

University Library

Author/Filing Title BURNS, S

Class Mark T

Please note that fines are charged on ALL
overdue items.

FOR REFERENCE ONLY



**RESISTANCE EXERCISE, POSTPRANDIAL TRIACYLGLYCEROL
METABOLISM AND C-REACTIVE PROTEIN**

by


Stephen Francis Burns

A Doctoral Thesis

Submitted in partial fulfilment of the requirements for the award of Doctor of
Philosophy of Loughborough University

May 2006

© by S.F. Burns (2006)

	Loughborough University Pilkington Library
Date	JAN 2007
Class	T
Acc No.	0403271436

Abstract

Elevated postprandial triacylglycerol (TAG) concentrations and increased C-reactive protein (CRP) concentrations are risk markers for cardiovascular disease. The effects of resistance exercise on postprandial TAG metabolism and CRP are uncertain. The studies reported here examined the influence of resistance exercise on postprandial lipaemia and CRP. All studies were approved by the university's Ethical Advisory Committee. Fifty-five male participants were recruited to five studies. The age, height and body mass of the participants were: 24.2 ± 3.8 yrs, 1.78 ± 0.06 m, 78.0 ± 10.8 kg (mean \pm SD).

The aim of the first study was to evaluate the hypothesis that a single session of resistance exercise would lower postprandial lipaemia. Participants consumed a test meal on two separate days. The afternoon prior to one test meal participants performed an 88 min bout of resistance exercise. The afternoon prior to the other test meal participants rested (control). The order of the trials was randomised. Plasma TAG concentration (measured for 6 h after of the consumption test meals) did not differ between trials.

Although a single session of resistance exercise failed to reduce postprandial lipaemia, evidence from epidemiological studies suggests that weight lifting is associated with a reduced risk of cardiovascular disease and studies of aerobic exercise suggest that energy expenditure is an important factor for reduced postprandial TAG concentrations. Therefore, study two employed multiple bouts of low intensity resistance exercise performed over a single day in an effort to increase the energy expenditure beyond that

elicited in study one. On day two of study two, plasma TAG concentrations were reduced on the exercise compared with the control trial: main effect of trial ($P=0.044$).

In contrast to the first two studies (which involved exercise testing on one day and postprandial testing on the next day), study three involved a one-day model (i.e. exercise and postprandial testing on the same day). Two trials were completed (exercise and control) in a randomised order. The morning of one trial participants performed a 90 min bout of resistance exercise and then consumed a test meal. The morning of the other trial participants rested (control) and consumed a test meal. Plasma TAG concentrations were higher during the exercise trial compared with the control trial: main effect of trial ($P=0.010$), trial x time interaction ($P=0.007$). These findings demonstrate that resistance exercise can evoke transient increases in postprandial TAG concentrations. This may be due to transient skeletal muscle damage (as indicated by elevated myoglobin concentrations) after resistance exercise which could inhibit TAG uptake into muscle.

If skeletal muscle damage is responsible for inhibiting TAG uptake into muscle then it follows that this effect might occur with aerobic exercise as well as with resistance exercise. Therefore, study four compared the effect of resistance exercise with that of downhill running – a form of aerobic exercise known to cause muscle damage – on postprandial TAG concentrations. Participants performed three, one-day trials in a randomised order as follows: 1) 90 min bout of resistance exercise + test meal, 2) 35 min downhill treadmill run + test meal, 3) rest (control) + test meal. Plasma TAG was measured for 5 h after consumption of the test meals. The incremental area under the

TAG concentration *versus* time curve differed between trials (control 3.51 ± 0.39 , resistance exercise 4.28 ± 0.58 , run 2.62 ± 0.50 mmol·5h·L⁻¹; $P=0.047$; mean \pm SE). However, myoglobin concentrations were not different between the resistance exercise and downhill running trials suggesting that muscle damage is not responsible for the elevation in postprandial TAG concentrations after resistance exercise.

Study five evaluated the effect of a high volume of resistance exercise on serum CRP. For one trial participants performed multiple bouts of low intensity resistance exercise; in a second trial participants rested (control). The order of trials was randomised. Serum CRP was measured before and for 29 h after the start of exercise. Serum CRP rose steeply after exercise but remained steady on the control trial: trial \times time interaction ($P=0.028$). The rise in CRP after resistance exercise could be related to contractile-induced or injury induced increases in interleukin-6 although this was not measured in study five.

In conclusion, a high volume of resistance exercise (inducing high energy expenditure) decreases postprandial TAG concentrations the next day. However, resistance exercise can cause a transient rise in postprandial TAG concentrations when a test meal is given early on in the post-exercise period. Whether this is related to skeletal muscle damage is unclear. Finally, multiple bouts of resistance exercise performed in a single day may cause a post-exercise increase in serum CRP concentrations.

Keywords: triacylglycerol, postprandial lipaemia, C-reactive protein, resistance exercise, weight lifting, cardiovascular disease

Acknowledgements

I would like to express my gratitude to the following people who have given me their help and support in completing this thesis:

My Supervisor, Dr. David Stensel, I am particularly indebted to for giving me the opportunity to undertake the thesis and for all the support he has given in helping me to see it to completion. David, has given me invaluable advice and guidance that will last me throughout my career. Also for coming in to perform cannulations on participants on so many early mornings!

My Director of Research, Dr. Mary Nevill, for her advice and guidance throughout my PhD.

The members of the Exercise and Health team, Mr. Masashi Miyashita and Mr. David Broom, for their support and help in completing this work, and their friendship. I am also indebted to Dr. Chihoko Ueda for her help on the long testing days and her friendship.

All the physiology staff and PhD students who have made my time at Loughborough easier and provided me with advice and assistance whenever I needed it.

Mr. Spencer Newport, Mrs. Gabriella Scanlon, Mr. Steve Alvey and Ms. Deborah Butler for their assistance in obtaining all the things I ever needed in the lab.

Veni and my family for their support, encouragement and confidence in me.

