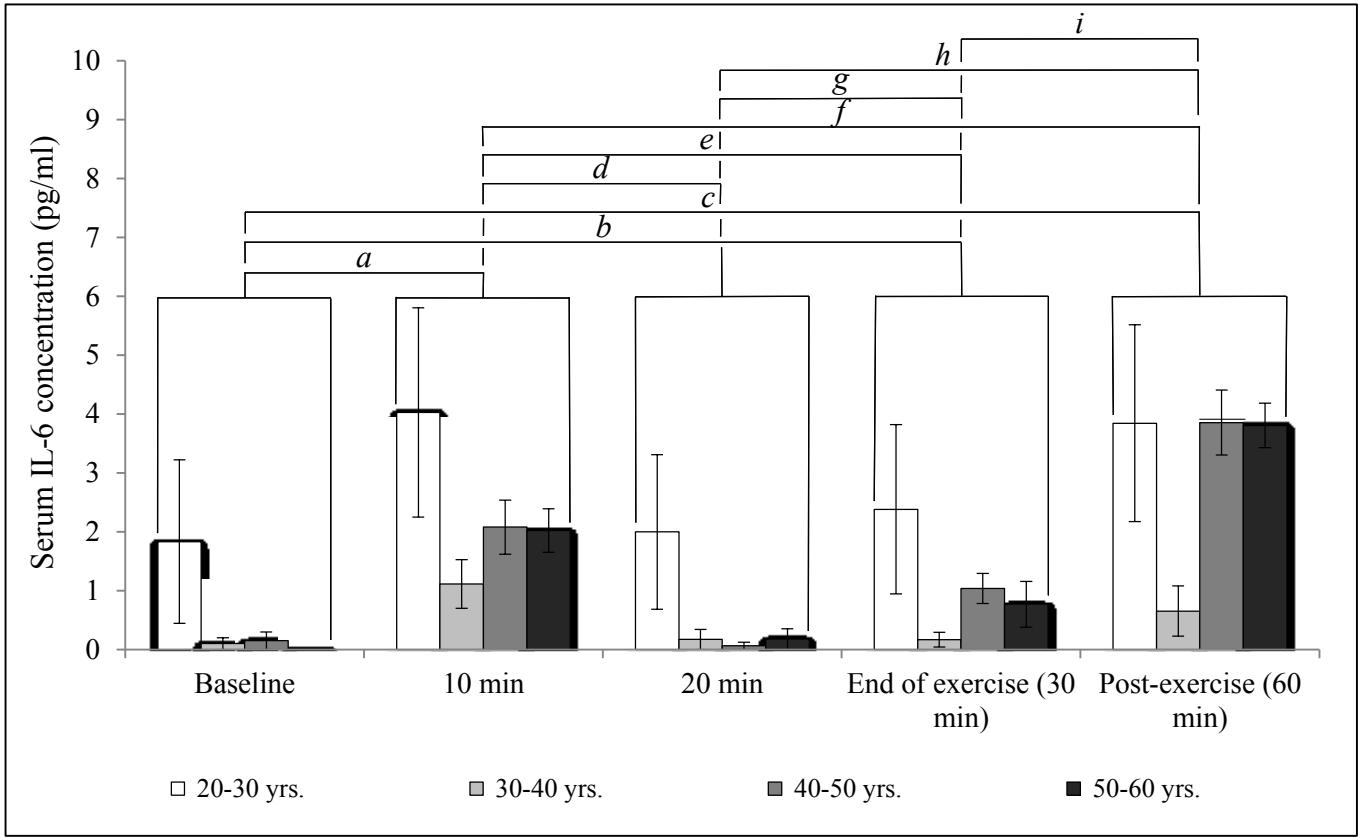


Figure 6.12. Systemic IL-6 response to heavy domain cycle ergometer exercise in 20 - 30, 30 - 40, 40 - 50 and 50 - 60 yr. old males. Significant effect of 'time' ($P < 0.001$). Post-hoc analyses, Baseline *vs.* 10 min ($P < 0.001$; *a*), End of exercise ($P = 0.04$; *b*), and Post-exercise (60 min) ($P < 0.001$; *c*), 10 min *vs.* 20 min ($P < 0.001$; *d*), End of exercise ($P < 0.001$; *e*) and Post-exercise (60 min) ($P < 0.001$; *f*), 20 min *vs.* End of exercise ($P = 0.004$; *g*), and Post-exercise (60 min) ($P < 0.001$; *h*), End of exercise *vs.* Post-exercise (60 min) ($P = 0.001$; *i*). Data are (mean ± SEM).

6.4.6.4. Systemic protein ratios

During heavy domain exercise the leptin to adiponectin ratio decreased by ~ 0.8 – 0.5-fold ($P = 0.004$; figure 6.13) and the GH to cortisol ratio increased by ~ 8.8 - 14-fold ($P < 0.001$; figure 6.14). The GH to cortisol ratio returned to baseline levels 60 min after cessation of exercise.

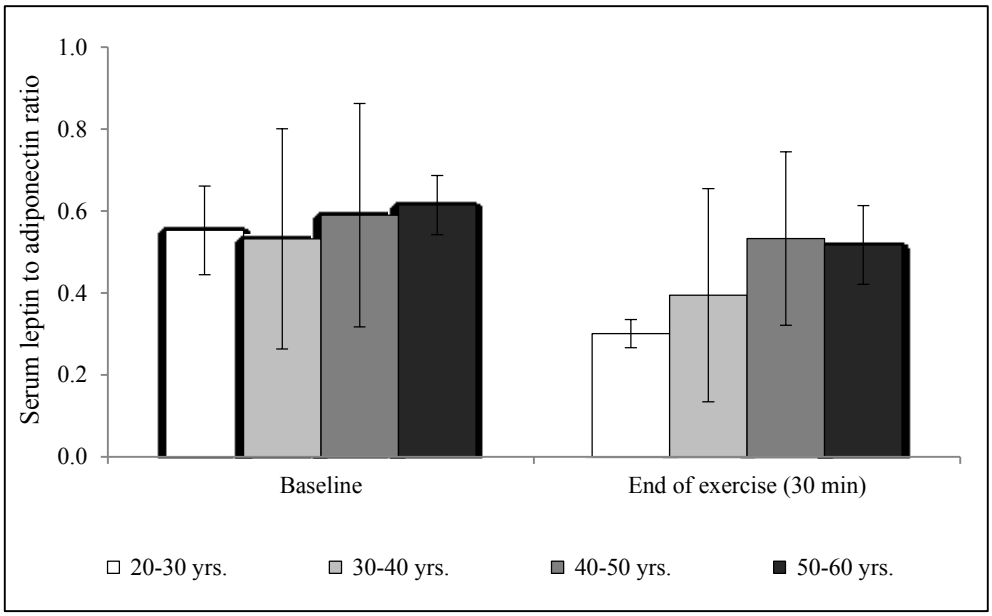


Figure 6.13. The leptin to adiponectin ratio response to heavy domain cycle ergometer exercise in 20 - 30, 30 - 40, 40 - 50 and 50 - 60 yr. old males. Data are (mean ± SEM).