Finally, a massive thank you to all the participants who gave their time and effort so freely in order that the studies described here could be completed.

PREFACE

The following parts of the work presented in this thesis have been published as follows:

Original articles

Burns SF, Corrie H, Holder E, Nightingale T, Stensel DJ (2005) A single session of resistance exercise does not reduce postprandial lipaemia. *Journal of Sports Sciences*. **23(3)**: 251-260.

Burns SF, Broom DR, Miyashita M, Ueda C, Stensel DJ (2006) Increased postprandial triacylglycerol concentrations following resistance exercise. *Medicine and Science in Sports and Exercise*. **38(3)**: 527-533.

Published communications

Burns SF, Miyashita M, Ueda C, Stensel DJ (2005) Increased postprandial lipaemia following a bout of resistance exercise. *Journal of Sports Sciences*. **23(11-12)**: 1183.

Burns SF, Ueda C, Stensel DJ (2006) The influence of multiple bouts of resistance exercise on postprandial triacylglycerol concentrations. *Medicine and Science in Sports and Exercise*. **38(5)**: S485.

TABLE OF CONTENTS

	Pg.
Abstract	i
Acknowledgements	v
Preface	vii
Table of contents	viii
List of tables	xiii
List of figures	xiv
List of abbreviations	xvii
CHAPTER 1	
INTRODUCTION	1
CHAPTER 2	
REVIEW OF LITERATURE	9
2.1 Atherosclerosis	9
2.2 Postprandial triacylglycerol metabolism and atherosclerosis	9
2.3 C-reactive protein and atherosclerosis	13
2.4 Influences on postprandial TAG metabolism	14
2.5 Postprandial TAG Assessment	16
2.6 Assessment of CRP	17
2.7 Aerobic exercise and postprandial TAG metabolism	17
2.8 Resistance exercise and cardiovascular disease	25
2.9 Resistance exercise and fasting TAG	27
2.10 Resistance exercise and postprandial TAG	28
2.11 Physical activity and CRP	32
2.12 Resistance exercise and CRP	33
2.13 Summary	36
CHAPTER 3	
GENERAL METHODS	37
3.1 Participants	37
3.2 Resting arterial blood pressure	37

3.3 Anthropometry	38
3.4 Repetition maximum tests	38
3.5 Control of diet and exercise	39
3.6 Measurement of expired air	39
3.7 Estimation of energy expenditure during weight lifting	40
3.8 The test meal	41
3.9 Blood sampling	43
3.10 Analytical methods	44
3.11 Data analysis	45
 CHAPTER 4	
THE EFFECT OF A SINGLE SESSION OF RESISTANCE EXERCISE ON POSTPRANDIAL LIPAEMIA	46
4.1 Introduction	46
4.2 Methods	48
4.2.1 Participants	48
4.2.2 Study design	48
4.2.3 Weight lifting protocol	49
4.2.4 Estimation of energy expenditure during weight lifting	50
4.2.5 Test meals	51
4.2.6 Haematocrit and haemoglobin	51
4.2.7 Data analysis	52
4.3 Results	54
4.3.1 Responses during weight lifting	54
4.3.2 Plasma concentrations in the fasted state	54
4.3.3 Postprandial plasma responses to the test meals	55
4.4 Discussion	60
 CHAPTER 5	
RESISTANCE EXERCISE AND POSTPRANDIAL TRIACYLGLYCEROL CONCENTRATIONS: INFLUENCE OF ENERGY EXPENDITURE	66
5.1 Introduction	66
5.2 Methods	68
5.2.1 Participants	68

5.2.2 Preliminary tests	68
5.2.3 Main Trials	69
5.2.4 Weight lifting protocol – day 1	69
5.2.5 Estimation of energy expenditure during weight lifting	70
5.2.6 Main trial protocol - day 2	71
5.2.7 Analytical Methods	71
5.2.8 Data Analysis	72
5.3 Results	76
5.3.1 Resistance exercise session	76
5.3.2 Plasma concentrations in the fasted state	76
5.3.3 Postprandial plasma responses	77
5.4 Discussion	84

CHAPTER 6

THE EFFECT OF A SINGLE SESSION OF RESISTANCE EXERCISE ON POSTPRANDIAL TRIACYLGLYCEROL RESPONSES IN A GROUP OF RESISTANCE TRAINED MALES

6.1 Introduction	92
6.2 Methods	95
6.2.1 Participants	95
6.2.2 Preliminary tests	95
6.2.3 Main Trials	96
6.2.4 Main trial protocol	96
6.2.5 Weight lifting protocol	97
6.2.6 Expired air collections during weight lifting	98
6.2.7 Analytical methods	98
6.2.8 Data analysis	98
6.3 Results	101
6.3.1 Resistance exercise session	101
6.3.2 Plasma concentrations in the fasted state	101
6.3.3 Postprandial plasma responses	102
6.4 Discussion	108

CHAPTER 7

A COMPARISON OF THE EFFECT OF RESISTANCE EXERCISE AND DOWNHILL TREADMILL RUNNING ON POSTPRANDIAL

TRIACYLGLYCEROL CONCENTRATIONS 113

7.1 Introduction 113

7.2 Methods 117

7.2.1 Participants 117

7.2.2 Preliminary tests 117

7.2.3 Twelve-repetition maximum tests 118

7.2.4 Submaximal treadmill test 118

7.2.5 Maximum oxygen uptake test 118

7.2.6 Main trials 119

7.2.7 Main trial protocol 119

7.2.8 Blood sampling for CRP 120

7.2.9 Weight lifting protocol 121

7.2.10 Estimation of energy expenditure during weight lifting 121

7.2.11 Downhill treadmill running 121

7.2.12 Analytical methods 122

7.2.13 Data analysis 123

7.3 Results 125

7.3.1 Resistance exercise session 125

7.3.2 Responses to treadmill running 125

7.3.3 Plasma concentrations in the fasted state 126

7.3.4 Postprandial plasma responses 126

7.3.5 Serum CRP concentrations 134

7.4 Discussion 137

CHAPTER 8

THE EFFECT OF RESISTANCE EXERCISE ON SERUM C-REACTIVE PROTEIN CONCENTRATIONS

144

8.1 Introduction 144

8.2 Methods 146

8.2.1 Participants 146

8.2.2 Preliminary tests 146

8.2.3 Main trials	147
8.2.4 Weight lifting protocol – day 1	147
8.2.5 Estimation of energy expenditure during weight lifting	148
8.2.6 Main trial protocol - day 2	148
8.2.7 Blood sampling for CRP	149
8.2.7 Analytical methods	149
8.2.8 Data analysis	149
8.3 Results	154
8.3.1 Resistance exercise session	154
8.3.2 Baseline serum concentrations	154
8.3.3. Changes in serum CRP	154
8.4 Discussion	158
 CHAPTER 9	
GENERAL DISCUSSION	165
9.1 Discussion	165
9.2 Further Research	176
 REFERENCES	179
 APPENDICES	
APPENDIX A	
APPENDIX B	
APPENDIX C	
APPENDIX D	

LIST OF TABLES

	Pg.
3.1 Ingredients of the test meal used in Study 1 (Chapter 4), administered per kg body mass.	42
3.2 Ingredients of the test meal used in Studies 2-5 (Chapters 5-8), administered per kg body mass.	42
3.3 Macronutrient content of the two test meals used in this thesis.	43
4.1 Physical characteristics of the participants.	48
4.2 Plasma concentrations in the fasted state.	54
4.3 Six-hour areas under the plasma concentration <i>versus</i> time curves after consumption of a test meal in the control and resistance exercise trials.	56
5.1 Physical characteristics of the participants.	68
5.2 Plasma concentrations in the fasted state on day 2.	77
6.1 Physical characteristics of the participants.	95
6.2 Plasma concentrations in the fasted state.	101
7.1 Physical characteristics of the participants.	117
7.2 Plasma concentrations in the fasted state.	126
7.3 Relationships between indices of TAG and insulin on the run trial.	133
7.4 Serum C-reactive protein (CRP) concentrations using a high sensitivity assay on the control and resistance exercise trials.	136
8.1 Physical characteristics of the participants.	146
8.2 Individual CRP data for nine participants on the control and resistance exercise trial.	157

LIST OF FIGURES

	Pg.
4.1 Schematic representation of the main trial protocol.	53
4.2 Total (A) and incremental (B) plasma triacylglycerol (TAG) concentrations in the fasted state (0 h) and for 6 h following consumption of a test meal in the control (◇) and resistance exercise (■) trials.	57
4.3 Relationship between fasting triacylglycerol (TAG) and total area under the TAG concentration <i>versus</i> time curve (AUC) for the control ($r = 0.95$, $P < 0.0005$) (A) and resistance exercise ($r = 0.93$, $P < 0.0005$) (B) trials.	58
4.4 Plasma non-esterified fatty acids (NEFA) (A), glucose (B) and insulin (C) concentrations in the fasted state (0 h) and for 6 h following consumption of a test meal in the control (◇) and resistance exercise (■) trials.	59
5.1 Schematic representation of one 45 minute bout of weight lifting.	73
5.2 Schematic representation of weight lifting protocol – day 1.	74
5.3 Schematic representation of the main trial protocol – day 2.	75
5.4 Postprandial total plasma triacylglycerol (TAG) concentrations for 6 h following consumption of a test meal in the control (◇) and resistance exercise (■) trials.	78
5.5 Relationship between fasting triacylglycerol (TAG) and incremental area under the TAG concentration <i>versus</i> time curve (IAUC) for the control ($r = 0.78$, $P < 0.0005$) (A) and resistance exercise ($r = 0.57$, $P = 0.004$) (B) trials.	79
5.6 Postprandial plasma insulin (A) and glucose (B) concentrations for 6 h following consumption of a test meal in the control (◇) and resistance exercise (■) trials.	81
5.7 Postprandial plasma non-esterified fatty acid (NEFA) (A) and 3-hydroxybutyrate (B) concentrations for 6 h following consumption of a test meal in the control (◇) and resistance exercise (■) trials.	83

6.1	Schematic representation of the main trial protocol.	100
6.2	Postprandial total plasma triacylglycerol (TAG) concentrations for 5 h following consumption of a test meal in the control (◇) and resistance exercise (■) trials.	103
6.3	Postprandial total plasma myoglobin concentrations for 5 h following consumption of a test meal in the control (◇) and resistance exercise (■) trials.	104
6.4	Postprandial plasma insulin (A) and glucose (B) concentrations for 5 h following consumption of a test meal in the control (◇) and resistance exercise (■) trials.	105
6.5	Postprandial plasma non-esterified fatty acid (NEFA) (A) and 3-hydroxybutyrate (B) concentrations for 5 h following consumption of a test meal in the control (◇) and resistance exercise (■) trials.	107
7.1	Schematic representation of the main trial protocol.	124
7.2	Postprandial total plasma triacylglycerol (TAG) concentrations for 5 h following consumption of a test meal in the control (◇), resistance exercise (■) and run (Δ) trials.	127
7.3	Postprandial total plasma myoglobin concentrations for 5 h following consumption of a test meal in the control (◇), resistance exercise (■) and run (Δ) trials.	128
7.4	(A) Relationship between adjusted peak triacylglycerol (TAG) (peak minus fasting concentrations) and adjusted peak myoglobin on the run trial ($r = 0.814$, $P=0.014$). $n = 8$. (B) Relationship between adjusted peak TAG (peak minus fasting concentrations) and adjusted peak myoglobin on the run trial ($r = 0.709$, $P=0.049$).	130
7.5	Postprandial plasma insulin (a) and glucose (b) concentrations for 5 h following consumption of a test meal in the control (◇), resistance exercise (■) and run (Δ) trials.	132
7.6	Serum CRP concentrations over 26 hours on the control (◇) and resistance exercise (■) trials.	135