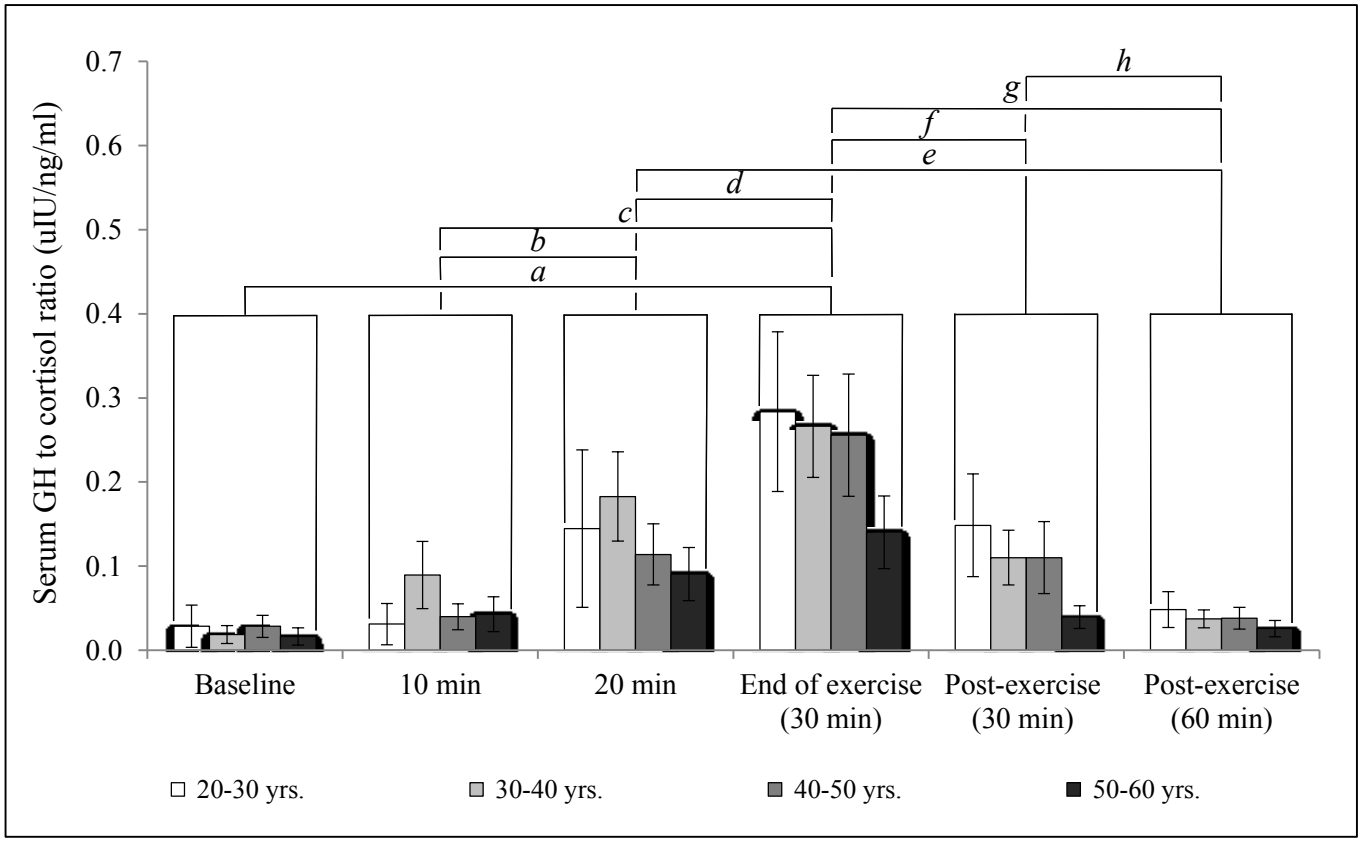


Figure 6.14. The GH to cortisol ratio response to heavy domain cycle ergometer exercise in 20 - 30, 30 - 40, 40 - 50 and 50 - 60 yr. old males. Significant effect of 'time' ($P < 0.001$). Post-hoc analyses, Baseline *vs.* End of exercise ($P < 0.001$; *a*), 10 min *vs.* 20 min ($P = 0.02$; *b*) and End of exercise ($P = 0.001$; *c*), 20 min *vs.* End of exercise ($P = 0.05$; *d*), and Post-exercise (60 min) ($P = 0.02$; *e*), End of exercise *vs.* Post-exercise (30 min) ($P < 0.001$; *f*) and Post-exercise (60 min) ($P < 0.001$; *g*), Post-exercise (30 min) *vs.* Post-exercise (60 min) ($P = 0.007$; *h*). Data are (mean ± SEM).

6.5. Discussion

6.5.1. Participant descriptive and cardio-respiratory data

As is commonly observed (Higginbotham et al., 1986; Ogawa et al., 1992) HRmax was reduced in the older participant groups. This is in contrast to similar values for body mass, stature, BMI, $\dot{V}O_2$max and POmax across the cohort. These data confirm a healthy heterogeneic population with a good level of aerobic fitness (Heyward 1998).

6.5.2. Heavy domain exercise trial comparison analyses

Previously, it was established that there were good correlations between $\dot{V}O_2$, HR and $\dot{V}O_2$/HR measured during the $\dot{V}O_2$max tests and during exercise trials for moderate, heavy and very heavy domain exercise in young participants (Chapter 3. Section 3.4.3. 'Exercise trial comparison analyses'). Correlations from the present study indicate agreement over the extended age range. No differences were found between HR and $\dot{V}O_2$ /HR. However, a significant difference in $\dot{V}O_2$ was found. This was due to the effect of the $\dot{V}O_2$ slow component; a gradual increase in $\dot{V}O_2$ apparent after 10 - 20 min in exercise above the GET (Jones et al., 1999). Although not significantly different, HR was also elevated in the heavy exercise trial compared to the $\dot{V}O_2$max test. This maybe due to the influence of cardio-vascular drift (Coyle and González-Alonso, 2001) where a progressive decline in stroke volume and pulmonary and systemic mean arterial pressures is accompanied by a parallel increase in HR in order to maintain cardiac output (Coyle and González-Alonso, 2001). The similarity in $\dot{V}O_2$/HR between the two tests indicates comparable increases in both $\dot{V}O_2$ and HR during the exercise trials.

6.5.3. Blood lactate and blood glucose analyses

Blood lactate concentrations increased during both maximal and heavy domain exercise. The concentration of BLa was greater in response to maximal exercise due to a greater anaerobic influence. The younger age groups appeared to have lower BLa concentrations at baseline than the older age groups (~ 0.5 *vs.* 1.3 mmol/l), and in response to both maximal (~ 8.1 *vs.* 10 mmol/l) and heavy domain (~ 2.3 *vs.* 3.1 mmol/l) exercise respectively, despite the similar relative physiological demands of the heavy domain

exercise bout. Lower skeletal muscle oxidative capacity maybe responsible for the higher BLa concentrations in the older individuals (Chisari et al., 2002). Blood glucose concentration is maintained within a narrow range. Deviations from this normal range (hypo/hyper-glycaemia) can have neurological consequences, such as light-headedness, confusion, and if unchecked, can result in coma. Fasted baseline BGlu concentrations were within the normal range (3.9 - 5.5 mmol/l) (Diabetes.co.uk, no date). Blood glucose increased during maximal exercise. However, while the data show a similar response in the two younger age groups, the increase was somewhat attenuated in the older age groups. The reason for this uncoupling is unclear. Blood glucose concentrations remained similar to baseline concentrations during heavy domain exercise. These variations in the BGlu response can broadly be explained by changes in the mechanisms of Glucose utilisation (GU) and Glucose production (GP) with exercise intensity (Marliss and Vranic, 2002).

6.5.4. Insulin, leptin and adiponectin adaptations

Fasting concentrations of leptin (Liu et al., 1999), adiponectin (Antuna-Puente et al., 2008) and insulin (Kahn et al., 1997) were within previously established normal ranges. During maximal exercise insulin increased, while during heavy domain exercise insulin decreased. Leptin decreased significantly also during heavy exercise. Exercise was without effect on adiponectin concentration. An increase in insulin during maximal exercise would promote glucose uptake in skeletal muscle, adipose tissue and the liver, and fatty acid uptake and lipogenesis in adipose tissue. Insulin reduces the release of glucose from the liver by inhibiting hepatic glycogenolysis and the expression of key gluconeogenic enzymes (Wahren and Ekberg, 2007). In addition to the primary role of maintaining glucose homeostasis, insulin mediates other cellular events including regulation of ion and amino-acid transport, lipid metabolism, glycogen synthesis, gene transcription and Messenger ribonucleic acid (mRNA) turnover, protein synthesis and degradation in liver and muscle, and Deoxyribonucleic acid (DNA) synthesis (Cheatham and Kahn, 1995). Impaired processing of pro-insulin to insulin has been suggested as a mechanism for the age-related decline in insulin release (Bryhni et al., 2010). Data from the present study show fasted baseline insulin concentrations to be lowest in the oldest age group and highest in the youngest age group, thus broadly supporting this hypothesis.

During maximal exercise insulin increased in a parallel manner in all age groups. The decrease in insulin during heavy domain exercise was most pronounced in the youngest age group. The contrasting insulin responses can be explained by the variation in the intensity of exercise and the relative requirements for BGlu (Marliss and Vranic, 2002). During heavy domain exercise characteristic of that in the present study there is an increase in demand for glucose by the exercising muscles. This promotes a response that increases GP to match GU precisely, and insulin secretion is inhibited to below fasting levels through α-adrenergic receptor activation (Robertson et al., 1976; Broadstone et al., 1987). Inhibition of insulin secretion increases GP by the liver by increasing sensitivity to glucagon (Vranic et al., 1976; Issekutz and Vranic, 1980; Zinker et al., 1994). The decrease in insulin with either no change or an increase in glucagon accounts for the corresponding increases in GP (Wasserman et al., 1989a; 1989b). Thus, the ratio of glucagon to insulin is the main determinant of GP during heavy domain exercise characteristic of that in the present study (Issekutz and Vranic, 1980; Wasserman et al., 1984). Maximal, exhaustive exercise, promotes rapid hepatic glycogenolysis. In contrast to the 'feedback' signal at lower exercise intensities, this has been suggested to be a beneficial 'feed forward' loop (Kjaer et al., 1986; 1993). It has been proposed that the relatively greater magnitude of the catecholamine response in maximal exercise, compared to that at lower intensity, is the prime regulator of GP (Marliss et al., 1991; 1992; Sigal et al., 1996; Marliss and Vranic, 2002; Kreisman et al., 2000; 2001; 2003). The result is that GP is increased above the rate of GU. Systemic insulin, however, remains relatively stable as high catecholamine concentrations (acting through a dominant α-adrenergic effect) prevent glucose stimulation of insulin secretion. Post-exercise, systemic insulin increases in response to the net increase in blood glucose as epinephrine and norepinephrine rapidly decline (Marliss et al., 1992; Sigal et al., 1996; Marliss and Vranic, 2002). Thus, exercise-induced changes in insulin abundance observed in the present study appear to be related to blood glucose homeostasis as described above, although further experiments would be required for confirmation, with no indication of insulin resistance.

Ageing is associated with increased adiposity, most notably central adiposity. Interestingly, although not significantly different, the highest baseline leptin concentrations were observed in the oldest age group who possessed the greatest BMI value. Similarly, this group possessed the highest baseline adiponectin concentrations, broadly suggesting that they maybe benefiting from the anti-inflammatory and anti-

atherogenic effects of this protein also. Systemic leptin concentrations have been linked to energy expenditure, and shown to decline as a result of both acute exercise (Duclos et al., 1999; Zaccaria et al., 2013; Rosa et al., 2012) and exercise training (Simsch et al., 2002; Olmedillas et al., 2011). This finding is supported by data from the present study in which leptin concentrations declined during heavy domain exercise. Further, heavy domain exercise resulted in a decrease in the leptin/adiponectin ratio, potentially offsetting the negative effects of increasing circulating leptin concentrations known to increase with accumulating adipose tissue mass. Age was without effect on the leptin/adiponectin ratio. Adiponectin has been shown to be inversely correlated with fasting insulin (Park et al., 2004). Our data support this and we note that adiponectin appears to inversely reflect the changes in insulin during heavy domain exercise. These findings support a role for insulin in blood glucose homeostasis during exercise where insulin resistance is not apparent, reflected by basal abundance of insulin, and show acute heavy domain exercise is able to bring about positive changes in the systemic abundance of adipokines leptin and adiponectin.