8.1	Schematic representation of one 45 minute bout of weight lifting.	151
8.2	Schematic representation of weight lifting protocol – day 1.	152
8.3	Schematic representation of the main trial protocol – day 2.	153
8.4	Serum C-reactive protein (CRP) concentrations in the control (◇) and resistance exercise (■) trials.	155

LIST OF ABBREVIATIONS

The following abbreviations are used throughout this thesis. Where they appear in the text they have always been defined in the first instance:

TAG – triacylglycerol

HDL – high density lipoprotein

LDL – low density lipoprotein

CRP – C-reactive protein

VLDL – very low density lipoprotein

BMI – body mass index

$\dot{V}O_2$ - rate of oxygen uptake

$\dot{V}CO_2$ - rate of carbon dioxide production

NEFA – non-esterified fatty acid(s)

SE – standard error

SD – standard deviation

ANOVA – analysis of variance

$\dot{V}O_{2\text{ max}}$ – maximum oxygen uptake

AUC - area under the concentration *versus* time curve

IAUC - incremental area under the concentration *versus* time curve

NS - non-significant

Chapter 1

Introduction

Cardiovascular disease, which includes arterial disease affecting the blood supply to the heart, brain or peripheral regions of the body, is the leading cause of death both in the United Kingdom and worldwide (British Heart Foundation, 2005; World Health Organisation, 2001). More than one in three people (38%) in the United Kingdom die from cardiovascular disease (British Heart Foundation, 2005). Cardiovascular diseases whether they affect the coronary, cerebral or peripheral arteries, share a common pathophysiology involving atherosclerosis - thickening of the arterial wall - and thrombosis - blood clot formation.

A number of risk factors can be related to the subsequent occurrence of cardiovascular disease. The role lipids and lipoproteins play as risk factors for cardiovascular disease is well documented (Levine *et al.*, 1995; Barter & Rye, 1996; Wood *et al.*, 1998). Assessment of lipoproteins is traditionally based on measurements made in fasted blood samples. However, people typically spend most of their lives in the postprandial (non-fasting) state and consumption of multiple meals throughout the day may represent a repeated metabolic challenge. Furthermore, postprandial lipid and lipoprotein levels may better reflect diurnal patterns than fasting values.

Changes to metabolism in the postprandial period can promote atherosclerosis due to disturbances in lipoprotein metabolism which encourage: a) an accumulation of triacylglycerol (TAG) rich lipoprotein remnants in the plasma; b) catabolism of high

density lipoprotein (HDL) cholesterol; c) formation of small, dense low density lipoprotein (LDL) particles which have an increased susceptibility to oxidation in the arterial intima (Cohn, 1998; Karpe & Hamsten, 1995). The co-existence of an elevated plasma TAG concentration, low HDL-cholesterol and abundance of small, dense LDL particles has been termed the atherogenic lipoprotein phenotype (Griffin & Zampelas, 1995). It appears that the enhanced TAG flux after ingestion of a meal is the driving force for the modification of LDL particles and the depletion of HDL-cholesterol. Furthermore, remnants of TAG-rich lipoproteins may directly infiltrate the arterial wall (Zilversmit, 1979).

Atherosclerosis bears many of the hallmarks of a chronic inflammatory disease (Ross, 1999). While much of the inflammatory activity in atherosclerosis is located within the arterial intima, there is evidence to suggest that it is reflected by a persistent, low-grade inflammation in the circulation (Willerson & Ridker, 2004). One marker of this low-grade inflammation is C-reactive protein (CRP) (Tracy, 1998; Yeh & Willerson, 2003). C-reactive protein is synthesised by the liver and primarily regulated by the cytokine (chemical messenger to promote an inflammatory immune response) interleukin-6. High serum concentrations of CRP have been found to correlate with the presence of sub-clinical cardiovascular disease and the risk of acute cardiovascular events (Kuller *et al.*, 1996; Tracy *et al.*, 1997a; Ridker *et al.*, 1998; Ridker *et al.*, 2000). Furthermore, CRP levels are positively associated with a number of classical cardiovascular disease risk factors such as age, body mass, systolic blood pressure and smoking, whilst negatively associated with others, such as HDL-cholesterol (Das 1985; Tracy *et al.*, 1997b; Pradhan *et al.*, 2001; Lemieux *et al.*, 2001; Rawson *et al.*, 2003). C-reactive protein may also have pro-inflammatory properties

(Pasceri *et al.*, 2000). However, the evidence for whether CRP is simply a marker of the inflammatory process, or if it plays an aetiological role in atherogenesis is unclear at the moment.

Physical inactivity is a modifiable risk factor for the subsequent occurrence of cardiovascular disease. Population studies have found that high levels of physical activity and high cardiorespiratory fitness are associated with a reduced risk of cardiovascular disease and coronary heart disease in particular (Morris *et al.*, 1953a; Morris *et al.*, 1953b; Paffenbarger *et al.*, 1978; Blair *et al.*, 1996). The ability of physical activity to reduce the risk of cardiovascular disease may, in part, be mediated through its effects on lipoproteins in the postprandial period. A large body of evidence now exists to suggest that a single bout of aerobic exercise, such as running or walking, may reduce postprandial TAG concentrations (Aldred *et al.*, 1994; Tsetsonis & Hardman, 1996a; Tsetsonis & Hardman, 1996b; Tsetsonis *et al.*, 1997; Gill *et al.*, 2003). This reduction appears to be transient, however, since a period of detraining leads to an elevation in postprandial TAG concentrations (Hardman *et al.*, 1998; Herd *et al.*, 1998). Therefore, exercise must be performed frequently for continued benefits. Furthermore, the energy expenditure of the exercise appears to be the primary determinant of the exercise-induced reduction in postprandial TAG concentrations (Gill *et al.*, 2002; Petitt & Cureton, 2003b; Tsetsonis & Hardman, 1996b). To date, however, studies investigating the effect of exercise on postprandial TAG concentrations have concentrated on the effects of aerobic exercise.