6.5.5. Anabolic adaptations

Baseline GH concentrations in the present study are similar to the 0.8 ng/ml (2.42 μIU/ml) baseline concentrations reported by Drobny et al., (1983). Elevation above this figure is likely to be due in part to the fasting state of the participants (Ho et al., 1988) and variation in peak pulsatile secretion with sample time. In the present study fasting IGF-I concentrations were within the normal range established previously, with the greatest concentration (~ 100 ng/ml greater) in the youngest age group (Yamamoto et al., 1991). Basal systemic IGF-I concentrations in 207 normal adults (103 males and 104 females), aged 21 to 80 years suggested a significant decline in basal IGF-I with age, which is linear in males (Yamamoto et al., 1991). Insulin-like growth factor-I is suggested to be primarily responsible for the anabolic response to exercise (Velloso, 2008). Growth hormone is the principal regulator of the hepatic synthesis of IGF-I and Insulin-like growth factor binding protein-3 (IGFBP-3), the major Insulin-like growth factor carrier and modulator of systemic IGF-I action (Frystyk, 2004; 2010). Local IGF-I may augment systemic concentrations of hepatic origin acting in a paracrine fashion (Adams, 2002; Berg and Bang, 2004; Nindl, 2010). The Growth hormone/Insulin-like growth factor-I (GH/IGF-I) axis exerts short- and long-term metabolic effects on exercise (Gibney et al., 2007; Widdowson et al., 2009). The marked exercise-induced GH response first described by

Roth et al., (1963) has since been well documented with regard to endurance, sprint and resistance exercise (Godfrey et al., 2003; Gibney et al., 2007; Gilbert et al., 2008). Endurance exercise above the Anaerobic threshold (ATh) is considered to elicit the most marked increase in GH (Pritzlaff et al., 1999; Godfrey et al., 2003; Gilbert et al., 2008; Wahl et al., 2010; 2013). The exercise-induced increase in systemic GH concentration is suggested to spare blood glucose by increasing gluconeogenesis and mobilising free fatty acids from adipose tissue (Hunter et al., 1965a; 1965b). However, it is thought that the latter maybe restricted during very strenuous exercise due to reduced blood flow to adipose tissue depots. Systemic IGF-I is involved in the feedback inhibition of GH release (Frystyk, 2004; 2010) and therefore enhancement of insulin action and glucose homeostasis (Clemmons, 2006b). Data from the present study support previous findings that endurance exercise above the ATh results in a significant increase in systemic GH concentration within the first 20 min (Felsing et al., 1992), and that the response is attenuated in middle-aged men (Gilbert et al., 2008). Both maximal and heavy domain exercise resulted in a significant increase in systemic GH in all groups. Similar to the findings of others, GH concentrations were significantly increased within 20 min of the onset of heavy domain exercise, peaked at the end of exercise and returned to baseline levels within 60 min (Lassarre et al., 1974; Raynaud et al., 1981; Viru et al., 1992; Zaldivar et al., 2006). The most significant variation occurred between GH concentrations at the end of exercise compared with those at baseline and post-exercise (60 min), which were highly comparable. Although post-hoc comparisons revealed no significant group differences, the GH response to both maximal and heavy domain exercise appears very much dampened in the older compared with that of the younger age groups.

In the present study maximal exercise induced a significant increase in systemic concentrations of IGF-I, which were progressively attenuated with increasing age. A significant increase in systemic concentrations of IGF-I was shown by De Palo et al., (2008) in young male participants (n = 20, mean age 19 ± 2 yrs.) in response to two exhaustive cycling exercise trials lasting 25 and 40 min. No significant exercise-induced increase in IGF-I was observed in response to heavy domain exercise. We note here again, similar to the response reported in Chapter 3., that the heavy domain exercise-induced peak in systemic GH abundance is not reflected in the IGF-I response, thus representing an uncoupling of the GH/IGF-I axis. This finding, also observed by Zaldivar et al., (2006), is interesting given the widely acknowledged regulatory links between GH and IGF-I. Frystyk (2010) suggested the uncoupling maybe due to the transient nature of the

exercise-induced systemic GH response.

It has been shown that systemic IGF-I abundance follows a similar age-related decline to that of GH (Corpas et al., 1993). Given the evidence that locally produced IGF-I maybe capable of augmenting systemic concentrations, it is hypothesised that the age-related reduction in systemic IGF-I shown here is also due to a reduced contribution of locally produced IGF-I. Further, one might also argue that this contributes to the reduced capacity for exercise-induced skeletal muscle hypertrophy in older individuals. Data from the present study suggests that neither acute maximal nor heavy domain exercise can compensate for the age-related decline in the systemic abundance of these two key anabolic proteins.

6.5.6. Catabolic and metabolic/inflammatory adaptations

Baseline systemic cortisol concentrations were comparable to those found by others (Silverman and Mazzeo, 1996; Del Corral et al., 1998; Hill et al., 2008; Wahl et al., 2010; Rosa et al., 2012). Increased basal systemic cortisol concentrations have been reported in older individuals (Pavlov et al., 1986; Van Cauter et al., 1996). However, data from the present study do not support this. Age-related temporal alterations in cortisol pulsatile secretion dynamics have also been reported (Sherman et al., 1985; Bergendahl et al., 2000). The small variation in the baseline systemic cortisol concentration data may reflect this.

The pulsatile secretion of cortisol, the end product of the Hypothalamic-pituitary-adrenal (HPA) axis, is tightly controlled via a complex regulatory mechanism incorporating negative feedback (Lightman et al., 2008; Russell et al., 2010). In addition, perceived or actual physical or psychological stress induces transient activation of HPA and cortisol secretion; the fight or flight response, an essential mechanism that promotes adaptation and survival via responses of the neural, cardio-vascular, autonomic, immune and metabolic systems (Charmandari et al., 2005). It is widely acknowledged that the HPA axis mediates the relationship between stressful life experiences and health outcomes, and evidence suggests that HPA axis activity contributes to biological ageing through inappropriate or excessive glucocorticoid secretion (Krøll, 2010; Aguilera, 2011; Lucassen et al., 2013). The effects of chronic hypercortisolism (excessive cortisol secretion) can be observed in Cushing's Disease. As such, hypercortisolism has been

linked with muscle weakness, increased central adiposity/obesity (Medline Plus, no date) and insulin resistance in otherwise healthy individuals (Cree et al., 2010). Currently, research suggests a bi-phasic model of glucocorticoid regulation in which the normal diurnal secretion profile of cortisol supports the activity of defense mechanisms, including innate immune inflammation, while greater, stress-induced, concentrations act to acutely suppress inflammation, thus preventing tissue injury from an excessive or prolonged inflammatory response (Yeager et al., 2011).

The $\dot{V}O_2$max test was without effect on systemic cortisol concentrations. During heavy domain exercise there was a reduction in systemic cortisol concentrations in all age groups. Although not supported by a significant effect of 'group' or 'group x time interaction', the greatest reduction in systemic cortisol concentration during exercise appeared to be in the youngest age group, with comparable further post-exercise reductions across age groups. Our findings support those of others (Rosa et al., 2011; 2012) who observed no increase in systemic cortisol abundance in response to exercise. During heavy domain exercise reported in our study, systemic cortisol decreased and GH increased. This promoted an increase in the ratio of GH to cortisol, theoretically promoting a systemic environment conducive to improving insulin sensitivity and gains in skeletal muscle mass.

It is well known that exercise is a potent stimulus for IL-6 (Pedersen and Febbraio, 2012). Systemic abundance of this cytokine has been shown to increase over 100-fold in response to 'strenuous' exercise (marathon running) of long duration (Ostrowski et al., 1999), although less dramatic increases are typical. The systemic concentration of IL-6 in resting, healthy individuals is ~ 1 pg/ml or lower (Brüünsgaard et al., 1997; Ostrowski et al., 1998a; Knudsen et al., 2008). The findings from the present study broadly support this. However, the baseline concentration in the youngest age group was ~ 2 pg/ml. The large error bars present at all time points in both maximal and heavy domain exercise highlight the great variability in the systemic abundance of IL-6 within this group. Both maximal and heavy domain exercise resulted in significant differences in systemic IL-6 abundance. During heavy domain exercise systemic IL-6 abundance varied significantly at all time points except at 20 min, which was not significantly different to baseline. The similarity between the secretion profile of all groups suggests the variability in the youngest age group is not as a result of the exercise trials, but reflective solely of the systemic state of individuals in this group. Fischer (2006) suggested that reduced basal

systemic IL-6 abundance, as well as in response to exercise, is a characteristic of the normal adaptation to training, whereas high basal systemic IL-6 abundance is closely associated with physical inactivity. Indeed, life-long endurance activity has been shown to be associated with a significant reduction in inflammatory markers C-reactive protein (CRP) and IL-6 (Mikkelsen et al., 2013). All of the participants recruited for the study were apparently healthy, with many engaged in some form of recreational physical activity/training. It is entirely possible that for some of the older participants physical activity has been a life-long pursuit; potentially indicated by their descriptive and cardio-respiratory measures. Therefore, it is also possible that the exercise-induced systemic abundance of IL-6 maybe reduced in these older groups compared with the potentially less trained, younger cohort. The finding of a significant 'group' x 'time' interaction ($P < 0.001$), and post-hoc 'Delta' analyses showing a significant impact of 'time' at post-exercise (60 min) ($P = 0.001$), highlights the significant difference in the post-exercise secretion profiles between the younger 20 - 40 yr. old and older 40 - 60 yr. old participants. Interleukin-6 concentrations in the 30 - 40 yr. old participants remained below 1 pg/ml, which was significantly lower than those in the 40 - 50 and 50 - 60 yr. old age groups ($P = 0.002$). These data suggest a potential uncoupling of the response during and post-exercise.

6.5.7. Summary and conclusion

In line with the objective of this chapter, to establish the impact of age (20 - 60 yrs.) on the systemic hormone and cytokine response to an acute, 30 min, heavy domain constant work-load cycle ergometer exercise intervention in recreationally active males, we analysed the systemic abundance of insulin, leptin, adiponectin, IL-6, IGF-I, cortisol and GH in four groups of recreationally active males at baseline and in response to heavy domain exercise. We hypothesised that ageing would be reflected in systemic hormone and cytokine temporal and/or abundance variations in the older participant groups. IL-6 displayed a similar bi-phasic profile, with an increase during exercise at 10 min and subsequently at 60 min post-exercise in all groups. A decline in both insulin and leptin was observed in all groups during exercise, while exercise was without effect on adiponectin. In all groups the peak in systemic GH occurred at the end of exercise, although the peak appeared to be somewhat depressed in the 40 - 60 yr. age range. Exercise was without effect on IGF-I, but concentrations were consistently higher in the 20 – 30 yr. age group. In all groups cortisol concentrations declined post-exercise. We

conclude that systemic IGF-I concentrations clearly decline between the 3rd and 6th decades of life, and that age (20 - 60 yrs.) is not reflected by temporal and/or abundance variations in systemic insulin, leptin, adiponectin, IL-6, GH, IGF-I or cortisol variations in response to an acute 30 min bout of heavy domain cycle ergometer exercise.

Chapter 7. Discussion and future directions

7.1. Achievement of overall aim and objectives

Our overall aim was to implement an empirically informed, palatable acute endurance exercise intervention that elicits beneficial hormonal responses with the potential for improved health/quality of life across the lifespan. We envisage such an intervention might eventually form an exercise component in a multimodal lifestyle approach incorporating physical activity (endurance/aerobic and strength/power), nutrition (optimum calorific quantity and nutritional makeup) and key pharmaceutical therapies targeted to improving the performance of activities of daily living in older individuals through improvements in cardio-respiratory fitness, skeletal muscle quality and function, and reductions in adiposity and systemic inflammation. Our objectives were: 1) to develop, with regard to the principles and techniques associated with oxygen uptake dynamics research, acute 'domain-based' constant work-load cycle ergometer exercise interventions of varying intensity, but equal total work done by varying exercise duration, 2) to establish the changes in the mRNA expression of selected hormones and cytokines that occur locally in skeletal muscle and subcutaneous adipose tissue and 3) systemically, as a function of our acute 'domain-based' constant work-load cycle ergometer exercise interventions in recreationally active young males, and 4) to establish the the impact of age (20 - 60 yrs.) on the systemic hormone and cytokine response to an acute heavy domain constant work-load cycle ergometer exercise intervention in recreationally active males.

In Chapter 3 we satisfied our first objective by comparing data collected from the cycle ergometer maximum rate of oxygen uptake ($\dot{V}O_2max$) test, conducted using principles and techniques associated with oxygen uptake dynamics research, with that from three acute 'domain-based' constant work-load cycle ergometer exercise trials of varying intensity, but equal work done. In support of this alternative approach, which takes into account skeletal muscle metabolic threshold events rather than assigning constant work-load exercise in groups of participants with reference to a percentage of the maximum rate of oxygen uptake (% $\dot{V}O_2max$), a percentage of heart rate at $\dot{V}O_2max$ (% HRmax), or a target Heart rate (HR) derivative of a % $\dot{V}O_2max$ value such as is the case with the Karvonen formula (Karvonen and Vuorimaa, 1988), we observed no significant differences between mean rate of oxygen uptake ($\dot{V}O_2$), HR and rate of oxygen uptake

divided by heart rate ($\dot{V}O_2$/HR) data obtained from the constant work-load cycle ergometer exercise trials and the $\dot{V}O_2$max tests. This indicates that the exercise trials were conducted at the same 'domain-based' intensity identified during the $\dot{V}O_2$max tests.

In Chapters 4 and 5 we satisfied our second and third objectives by establishing the changes in Messenger ribonucleic acid (mRNA) expression in skeletal muscle and subcutaneous adipose tissue and systemic abundance, respectively, of selected hormones and cytokines with suggested key anabolic/catabolic/metabolic and inflammatory roles resulting from our alternative, exercise 'domain-based' approach. We discussed our findings within the context of mechanisms behind the hormone and cytokine responses to endurance exercise suggested in the existing literature. We suggest that our findings in young and apparently healthy participants appear predominantly to reflect mechanisms of metabolic control responding to the demands of the exercise trials.

In Chapter 6 we satisfied our final objective and broadly achieved our overall aim. We established how the systemic abundance of selected hormones and cytokines with suggested key anabolic/catabolic/metabolic and inflammatory roles varied in response to an acute 30 min heavy domain constant work-load cycle ergometer exercise intervention. It has been suggested that the beneficial effects of endurance exercise are due to the anti-inflammatory effects of regular exercise, through a reduction in fat mass and/or induction of an anti-inflammatory environment with each acute bout of exercise. We therefore suggest that heavy domain exercise may represent an effective endurance exercise component in a multimodal lifestyle approach targeted to improving the performance of activities of daily living in older individuals through improvements in cardio-respiratory fitness, skeletal muscle quality and function, and reductions in adiposity and systemic inflammation. Again, we discussed our findings within the context of pertinent existing literature, and again, our findings in apparently healthy participants between 20 and 60 yrs. age appear predominantly to reflect mechanisms of metabolic control responding to the demands of the exercise trials. However, our data also appear to show that the systemic abundance of markers of anabolism Insulin-like growth factor-I (IGF-I) and Growth hormone (GH) decline during this period of life, both basally and in response to acute exercise.

7.2. General discussion

Over the last 15 - 20 years, research propelled by the significant socio-economic issues presented by obesity and an ageing population (World Health Organization, 2000; 2012; 2013) has identified links between physical inactivity, metabolism and disease (Egan and Zierath, 2013; Pillon et al., 2013; Weigert et al., 2013; Wunderlich et al., 2013; Bosma, 2014). Our increasingly less active lifestyles and opportunities to obtain sustenance with almost no effort facilitate over-nutrition and obesity, resulting in dysregulation of metabolism and the progression of a chronic inflammatory state (immunometabolism) (Ferrante, 2013; Mathis and Shoelson, 2011) leading to insulin resistance and Type 2 diabetes mellitus (T2DM) (Sarvas et al., 2013). As we age a similar inflammatory process (inflammageing) (Degens, 2010; Cevenini et al., 2013; Franceschi and Campisi, 2014) contributes to the decline in the skeletal muscle mass (sarcopenia) and strength (dynapenia), eroding functional capability and quality of life. This appears to be further compounded by a progressive decline in anabolic potential, and therefore the ability to affect positive change. Through the mechanism of excitation-transcription coupling (Gundersen, 2011), skeletal muscle functions as a secretory organ. This provides the basis for a new paradigm for understanding the communication between skeletal muscle and other tissues and organs such as adipose tissue and the liver, and explains why a lack of physical activity appears to be involved in a network of diseases termed 'The diseasome of physical inactivity' (Pedersen, 2009). Exercise appears to be an effective therapy to correct metabolic function and reduce ectopic excess nutrient storage (Bosma, 2014), thereby reducing the associated low-grade systemic and tissue specific inflammation consequent to over-nutrition in obesity, sarcopenic obesity and dynapenic obesity (Egan and Zierath, 2013).

7.2.1. Exercise trial design

In contrast to traditional approach in which exercise intensity is expressed in terms of a % $\dot{V}O_2$max, we favoured an approach more closely aligned with oxygen uptake dynamics research, and the concept of exercise domains (Whipp et al., 2005) as described in detail in Chapter 2 'General methods'. Section 2.5.1. 'Methodological considerations: an oxygen uptake dynamics approach'. It is our view that the former could result in markedly different physiological stress characteristics at what would appear to be identical relative exercise intensities. Here we have demonstrated the precision with which constant work-load exercise can be assigned, with reference to the profiles of the muscle metabolic and pulmonary gas exchange responses, from maximal incremental exercise in a domain-

based manner in males between 20 and 60 yrs. of age. It is suggested that this should become the preferred method for assigning constant work-load exercise as it facilitates a closer alignment of groups of participants in terms of physiological stress, therefore excluding potentially confounding variables. This is particularly important given the mounting evidence linking the exercise-induced hormone and cytokine response with the complex mechanisms of metabolic control. In the present work two independent studies were linked by the inclusion of heavy domain constant work-load cycle ergometry trials. A schematic (figure 7.1.) describing the relationship between the exercise studies performed and the presentation of work throughout the thesis is presented in section 7.3. 'Exercise studies and presentation of work schematic'. Exercise duration was set to ensure equal total work done between exercise trials in the moderate and heavy domains in study 1, and exercise trials in the heavy and very heavy domains in study 2. Statistical analyses indicated the pooling of data from the two heavy domain exercise trials was appropriate, and that the participants could be considered as a single representative group.

7.2.2. Exercise intensity and local and systemic cytokine responses

An emerging theory suggests skeletal muscle has a physiological function as a sensor and responder to stress and that Interleukin-6 (IL-6) gene expression in muscle responds to a wide variety of internal and external stressors (Welc and Clanton, 2013; Bustamante et al., 2014). In the context of exercise, the requirement for energy represents a potent stressor (Pedersen, 2012). Exercise intensity influences the relative contributions from carbohydrate and lipid sources, and circulating (extra-muscular) and intramuscular fuel stores, to adenosine triphosphate provision (Coyle, 1995; Houten and Wanders, 2010; Egan and Zierath, 2013). It has been suggested that IL-6 expression mediates the response to this stress and increased expression mainly stimulates skeletal muscle glucose uptake, but also the mobilisation of substrate in anticipation of a prolonged exercise bout (Pal et al., 2014). Our data support this and show that even relatively short duration endurance exercise increases both expression of the IL-6 gene in skeletal muscle and systemic abundance of the protein. We have shown that under conditions of equal work done, acute very heavy domain exercise, requiring the most forceful contractions of all of the acute exercise trials investigated in the present work, stimulated the greatest increase in IL-6 mRNA expression in skeletal muscle. Acute moderate domain exercise, requiring the least forceful contractions, promoted the greatest systemic accumulation of IL-6. Given the variation in exercise duration between these two trials, we speculate that although

moderate exercise appears to induce a lesser rate of IL-6 transcription and release, the greater exercise duration facilitates systemic accumulation of the protein. Given the evidence indicating a role for IL-6 in lipid homeostasis and metabolism (Lyngso et al., 2002; van Hall et al., 2003; Petersen et al., 2005), we further speculate that systemic accumulation of IL-6 promotes greater access to adipose tissue depots, including those within skeletal muscle as an intermediary step (Kelly et al., 2004; Al-Khalili et al., 2006; Carey et al., 2006; Wolsk et al., 2010), and therefore mobilisation of fuel via endocrine and/or paracrine signalling for prolonged exercise bouts.