The evidence to suggest that exercise may reduce serum CRP is conflicting. The majority of cross-sectional studies, although not providing information with regard to

a possible causal relationship, demonstrate an inverse relationship between physical activity or physical fitness and serum CRP (Aronson *et al.*, 2004; Dufaux *et al.*, 1984; LaMonte *et al.*, 2005; Albert *et al.*, 2004; Katja *et al.*, 2005). The influence of exercise training on serum CRP concentrations is more conflicting. Several studies have shown a reduction in CRP after a period of aerobic exercise training (Tisi *et al.*, 1997; Mattusch *et al.*, 2000; Smith *et al.*, 1999), but other studies have shown no benefit of exercise training on CRP (Marcell *et al.*, 2005; Hammett *et al.*, 2004; Hammett *et al.*, 2006). Differences in length of training or health of the participants may account for some of these differences. However, some of these training studies were non-randomised and did not include a control group (Mattusch *et al.*, 2000; Smith *et al.*, 1999) making it difficult to establish causality.

The effect of an acute bout of exercise on CRP conflicts with the evidence provided by the majority of cross-sectional and training studies. Several studies have shown an increase in CRP after strenuous exercise (Weight *et al.*, 1991; Taylor *et al.*, 1987; Siegel *et al.*, 2001; Fallon *et al.*, 2001). However, a single bout of moderate exercise has been shown to have no effect on CRP (Murtagh *et al.*, 2005). It is possible that CRP increases with exercise are proportional to the volume/intensity of activity and the extent of muscle injury (Strachan *et al.*, 1984). However, as with exercise and postprandial TAG concentrations, the majority of studies on the effect of physical activity on serum levels of CRP have used aerobic activities as the mode of exercise.

The health benefits of resistance exercise have been highlighted in recent reports on physical activity and health both in the United Kingdom and the United States (US Department of Health and Human Services, 1996; Department of Health, 2004).

Resistance training has been defined as training designed to increase strength, power and muscular endurance (Wilmore & Costill, 1999; Kraemer et al., 2002). However, many different variables can be manipulated within a resistance exercise programme in order to achieve these outcomes. Guidelines for frequency, volume and intensity of resistance exercise needed to increase muscle strength and hypertrophy have been published (Kraemer *et al.*, 2002; Kraemer & Ratamess, 2004). These reports and guidelines have focused on the role of resistance training in increasing muscle strength, power and mobility, its ability to help prevent falls in older adults and its use in maximising bone density for osteoporosis prevention. The role resistance exercise may play in the prevention of cardiovascular disease or in the reduction of risk markers for cardiovascular disease has been less well investigated.

There is, nonetheless, evidence to suggest that resistance training has the ability to improve endurance capacity and performance, at least in older adults. Ades and colleagues (1996) randomised 24 healthy older adults aged 65 to 79 years to 12 weeks of resistance training or a sedentary control group. A significant improvement in submaximal walking time was seen in the resistance training group in comparison to the controls. Brochu and co-workers (2002) randomised 30 elderly women with diagnosed coronary heart disease to either six months of resistance training or a control group performing stretching and flexibility exercises. A 26% significant improvement in endurance performance as measured by a functional task test and a 15% improvement in distance covered over a six minute walk test was seen in the resistance training group only. These studies suggest that for older people maintaining sufficient strength is important as it affects their mobility which can then lead to a reduced cardiovascular capacity.

Epidemiological studies provide further evidence for the potential for resistance exercise to have favourable effects on cardiovascular disease. Paffenbarger & Hale (1975) found that San Francisco dock workers engaged in heavy work had a reduced risk of coronary heart disease than co-workers involved in lighter duties. Further evidence for a role of resistance exercise in the prevention of coronary heart disease is provided by Tanasescu and colleagues (2002) who followed a cohort of male health professionals in the United States for 12 years. Those health professionals who trained with weights for more than 30 minutes each week had a 23% reduction in risk for coronary heart disease even after correction for other risk factors and other types of physical activity. Finally, Jurca and co-workers (2004) tested 8,570 men in the United States for cardiorespiratory fitness and age-specific muscular strength. They found significant inverse associations between muscular strength and the prevalence of the metabolic syndrome (characterised by abdominal obesity and disorders of lipid and carbohydrate metabolism) amongst men with low and moderate cardiorespiratory fitness.

Several cross-sectional and training studies have investigated the relationship between resistance exercise and fasting TAG concentrations with diverging results (Banz *et al.*, 2003; Behall *et al.*, 2003, Elliot *et al.*, 2002; Goldberg *et al.*, 1984; LeMura *et al.*, 2000; Prabhakaran *et al.*, 1999). Differences in participants, the length and volume of training and time of measurement since the end of the last bout of exercise may account for some of these differences. However, only two published studies to date have investigated the acute effects of resistance exercise on postprandial TAG concentrations (Pettit *et al.*, 2003a; Shannon *et al.*, 2005). Both of these studies were

published after the investigations in this thesis began. As with investigations on fasting TAG concentrations these studies conflict in their findings with one showing a reduction (Petitt *et al.*, 2003) and the other showing no reduction in postprandial TAG concentrations after exercise (Shannon *et al.*, 2005).

To date, only one study has examined the effect of resistance training on CRP concentrations (Castaneda *et al.*, 2004), showing a reduction in CRP after 12 weeks of training. Two studies have reported on the effect of a single bout of resistance exercise on serum CRP concentrations (Nosaka & Clarkson, 1996; Paulsen *et al.*, 2005). Both studies employed maximal eccentric contractions in isolated muscle groups to examine inflammatory markers after exercise. These studies conflict with one showing no change in CRP (Nosaka & Clarkson, 1996) and the other showing a significant increase in serum CRP (Paulsen *et al.*, 2005) post exercise. Differences in the volume of exercise performed and the muscle mass involved may explain the conflicting findings in these two studies. Although there are few studies to draw data from, as with aerobic exercise, the effects of resistance training on CRP may differ from the effects of a single session of resistance exercise. Certainly if a bout of resistance exercise were strenuous enough and caused muscle damage CRP concentrations could increase post exercise.