We suggest the increased expression of Suppressor of cytokine signalling-3 (SOCS3) mRNA in skeletal muscle is in response to the exercise-induced increase in skeletal muscle IL-6 mRNA and systemic concentrations of IL-6 (Rieusset et al., 2004; Carey et al., 2006; Holmes et al., 2008). In this context the elevation of SOCS3 functions to prevent the effects of a chronic IL-6 response to exercise by negative feedback inhibition of the Janus kinase (JAK)/Signal transducer and activator of transcription (STAT) signalling pathway (Babon and Nicola, 2012). In contrast to the contribution of chronically elevated SOCS3 to leptin and insulin resistance, the transient nature of the SOCS3 response to exercise-induced IL-6 is suggested to have limited effect on the beneficial metabolic and insulin sensitising effects of endurance exercise (Sarvas et al., 2013). Interestingly, a reduction in SOCS3 protein was found in the skeletal muscle of older (~ 67 yrs.) compared to that of younger (~ 20 yrs.) individuals 2 hrs. after an acute bout of resistance training (Trenerry et al., 2008). The implications of this remain to be fully elucidated. However, the authors speculate that this signalling potentiation by IL-6 maybe involved in impaired muscle regenerative capacity observed in older adults through the establishment of a pro-inflammatory state. It is possible that this mechanism is responsible for the increased systemic concentration of IL-6 from 40 - 60 yr. old males at 60 min post-heavy exercise in comparison to that from the 20 - 40 yr. old participants, indicated by our data.

In contrast to IL-6, the exercise-induced increase in skeletal muscle Tumour necrosis factor-alpha (TNFα) mRNA is suggested to be very small and to result in little if any measurable increase in systemic concentrations, indicating a local role in skeletal muscle (Steensberg et al., 2002; Febbraio et al., 2003). Exercise is suggested to promote an anti-inflammatory environment (Pedersen and Hoffman-Goetz, 2000; Brandt and Pedersen, 2010) due, in part, to IL-6 inhibition of Interleukin-1 (IL-1), TNFα, and Interleukin-8

(IL-8) expression and systemic abundance (Ostrowski et al., 1999; Starkie et al., 2003; Steensberg et al., 2003). However, our data show a significant increase in the expression of TNFα mRNA in response to acute very heavy domain exercise. These findings add support to those from others who have also shown an increase in skeletal muscle TNFα mRNA in response to endurance exercise above ~ 70 % $\dot{V}O_2$max (Nieman et al., 2003; 2005; Louis et al., 2007). Unfortunately, we are unable to identify the exact cellular source of the TNFα mRNA due to the use of homogenate, which in addition to skeletal muscle tissue may also include inflammatory cells. This does however lend itself to skeletal muscle repair and regeneration as a plausible explanation for the increased expression of this pro-inflammatory cytokine, as a local immune response shortly after the cessation of exercise forms the initial step in the regeneration process and activates Muscle satellite cells (MuSCs) (Seene and Kaasik, 2013; Madaro and Bouché, 2014). Interestingly, data from Al-Shanti et al., (2008) from the murine C2C12 muscle cell line, suggests TNF-α and IL-6 function positively and potentially also co-operatively with the Insulin-like growth factor system to achieve the maximal beneficial effect on skeletal myoblast numbers. We speculate that these data may indicate TNFα and IL-6 working synergistically to enhance skeletal muscle regeneration following very heavy exercise. However, it must be remembered that elevated TNFα has been shown to play a direct pathogenic role in conditions associated with skeletal muscle insulin resistance (Hotamisligil et al., 1996; Plomgaard et al., 2005; Bouzakri and Zierath, 2007), dyslipidemia, the development of lipid-induced insulin resistance (Steinberg et al., 2006; Plomgaard et al., 2008; Steinberg et al., 2009) and has been identified as a biomarker of peripheral muscle fatigue (Finsterer, 2012) and inflammation (Zelová and Hošek, 2013). Although highly speculative, considering these findings collectively, it is counter-intuitive to advocate a therapeutic role for endurance exercise in addressing age-related skeletal muscle functional decline, obesity or systemic inflammation at an intensity shown to promote an increase in expression of TNFα.

7.2.3. Exercise and the Growth hormone/Insulin-like growth factor-I axis

The present work addresses, in part, the absence of exercise dose-response reference data relating to systemic hormone and cytokine adaptations to acute endurance exercise in apparently healthy males between 20 – 60 yrs. of age. Our data support the view that the systemic abundance of markers of anabolism decline during this period of life (Baumgartner et al., 1999; Chahal and Drake, 2007; Giovannini et al., 2008), both basally and in response to acute exercise. Universally, systemic IGF-I concentrations were greatest in the youngest age group. This group demonstrated the greatest acute increase in response to maximal exercise and maintained the greatest concentration in response to acute heavy exercise. Further, our data suggest the decline in IGF-I becomes significant after 40 yrs. of age. In all studies GH peaked at the end of exercise and returned approximately to baseline concentrations by 60 min post-exercise. Although not statistically significant, our data show a decline in the exercise-induced peak in the systemic concentrations of GH. Similar to the decline in IGF-I, this is most apparent after 40 yrs. of age. The reduction in the GH to cortisol ratio peak at the end of heavy domain exercise in the 50 - 60 yr. age group further supports this. Systemically, our data show an uncoupling of the GH/IGF-I axis during exercise at sub-maximal intensities, similar to that observed by Zaldivar et al., (2006), where the exercise-induced peak in systemic GH abundance is not reflected in the IGF-I response. This has been suggested to be due to the transient nature of the exercise-induced GH response (Frystyk, 2010) and emphasises a metabolic rather than anabolic role (Møller and Jørgensen, 2009).

Interestingly, and in addition to increased systemic IL-6, GH and insulin concentrations, our data show significant increases in systemic IGF-I concentrations in all studies regardless of age in response to maximal exercise, supporting the findings of others (De Palo et al., 2008; Zebrowska et al., 2009). In contrast, but also regardless of age, no significant increases in systemic IGF-I, or IGF-I and Insulin-like growth factor-I receptor (IGF-IR) mRNA expression were observed in response to sub-maximal exercise. Increased expression of IGF-I in skeletal muscle has been shown with unchanged systemic levels (LeRoith et al., 2001; Velloso, 2008; Gatti et al., 2012) suggesting that local IGF-I may augment systemic concentrations (Adams, 2002; Berg and Bang, 2004; Nindl, 2010). This suggests the possibility for potential anabolic gain from acute endurance exercise at intensities that elicit maximal oxygen uptake in individuals of less than 60 yrs. of age. High intensity exercise training has recently been advocated as a

potential therapy with beneficial effects on glycaemic control, as an alternative to low intensity high volume training (Adams, 2013; Roberts et al., 2013; Wahl et al., 2013), and this maybe an avenue worthy of further investigation.

7.3. Exercise studies and presentation of work schematic

A schematic representation of the relationship between the exercise studies performed and the presentation of work throughout the thesis is provided in figure 7.1. For additional information pertaining to the exercise trial timelines and exercise trial protocols, the reader is directed to Chapter 2. Section 2.5.1.8.1. ‘Overview of exercise studies’.

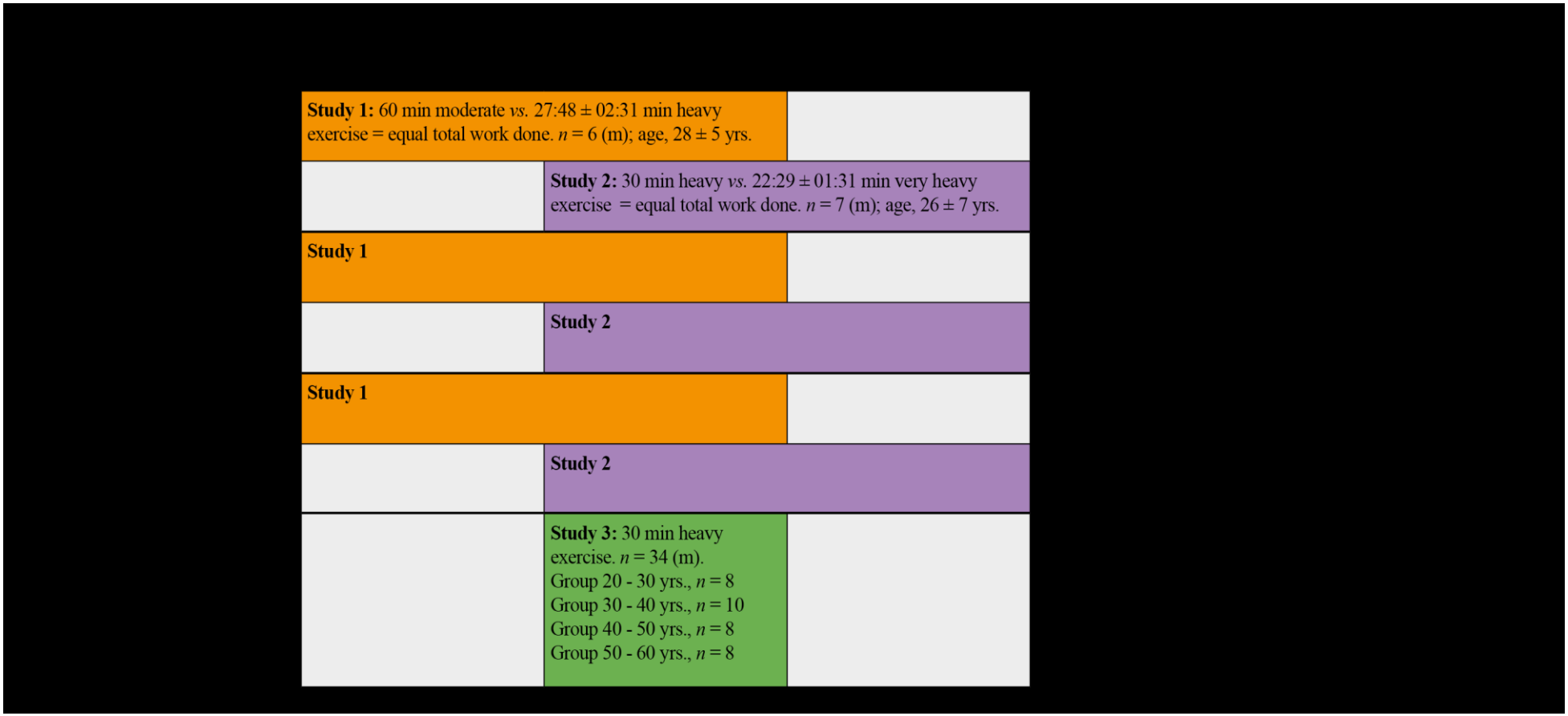


Figure 7.1. Schematic describing the relationship between the exercise studies performed and the presentation of work throughout the thesis. Heavy domain exercise trials link all three studies. Sd - significantly different, NS - no significant differences, B – baseline, MAX - $\dot{V}O_2$ max test, S↑ - significant increase, S↓ - significant decrease, m – male.