In summary there is good evidence that resistance training can positively influence endurance capacity and performance, at least in older adults. Evidence from epidemiological studies shows the potential for resistance exercise to reduce cardiovascular disease (or at least coronary heart disease). However, there is still uncertainty with respect to which cardiovascular disease risk factors resistance

exercise affects and the mechanisms through which it does this. Moreover, the effect of resistance exercise on either fasting or postprandial TAG metabolism or its effects on CRP are unclear. With the paucity of information and conflicting evidence on the effect of resistance exercise on postprandial TAG concentrations and serum CRP concentrations, the aim of the studies presented in this thesis was to examine this topic. The main hypothesis of these studies is that if energy expenditure from resistance exercise is sufficiently high then a reduction in postprandial TAG concentrations will be seen. A secondary hypothesis is that completing a high volume session of resistance exercise will produce an acute increase in serum CRP concentrations. Young healthy males were chosen to participate in these studies as this group would be able to complete the large volumes of resistance exercise necessary to examine the area.

Chapter 2

Review of Literature

2.1 Atherosclerosis

Cardiovascular diseases whether they affect the coronary, cerebral or peripheral arteries, share a common pathophysiology involving atherosclerosis and thrombosis. Atherosclerosis involves the thickening of the arteries caused by the development of lesions, or plaques, in the arterial walls. The atherosclerotic plaque is a lipid core, mainly cholesterol, in the intima of the arterial wall, covered by a fibrous cap. The atherosclerotic plaque protrudes into the arterial lumen and in some cases the cap of the plaque may eventually rupture and a blood clot forms (thrombus). The thrombus may occlude blood flow at the site of rupture or become loose and be carried to other sites where it can lodge and obstruct blood flow. This can lead to a myocardial infarction (heart attack) or ischaemic stroke in the brain. Narrowing of arteries outside the myocardium or brain by plaques can lead to peripheral vascular disease (Berliner *et al.*, 1997).

2.2 Postprandial triacylglycerol metabolism and atherosclerosis

Triacylglycerol flux through the plasma after the ingestion of a meal is a major determinant of HDL-cholesterol concentration and a determinant of LDL particle size. In the postprandial period the TAG-rich lipoproteins, intestinally derived chylomicrons and hepatically derived very low density lipoproteins (VLDL), compete for clearance by the same lipolytic pathway (Brunzell *et al.*, 1973; Bjorkegren *et al.*, 1996). Chylomicrons and VLDL are both hydrolysed by the enzyme lipoprotein lipase as they pass through tissues which express this enzyme. This process has been termed

the 'common saturable removal mechanism.' This leads to an accumulation of circulating TAG-rich lipoproteins in the plasma.

An inverse relationship is usually observed between plasma TAG and HDL-cholesterol concentrations: the higher the TAG concentration the lower the HDL-cholesterol concentration (Patsch *et al.*, 1983; Patsch *et al.*, 1984; Miesenbock *et al.*, 1992). When the TAG-rich lipoprotein concentration is high, such as in the postprandial period, circulating cholesteryl ester-transfer protein catalyses the exchange of lipids between lipoprotein particles. Esterified cholesterol from HDL and LDL is transferred to TAG-rich lipoproteins with TAG transfer in the opposite direction. The HDL particles are now enriched with TAG and are hydrolysed by hepatic lipase, resulting in small cholesteryl-ester depleted HDL particles, which are removed from the circulation at an enhanced rate (Rashid *et al.*, 2002).

Small, dense LDL are formed as the TAG entering the LDL particle is also hydrolysed by hepatic lipase and the core volume of the particle reduced (Griffin, 1997). Small dense LDL has a lower affinity for the LDL receptor expressed in most tissues than normal buoyant LDL. It is thought that one consequence of this is that small, dense LDL more avidly binds to and enters the arterial intima for oxidative modification. A large number of case-control studies have demonstrated a strong association between small, dense LDL and atherosclerosis or cardiovascular disease (Austin *et al.*, 1988; Griffin *et al.*, 1994; Stampfer *et al.*, 1996; Skoglund-Andersson *et al.*, 1999).

The postprandial accumulation of TAG-rich lipoproteins may also be important for reasons other than its effects on HDL and LDL particles. Transfer of esterified cholesterol from HDL and LDL in the postprandial period leads to TAG depleted but cholesteryl-ester enriched remnants of the TAG-rich lipoproteins. These particles are potentially highly atherogenic (Zilversmit, 1979).

There is good evidence to support a potential link between postprandial TAG metabolism and atherosclerosis. Case control studies support an association between postprandial TAG concentrations and coronary heart disease (Simpson *et al.*, 1990; Groot *et al.*, 1991; Patsch *et al.*, 1992; Meyer *et al.*, 1996). Patsch and colleagues (1992) in a study investigating 61 male normolipidaemic subjects with severe coronary heart disease and 40 control subjects as verified by angiography found patients with coronary heart disease exhibited a higher and longer postprandial TAG response compared to controls. Multivariate logistic-regression analysis, including HDL-cholesterol, revealed that postprandial TAG concentrations were independently associated with the disease. Notably, postprandial TAG concentrations at six hours predicted the presence or absence of coronary heart disease with an accuracy (68%) which was higher than that of HDL₂-cholesterol (64%) (the main subfraction of HDL-cholesterol). In another study, postprandial TAG response was again pronounced and late in normolipidaemic patients with severe coronary atherosclerosis compared to matched controls (Groot *et al.*, 1991).

Whilst case control studies support a potential link between postprandial TAG concentrations and coronary heart disease they provide retrospective information and are subject to bias in the selection of control groups. However, at least two

prospective studies (Stensvold *et al.*, 1993; Stampfer *et al.*, 1996) have found an association between postprandial TAG concentrations and risk of coronary heart disease. Stampfer and colleagues (1996) followed 14,916 men in the U.S. over seven years. Myocardial infarction was diagnosed over the seven years. After adjustment for lipids and other coronary heart disease risk factors a significant increase in the relative risk (1.4) for a myocardial infarction was seen with each $1.13 \text{ mmol}\cdot\text{L}^{-1}$ increase in postprandial TAG concentrations. This association between TAG and myocardial infarction was linear across the distribution with men in the highest quintile having 2.5 times the risk of men in the lowest quintile. Stensvold and co-workers saw similar results in 50,000 men and women followed over 14 years. After adjustment for a number of other risk factors a significant increase in the relative risk (4.7) of death from coronary heart disease was seen between those persons with a TAG concentration $\geq 3.5 \text{ mmol}\cdot\text{L}^{-1}$ and those with a TAG concentration of $\leq 1.5 \text{ mmol}\cdot\text{L}^{-1}$.

Postprandial TAG concentrations are a potential discriminator not only for the presence of severe coronary heart disease but for early atherosclerosis as well. Ryu *et al.* (1992) studied 47 moderately hypercholesterolaemic subjects (including 23 women) and found peak postprandial TAG response was significantly correlated with carotid artery intima-media thickness (a marker for early atherosclerosis). Similar results have been observed by Sharrett and colleagues (1995) and Karpe and co-workers (1998). It has also been found that remnant TAG-rich lipoproteins are related to intima-media thickness of the carotid artery intima-media thickness independently of plasma TAG and LDL-cholesterol (Karpe *et al.*, 2001).

2.3 C-reactive protein and atherosclerosis

Atherosclerosis bears many of the hallmarks of a chronic inflammatory disease (Ross, 1999). One stimulus for the inflammatory response is oxidised LDL although several others have been proposed (Berliner *et al.*, 1997). The inflammatory response attracts cells of the immune system, monocytes and lymphocytes, to the artery wall, which infiltrate the arterial intima and differentiate into macrophages (white blood cells with scavenger properties that collect at infection sites to remove foreign bodies) that take up oxidised LDL (Witztum & Berliner, 1998). Eventually the macrophages become excessively laden with cholesterol and die through necrosis (uncontrolled cell death) and apoptosis (programmed cell death). The lipid within the macrophages is deposited within the core of the developing plaque.

While much of the inflammatory activity in atherosclerosis is located within the arterial intima, there is evidence to suggest that it is reflected by a persistent, low-grade inflammation in the circulation (Libby *et al.*, 2002; Willerson & Ridker, 2004). This is likely to be the result of a 'spilling over' of inflammatory molecules from the vessel wall into the circulation. These molecules then act on the liver to induce the secretion of certain acute phase proteins. One of these is CRP. It is unclear, presently, whether CRP is simply a marker of the inflammatory process, or if it plays an aetiological role in atherogenesis.

As with postprandial TAG concentrations there is good evidence to support a link between CRP and cardiovascular disease. Ridker and colleagues (2000) followed 28,263 healthy post-menopausal women over three years in a prospective study. They found high sensitivity CRP (see Section 2.6, Pg.17) was the strongest univariate

predictor of the risk of a cardiovascular event from 12 different markers of inflammation. The relative risk for women in the highest quartile of CRP as compared to the lowest quartile of CRP was 4.4. Similarly, CRP has been shown to be a strong predictor of myocardial infarction in a prospective study of 14,916 men (Ridker *et al.*, 1998). However, it should be noted that prediction of risk of a future myocardial infarction was significantly better when CRP was incorporated in analyses alongside lipid parameters than when either CRP or lipids alone were used. C-reactive protein has also been shown to be significantly associated with the degree of carotid atherosclerosis as measured by intima-media thickness in males (Blackburn *et al.*, 2001).

2.4 Influences on postprandial TAG metabolism

A number of factors influence the magnitude of the postprandial response in an individual. The postprandial TAG response to a test meal is greater in older individuals. In a study by Cohn *et al.* (1988) younger subjects exhibited a TAG response 40% lower than older subjects. Several studies have also demonstrated that women exhibit lower postprandial lipaemia than men (Kashyap *et al.*, 1983; Tollin *et al.*, 1985; Cohn *et al.*, 1988; Ohta *et al.*, 1992).

Preceding dietary intake can substantially influence postprandial TAG assessment. Supplementation with long-chain ω -3 polyunsaturated fatty acids leads to a decrease in both fasting and postprandial TAG concentrations and a decreased residence time in the circulation of the TAG-rich lipoproteins, even in doses as low as 1 g per day (Roche & Gibney, 1996; Roche & Gibney, 2000; Volek *et al.*, 2000). A background diet rich in ω -6 polyunsaturates also appears to attenuate postprandial responses

(Weintraub *et al.*, 1988; Bergeron & Havel, 1995). Although the TAG lowering effects are not as marked as when long-chain ω -3 polyunsaturates are fed, the ω -6 fatty acids have been shown to reduce the accumulation of VLDL particles in the postprandial state. Diets high in monounsaturated fatty acids seem to affect the pattern rather than the magnitude of the postprandial response in comparison with diets rich in saturated fats (Zampelas *et al.*, 1998; Roche *et al.*, 1998).

Low-fat, high-carbohydrate diets have a tendency to increase fasting and postprandial TAG (Parks, 2001). The mechanism could involve either increased TAG production and/or reduced TAG catabolism in peripheral tissues. Interestingly, Koutsari and colleagues showed that the postprandial hypertriglyceridaemic effect of a low-fat, high-carbohydrate diet can be counteracted by moderate intensity aerobic exercise (Koutsari *et al.*, 2001a; Koutsari *et al.*, 2001b). The inclusion of ω -3 polyunsaturates can also counteract the effect of a low-fat, high-carbohydrate diet (Roche & Gibney, 1999).