7.4. Limitations

The following is a discussion of what could be perceived as limitations of the work presented in this thesis. Limitations specific to each data chapter are first summarised and then discussed.

Chapter 3 limitations: 1) a departure from traditional methods of aligning exercise intensity i.e. % $\dot{V}O_2$max, % HRmax, or a similar method such as by use of the Karvonen formula (Karvonen and Vuorimaa, 1988), in favour of an exercise 'domain-based' approach, 2) the division of work into two independent studies, linked by the inclusion of heavy domain constant work-load cycle ergometry trials, 3) generalisation, based on the findings of Pringle and Jones (2002), rather than direct measurement of Maximal lactate steady state (MLSS) to define the boundary between heavy and very heavy domain exercise, 4) deviation from The British Association of Sport and Exercise Sciences (BASES) and American College of Sports Medicine (ACSM) criteria for successful determination of $\dot{V}O_2$max, and 5) the lack of a verification stage to indicate conclusively attainment of $\dot{V}O_2$max.

By departing from traditional methods of aligning exercise intensity and adopting methods common to oxygen uptake dynamics research, we have been able to specify exercise intensity and measure the resulting variation in hormone and cytokine responses in our participants with regard to common threshold events in their individual muscle metabolic and pulmonary gas exchange responses. We feel that skeletal muscle metabolic threshold events such as the Anaerobic threshold (ATh), or in oxygen uptake dynamics research terminology the Gas exchange threshold (GET), maybe important in defining the hormone and cytokine response to exercise, and consequently this approach may help clarify the many and varied roles these proteins play. Simply aligning exercise intensity in groups of participants by % $\dot{V}O_2$max, % HRmax, or a target HR derivative of a % $\dot{V}O_2$ max value, as is the case with the Karvonen formula (Karvonen and Vuorimaa, 1988), could result in markedly different physiological stress characteristics at what would appear to be identical relative exercise intensities, i.e. exercise at 70 % $\dot{V}O_2$max, in a group of participants varying in aerobic fitness might mean that some participants are working below their ATh while others maybe working above their ATh.

The division of work into two independent studies, linked by the inclusion of heavy domain constant work-load cycle ergometry trials allowed us to implement a protocol which would otherwise have been highly demanding, potentially influencing adherence to and completion, and ethically questionable in terms of the invasive nature of the procedures for a single study group. The results of the statistical tests comparing data from the two participant groups described previously indicate that the decision to proceed with this study design has not compromised the integrity of the findings. Similarly, direct measurement of MLSS, conclusively defining the boundary between the heavy and very heavy exercise domains would have significantly increased the demand on the participants. Informed by the findings of Pringle and Jones (2002) described previously we chose not to measure MLSS directly, but to perform the heavy domain exercise trial at a power output which elicited a 30 % Δ response and the very heavy domain exercise trial at a power output which elicited a 60 % Δ response, thus avoiding the MLSS and placing the respective trials firmly within each exercise domain.

We chose to deviate from established BASES and ACSM guidelines for the successful determination of $\dot{V}O_2max$ in favour of a set of criteria determined by ourselves with reference to the existing literature. Our reasons for this were two-fold. Firstly, due to the use of a breath-by-breath expired gas analysis system, we were confident in our ability to identify our primary criterion, a plateau in the $\dot{V}O_2$ data with increasing work-load. Secondly, in the face of literature criticising secondary deterministic criteria, it appears contrary to the scientific process to fail to acknowledge limitations and not to push for improvement in methods and techniques. Given our confidence in being able to accurately determine $\dot{V}O_2max$, we felt that implementing a verification stage to indicate conclusively attainment of $\dot{V}O_2max$ was not required and would be ethically questionable when testing the older participant groups.

Chapter 4 limitations: 1) timing of biopsy sampling immediately post-exercise may provide an incomplete picture of mRNA expression, 2) the presence of cells such as smooth muscle cells, fibroblasts, endothelial cells, and macrophages in the bipotic material, which may have contributed to mRNA expression data, and 3) the measurement of the changes in mRNA expression and not the rate of gene transcription.

A single biopsy collected immediately post-exercise will not provide a complete picture of the exercise-induced changes in mRNA expression. As previously discussed, it is entirely possible that the timing of the biopsy, immediately post exercise, is the reason no significant changes in mRNA expression were detected in subcutaneous adipose tissue. However, our data document the changes in skeletal muscle relative to a lack of change in subcutaneous adipose tissue immediately post-exercise. Further post-exercise biopsies may have provided interesting information and clarified our understanding of the temporal characteristics of the exercise response in these tissues. Due to the sample method, it is entirely possible that the changes in gene expression observed in the present study may be due, at least in part, to increased mRNA expression in cells other than skeletal muscle myocytes. However, it is therefore logical to assume that these cells make an important contribution to the local hormone and cytokine milieu, which we feel justifies the inclusion and the use of the biopsy technique. It is important to acknowledge that we measured changes in mRNA expression and not the rate of gene transcription. It is entirely possible that data reflect an increase in transcription and/or increases in mRNA stability, or a combination of both.

Chapter 5 limitations: 1) timing of blood sampling may provide an incomplete picture of the changes in systemic abundance of the selected hormones and cytokines, 2) the influence of natural circadian variation and the pulsatile nature of GH and cortisol secretions, and 3) the effect of fasting on systemic hormone and cytokine abundance.

Blood samples were taken at baseline, immediately post-exercise, and at 60 minutes and 24 hrs. post-exercise. To gain a complete picture of the changes in the systemic abundance profiles of the selected hormones and cytokines in response to the exercise trials over the 24 hr. period a far greater frequency of sampling would be required. Quite apart form the cost implication, an analysis of this depth was not possible within the scope of the thesis. However, our data document the changes in the systemic abundance profiles of the selected hormones and cytokines relative to each other in response to the exercise trials, and have provided some interesting information and clarified our understanding of the temporal characteristics of the exercise response. As far as is reasonably practicable we have controlled for circadian effects and the pulsatile nature of GH and cortisol secretions from the anterior pituitary and the adrenal glands, respectively, by ensuring the homogeneity of our participants and that all tests were performed in a similar physiological state, e.g. rested, fasted, euhydrated and at the same time of day. However,

the changes in the GH and cortisol systemic abundance profiles will reflect not only the GH and cortisol response to the exercise trials, but also the underlying natural circadian and pulsatile secretion dynamics. Future investigations into the GH and cortisol response to exercise could choose to first assess the 'normal' 24 hr. GH and cortisol secretion dynamics as a control measure prior to an acute exercise bout. All of the data collected was from participants in the fasted state. Evidence suggests that compared to the fed state, acute exercise in the fasted state promotes altered metabolic regulation, and is therefore both an important methodological consideration with a potentially significant impact on the results. It is therefore probable that the systemic hormone and cytokine responses reflect what is suggested in the literature to be a predisposition to metabolic control favouring pathways geared towards mobilizing and utilising stored fat. Future investigations could choose to control nutrient intake by supplying a standardised meal prior to data collection. The conclusions drawn from the data presented in this chapter, e.g. the likelihood that IL-6 functions metabolically during sub-maximal exercise to mobilise fat stores, are suggested with reference to the evidence presented in the literature. Within the scope of a single study, or indeed this thesis, it is not possible to examine completely the activation of the intricate signaling mechanisms that would provide proof beyond doubt. Future investigations could choose to focus on examining in detail downstream signalling events that may provide such evidence.

Chapter 6 limitations: 1) the use of recreationally active and apparently healthy participants between 20 and 60 yrs. age, 2) the lack of an assessment of habitual physical activity as a measure of participant training status, and 3) the use of alternative equipment for the determination of Blood lactate (BLa) and Blood glucose (BGlu) in 40 – 60 yr. old *vs.* 20 – 40 yr. old participants.

Recreationally active and apparently healthy participants between 20 and 60 yrs. age. were recruited for the work presented in Chapter 6. We felt that by employing a study design in which participants were arranged into four groups, with each group displaying variations in systemic hormone and cytokine abundance characteristic of healthy ageing between the 3rd and 6th decades of life, would allow us to augment the local and systemic data gathered previously (Chapters 4 and 5, respectively) in 18 – 40 yr. old participants while potentially highlighting early age-related changes in hormone and cytokine abundance. The ethical and methodological constraints of maximal exercise testing in older individuals were also considered.

Levels of habitual physical activity were not quantified in our recreationally active participants. It is probable that the exercise-induced systemic abundance of the hormones and cytokines was influenced by habitual physical activity or 'training'. For some of the older participants physical activity may have been a life-long pursuit, and this may have had a disproportionate influence on exercise-induced systemic hormone and cytokine responses in comparison to the younger, potentially less 'trained', participants. Future investigations may choose to quantify participant habitual physical activity, e.g. through the use of questionnaires (Baecke et al., 1982). Quantification of habitual physical activity would have allowed basic information regarding the activity levels of each of the participant age groups to be compiled and a comparison between them performed. Such as comparison may have informed the discussion of the data and the conclusions drawn.

Due to the relocation of laboratory facilities, two different analysers had to be employed in the measurement of BLa and BGlu during data collection (equipment described previously in Chapter 2. Sections 2.6.2.1. 'Blood lactate' and 2.6.2.2. 'Blood glucose', respectively). Despite performing daily calibration checks to ensure accurate readings, the lower BLa concentrations at baseline measured in the younger participants in response to both maximal and heavy domain exercise, despite the similar relative physiological demands of heavy exercise, maybe due to the unavoidable substitution of equipment and variation in measurement methods.

Notwithstanding the limitations discussed above, we feel that the data presented in this thesis adds clarity to the research regarding the hormone and cytokine response to endurance exercise, and highlights what we feel is an important methodological consideration in this field of research. Specifically, the alignment of exercise intensity with regard to skeletal muscle metabolic threshold events, given the important endocrine activity of the tissue, rather than by reference to % $\dot{V}O_2$max, % HRmax or a similar method such as by use of the Karvonen formula (Karvonen and Vuorimaa, 1988), which could result in markedly different physiological stress characteristics at what would appear to be identical relative exercise intensities (Whipp et al., 2005).

7.5. Conclusion

We adopted techniques and methods associated with oxygen uptake dynamics research and, based on comparisons of cardio-respiratory data, successfully aligned exercise intensity relative to the skeletal muscle metabolic threshold events characteristic of exercise in the moderate, heavy and very heavy exercise domains. In comparison to equal work done moderate (80 % GET) exercise, heavy (30 % Δ) exercise, promotes a favourable exercise-induced systemic change in the GH to cortisol ratio while avoiding the potentially negative inflammatory consequences of increased skeletal muscle TNFα mRNA expression, which we have shown occurs in response to very heavy (60 % Δ) exercise. Although skeletal muscle IL-6 mRNA is expressed at a similar level in equal work done heavy and very heavy exercise, we suggest that the greater duration of the former promotes systemic IL-6 accumulation with the metabolic role of mobilising free fatty acids from adipose tissue with the potential for positive effects on adipose tissue mass and associated inflammation. Further, heavy exercise of 30 min duration is palatable in males up to 60 yrs. age, and whilst the systemic abundance of IGF-I at baseline clearly declines between the 3rd and 6th decades of life, increasing age was not reflected by significant exercise-induced variations in systemic insulin, leptin, adiponectin, IL-6, GH, IGF-I or cortisol. We suggest the lack of age-related significant variations in the systemic abundance of the selected hormones an cytokines analysed is due to the health and fitness of our our recreationally active participants.

It has been suggested that the beneficial effects of endurance exercise are due to the anti-inflammatory effects of regular exercise, through a reduction in fat mass and/or induction of an anti-inflammatory environment with each acute bout of exercise. Our overall aim was to implement an empirically informed, palatable acute endurance exercise intervention that elicits beneficial hormonal responses with the potential for improved health/quality of life across the lifespan. We achieved this aim by implementing a bout of heavy domain exercise in recreationally active apparently healthy males of 20 – 60 yrs. age. However, a great deal of further work would clearly be required to identify and optimise an endurance exercise intervention for older individuals. We envisage such an intervention might eventually form an important exercise component in an multimodal lifestyle approach incorporating physical activity (endurance/aerobic and strength/power), nutrition (optimum calorific quantity and nutritional makeup) and key pharmaceutical therapies targeted to improving the performance of activities of daily living in older individuals

through improvements in cardio-respiratory fitness, skeletal muscle quality and function, and reductions in adiposity and systemic inflammation.

Finally, it is our opinion that given the evidence for skeletal muscle endocrine activity and adipose tissue cross-talk in order to satisfy metabolic demand during and post-exercise, exercise-induced hormone and cytokine research should adopt the techniques and methods associated with oxygen uptake dynamics research, which ensure alignment of relative exercise intensity with regard to skeletal muscle metabolic threshold events.

7.6. Future directions

Given that recent evidence suggests major roles for exercise-induced myokines and adipokines in metabolic regulation, future investigations should be performed with metabolic-demand-driven hypotheses in accordance with a central guiding principle that exercise places demands on the body that ultimately benefits health. The benefits of exercise are realised through: (a) impedance of the propagation of adipose tissue mediated inflammation and associated co-morbidities by control of adipose tissue mass, and (b) positive effects on the functional capacity of skeletal muscle, determined largely by exercise stimulus.

1) The experimental design in the present work provided the opportunity to directly compare exercise intensity (contractile force) in the completion of equal work done exercise trials, through manipulation of exercise trial duration. We were therefore unable to draw conclusions regarding the effect of exercise duration. Further work should be performed to explore the effect of varying exercise duration intra-domain. We propose three initial consolidation studies. Cycle ergometer exercise at each of the three exercise intensities with a second trial increasing the duration by 50 % as follows: 60 and 90 min at moderate (80 % GET), 30 and 45 min at heavy (30 % Δ), and 15 and 22 min at very heavy (60 % Δ).

2) We suggest addressing the translation from acute exercise-induced responses to chronic exercise training-induced adaptations, comparing the benefits of endurance and resistance training in sedentary young-adult and older participants. We propose a cross-over training study to investigate the effects of 12 weeks of endurance and 12 weeks of resistance training in apparently healthy sedentary males (n = 20 in each age group 20 -

30, 30 - 40, 40 - 50, 50 - 60, 60 – 70 yrs. of age). Participants would be randomly assigned within each age group to experimental group 1 or 2, and progress through the study as shown in figure 7.2. The findings may help to identify the extent to which relevant and supervised exercise interventions might impact health over an extended age range.

3) Given ethical constraints, it is not feasible to investigate mechanisms *in vivo*, and therefore the model of acute and chronic adaptations should be extended to incorporate cell culture and/or animal studies. Studies in human cells cultured from bioptic material obtained in 1 and 2 above could be used to compare and contrast stem cell behavior (growth, differentiation, survival, senescence, injury repair) across the age groups, pre- and post- acute and chronic interventions. The impact of serum (acute and chronic) from across the age groups could also be investigated. Specific questions would further the work of Al-Shanti et al., (2008; 2012) and the relationships between IL-6, TNFα and IGF-I, given the hypothesised effects of IL-6 on satellite cell mobilisation and hypertrophy (Serrano et al., 2008; McKay et al., 2009; Washington et al., 2011; Pedersen and Febbraio, 2012).

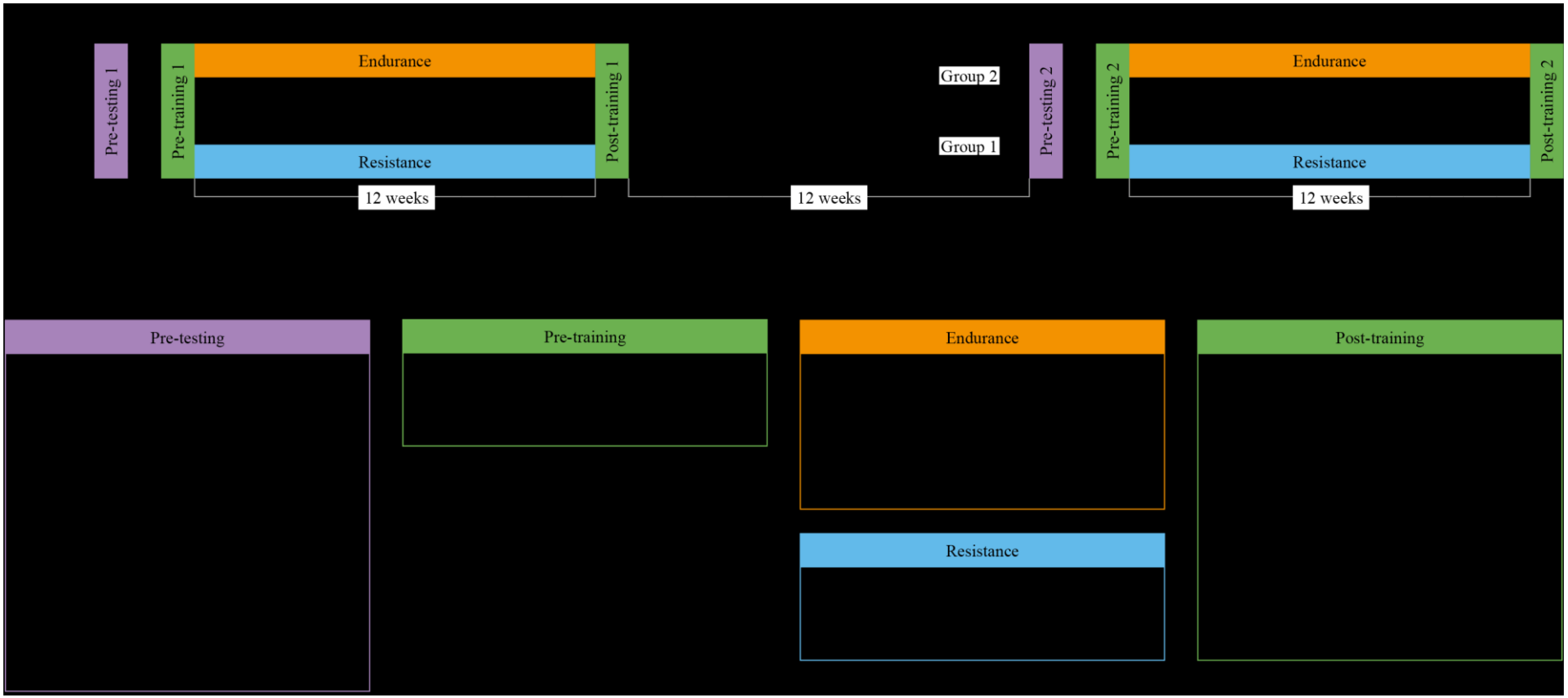


Figure 7.2. A cross-over training study to investigate the effects of 12 weeks of endurance and 12 weeks of resistance training. DEXA - Dual-energy X-ray absorptiometry, MRI - Magnetic resonance imaging, ADL – Activities of daily living, RM – Repetition maximum, SM&A - Skeletal muscle and subcutaneous adipose tissue biopsy.

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Chapter 9. Appendices

Appendix A: Chemicals and reagents

Chemical/reagent	Manufacturer/supplier
Betadine surgical scrub	Purdue Pharma LP, Stamford, Connecticut, USA.
Chloroform (99 %), C2432-500ml	Sigma-Aldrich Company Ltd., Poole, UK.
DEPC treated water, 75-0024	Invitrogen, Paisley, UK.
ELISA kits:	
IL-6, 850.035.096	IDS Ltd., Tyne and Wear, UK.
IL-1α, 583301	Cayman Chemical Company, Michigan, USA.
IL-1β, 583311	Cayman Chemical Company, Michigan, USA.
TNFα, 589201	Cayman Chemical Company, Michigan, USA.
Cortisol, DX-EIA-1887	IDS Ltd., Tyne and Wear, UK.
IL-6, BMS213INST	Bender MedSystems GmbH, Vienna, Austria.
GH, DX-EIA-3552	IDS Ltd., Tyne and Wear, UK.
IGF-I, EL2010	Oxford Biosystems Oxford, UK.
Leptin, BMS2039INST	Bender MedSystems GmbH, Vienna, Austria.
Adiponectin, DEE009	Demeditec Diagnostics GmbH, Kiel, Germany.
Insulin, KAP1251	BioSource Europe S.A., Nivelles, Belgium.
Ethanol (96 %), 10476	VWR International Ltd., Lutterworth, UK.
Isopropanol (99 %), I9516-500ml	Sigma-Aldrich Company Ltd., Poole, UK.
Lidocaine (2 %), PL01502/0021R	Hamlin Pharmaceuticals Ltd., Gloucester, UK.
Oligonucleotide synthesis:	Sigma-Genosys Ltd., Suffolk, UK.

	QIAGEN, West Sussex, UK.
Phosphate Buffered Saline, BR0014G	OXOID, Basingstoke, UK.
Power SYBR Green RNA-to-C_T 1-Step Kit, -4389986	Applied Biosystems, Warrington, UK.
RNase zap, AM9780	Applied Biosystems, Warrington, UK.
The RNA storage solution, AM7001	Applied Biosystems, Warrington, UK.
Trizol (TRI®) Reagent Solution, AM9738	Applied Biosystems, Warrington, UK.

Appendix B: Equipment

General laboratory plastic and glassware:

Jencons PLS, Leighton Buzzard, UK.
VWR International Ltd., Lutterworth, UK.
Fisher Scientific Inc., Loughborough, UK.

Equipment	Manufacturer/supplier
Aerosol resistant (ART®) pipette tips	Molecular BioProducts Inc., San Diego, CA, USA.
Auto. 96-well plate reader, ELx800UV	BIO-TEK Instruments Inc., Winooski, USA.
Blood glucose analyser 201+,120713	HemoCue AB, Ängelholm, Sweden.
Level 2 controls, 146.002.002	HemoCue AB, Ängelholm, Sweden.
Microcuvetes, Glucose 201+, 110716	HemoCue AB, Ängelholm, Sweden.
Blood glucose analyser, Accutrend GC	Roche Diagnostics, East Sussex, UK.
Glucose reagent strips, Accutest	Roche Diagnostics, East Sussex, UK.
Blood lactate analyser, Lactate Pro	Akray KDK, Koyota, Japan.
Lactate Pro reagent Strips	Akray KDK, Koyota, Japan.
Blood lactate analyser, YSI 1500 Sport	YSI Inc., Yellow Springs, Ohio, USA.
Bio. safety cabinet, class II, NU-437-400E	NuAire Inc., Plymouth, MN, USA.
Biotech photometer, WPA Biowave II	Biochrom Ltd., Cambridge, UK.
Biopsy conchotome Weil-Blakesley 52-030-50	Gebrüder Zepf Medizintechnik, Tüttlingen, Germany.
Cardio-pulmonary testing system, K4 b^2	COSMED, Rome, Italy.
Cryovials, 1.2 ml, CRY-100-015A	Fisher Scientific Inc., Loughborough, UK.
Cycle ergometer, Ergoline ER 800	Jaeger, GmbH, Würzburg, Germany.

Disposable prep razor, UN2000	Universal Hospital Supplies, Enfield, UK.
DNA Engine Peltier Thermal Cycler - with Chromo4 RT-PCR Detector	BioRad Laboratories Ltd., Hemel Hempstead, UK.
PC laptop, Samsung M50	Samsung, Seoul, South Korea.
Fluid aspiration system 401134	Vaccusafe Comfort, INTEGRA Biosciences AG, Chur, Switzerland.
Freezer storage:	
-80 °C, NuAire, NU9668	NuAire Inc., Plymouth MN, USA.
-80 °C, Revco Ultima, RFV-104-020L	Fisher Scientific, Loughborough, UK.
Heart rate monitor, Accurex Plus	Polar Electro Oy, Kempele, Finland.
Hypodermic needle, 21G, NN-2138R	Terumo Europe, Leuven, Belgium.
Hypodermic needle, 25G, NN-2516R	Terumo Europe, Leuven, Belgium.
Ice flaker, Scotsman AF100	Frimont, Milan, Italy.
Magnetic stirrer/hotplate, IKA Combimag RET	IKA-Werke GmbH & Co. KG, Staufen, Germany.
Micro-centrifuge, Galaxy 16DH, 521-2841	VWR International Ltd., Lutterworth, UK.
Micro-centrifuge tubes, 0.5 ml, 0030 121.023	Eppendorf AG. Hamburg, Germany.
Micro-centrifuge tubes, 1.5 ml, 0030 120.086	Eppendorf AG. Hamburg, Germany.
Micro-centrifuge tubes, 2 ml, 0030 120.094	Eppendorf AG. Hamburg, Germany.
Optical film sealing kit, MSO-1001	Bio-Rad Laboratories Ltd., Hemel Hempstead, UK.
Orbital shaker, Vibrax VXR	IKA-Werke GmbH & Co. KG, Staufen, Germany.
RT-PCR Plates, 96-well, HSP-9655	Bio-Rad Laboratories Ltd., Hemel Hempstead, UK.
pH meter, 410A	Thermo Electron Corporation, Beverly, MA, USA.
Pipette, 12-channel, 5-50 µl, Rainin L12-50	Anachem Ltd., Luton, UK.
Pipette tips, 2-250 µl, Rainin SS-L250	Anachem Ltd., Luton, UK.
Pipette, 12-channel, 50-300 µl, Rainin L12-300	Anachem Ltd., Luton, UK.

Pipette tips, 30-300 µl, Rainin SS-L300	Anachem Ltd., Luton, UK.
Pipettes, single channel, 10 µl – 5 ml	Gilson Inc., Middleton, WI, USA.
Pipette tips, 10 µl – 5 ml	Fisher Scientific UK Ltd., Loughborough, UK.
Pipette tips, RNase free, barrier, 10 µl 1 ml	ART Micropoint, Molecular BioProducts Inc., San Diego, CA, USA.
Pre-injection wipes, Sterets, 00766691	Seton Healthcare Group plc., Oldham, UK.
Refrigerated Centrifuge, 3K10	SIGMA Laborzentrifugen GmbH, Osterode am Harz, Germany.
Refrigerator storage:	
4 °C, Biocold, 2224	Scientific laboratory Supplies, Nottingham, UK.
S-Monovette tubes, Serum Z/9ml, 02.1063.001	Sarstedt AG & Co, Germany.
Safety lancets, Haemolance Plus, 7568	HTL Strefa, Inc., Marietta, GA, USA.
Scales, SECA beam balance, 709	SECA, Hamburg, Germany.
Serological pipette filler, Powerpette Pro, -266-185	Jencons PLS, Leighton Buzzard, UK.
Serological pipettes, 2 – 25 ml	Fisher Scientific UK Ltd., Loughborough, UK.
Stadiometer, Harpenden with – Veeder-Root high speed counter	Holtain Ltd, Crymych, Wales.
Sterile adhesive strips, 3M Steri-Strip	3M Healthcare, St. Paul, MN, USA.
Sterile compressive dressing:	
Self-Adherent Wrap, 3M Coban, 1584	3M Healthcare, St. Paul, MN, USA.
Gauze swab, Vernaid, 28919	Vernon-Carus ltd., Preston, UK.
Sterile container, 150 ml, FB51778	Fisher Scientific Inc., Loughborough, UK.
Sterile disposable scalpel, Size 11	Swann-Morton, General Medical, Aldershot, UK.
Sterile dressing, Mepore Ultra	Mölnlycke Health Care AB, Göteborg, Sweden.

Sterile procedure pack, Vernaid, 28853	Vernon-Carus ltd., Preston, UK.
Syringe, 5 ml, SS-05ES	Terumo Europe, Leuven, Belgium.
Syringe, 10 ml, SS-10ES	Terumo Europe, Leuven, Belgium.
Venous cannula, 22G, Venflon IV Blue	Beckton Dickinson (BD) Biosciences, Oxford, UK.
Vortex mixer, Vortex 3, 3340000	IKA-Werke GmbH & Co. KG, Staufen, Germany.
Water purification system, RIOS3	Millipore, Billerica, MA, USA.

Appendix C: Solutions

Solutions were prepared with dH_2O unless otherwise indicated.

Ethanol (75 % solution)

Ethanol 96 % v/v

- Add 100 ml of 96 % v/v ethanol to 22 ml dH_2O.

Appendix D: Gases

Gases were purchased from the British Oxygen Company (BOC) Ltd, Guildford, UK unless otherwise specified.

K4 b^2 calibration gas - O_2 (16 %)/CO_2 (5 %) N_2 balance.

Appendix E: Software

Software	Version	Designer/Supplier
Opticon Monitor	3.1.32	MJ Geneworks Inc., Waltham, Massachusetts, USA.
IBM SPSS Statistics	19 - 21	IBM Corporation, New York, USA.
K4 b^2 PC software	8.0b	COSMED, Rome, Italy.
Beacon Designer	5.11	PREMIER Biosoft International, California, USA.
NCBI Genbank database		NCBI, Maryland, USA.
Microsoft Office for Mac:		
Microsoft Excel for Mac	2011	Microsoft Corporation, Redmond, WA, USA.
Microsoft Word for Mac	2011	Microsoft Corporation, Redmond, WA, USA.