The post-absorptive plasma TAG concentration is a determinant of the magnitude of postprandial lipaemia. The TAG-rich lipoproteins are cleared by the same pathway and so a high fasting concentration of VLDL will increase competition for clearance. A positive relationship has been demonstrated between fasting TAG concentrations and postprandial lipaemia (Patsch *et al.*, 1983; Cohn *et al.*, 1988; Potts *et al.*, 1994). However, despite the close relationship between fasting and postprandial TAG concentrations, the postprandial TAG response is often independent of basal TAG concentrations. This has been demonstrated in studies where some participants

exhibited higher postprandial lipaemia than others with similar fasting TAG concentrations (Merrill *et al.*, 1989; Cohen *et al.*, 1989).

2.5 Postprandial TAG Assessment

In assessing an individual's TAG metabolic capacity most studies have used some kind of fat challenge which is administered either orally or intravenously. The studies in this thesis all use an oral fat challenge. The oral fat challenge is sometimes referred to as the oral fat tolerance test or high fat meal. Throughout this thesis I have used the term test meal for simplicity and also because typically the meal administered contains not only fat but also substantial amounts of carbohydrate and protein. The term test meal reflects this.

As no standardised test meal to assess an individual's TAG metabolic capacity has been developed comparison of results between studies is made more difficult. However, a small number of studies have attempted to assess the reproducibility of the test meal (Cohen *et al.*, 1988; Brown *et al.*, 1992; Ryu *et al.*, 1992; Gill *et al.*, 2005). As a number of different factors have already been outlined which are known to affect the magnitude of the postprandial response it is important to control for these when assessing reproducibility of the test meal. Gill and co-workers (2005) completed a well controlled study assessing the reproducibility of a test meal in seven men. Prior to each test meal, which was separated by a one week interval, male participants controlled their diet for 48 hours, abstained from alcohol for 24 hours and also refrained from physical activity. There was no significant difference in TAG responses after the two meals in men. This study would suggest that, for men, with adequate control of preceding lifestyle, the reproducibility of a test meal is high.

2.6 Assessment of CRP

Recent data are available that allow clinicians to interpret CRP concentrations either in terms of population-based quintiles or in terms of simple clinical cut-off points (Ridker, 2003). Although the former approach demonstrates the linear relationship between inflammation and vascular disease, the latter approach has greater clinical appeal. These clinical cut-off points representing low-, moderate- and high-risk groups for a future cardiovascular event correspond to CRP concentrations of <1 , 1 to 3 and $>3 \text{ mg}\cdot\text{L}^{-1}$ (Ridker, 2003; Pearson *et al.*, 2003). Traditional assays for CRP do not have adequate sensitivity to detect concentrations within all the clinical cut-off points and are unsuitable for the purpose of predicting coronary events in apparently healthy individuals. These routine automated assays for CRP quantification have limits of detection between $3\text{--}8 \text{ mg}\cdot\text{L}^{-1}$ (Roberts *et al.* 2001). Therefore, a number of high sensitivity CRP assays have been developed in order to help predict the risk of future coronary events in apparently healthy individuals (Roberts *et al.* 2001). These assays have improved sensitivity and precision at low concentrations of CRP and limits of detection are much lower ($<0.5 \text{ mg}\cdot\text{L}^{-1}$) than seen in traditional assays. The healthy young males tested in this thesis were free of any symptoms of cardiovascular disease and so a high sensitivity CRP assay was used to assess concentrations in the studies presented here.

2.7 Aerobic exercise and postprandial TAG metabolism

It has been recognised for some time that endurance trained individuals exhibit lower fasting TAG concentrations than control individuals who are untrained (Martin *et al.*, 1977; Lehtonen & Viikari, 1978; Vodak *et al.*, 1980; Marti *et al.*, 1991). Furthermore

there is evidence to show that these individuals have lower postprandial TAG responses and that this may be caused by improved TAG removal from the circulation (Cohen *et al.*, 1989; Merrill *et al.*, 1989; Sady *et al.*, 1988; Podl *et al.*, 1994). Whilst interesting these studies do not take into account natural selection biases and cannot, therefore, establish the direction of causality. Fundamentally, those individuals with genetic traits that may lead to an advantage in exercise, such as low fat mass, may be predisposed to do exercise. Therefore it may be these natural traits which lead to reduced TAG concentrations, and/or enhanced clearance, and not regular exercise.

Training studies, which help to establish the direction of causality, have shown a reduction in postprandial lipaemia post training (Altekruse & Wilmore, 1973; Zauner & Benson, 1977). However, Aldred colleagues (1995) failed to see a reduction in the postprandial TAG response after a twelve week walking programme in middle aged women when a test meal was given two days after the last exercise bout. This suggests that in the absence of an acute exercise bout postprandial lipaemia is not reduced. Indeed, the majority of evidence supports the fact that a single session of aerobic exercise can reduce postprandial lipaemia (Aldred *et al.*, 1994; Tsetsonis & Hardman, 1996a; Tsetsonis & Hardman, 1996b; Tsetsonis *et al.*, 1997; Gill *et al.*, 2003a). Moreover, several studies have shown that a period of detraining leads to a prompt elevation in postprandial lipaemia (Hardman *et al.*, 1998; Herd *et al.*, 1998; Gill *et al.*, 2003b). This suggests that exercise needs to be regular in order for it to be effective in reducing postprandial lipaemia. This is relevant from a public health standpoint, as current exercise recommendations are to perform exercise on most, if not all days of the week (US Department of Health and Human Services, 1996; Department of Health, 2004).

Two primary mechanisms have been proposed to explain the reduction in TAG after a bout of aerobic exercise. One is an increase in skeletal muscle lipoprotein lipase activity (Lithell *et al.*, 1979; Seip *et al.*, 1995; Seip *et al.*, 1997; Seip & Semenkovich, 1998; Gill *et al.*, 2003a). Lipoprotein lipase, which is located on the capillary endothelium of muscle, adipose, heart and other tissues, may enhance the uptake of TAG into the previously exercised muscle in response to the energy debt induced by a bout of exercise. The second is a reduced rate of endogenous VLDL secretion from the liver (Malkova *et al.*, 2000; Gill *et al.* 2001b). A reduction in circulating hepatic VLDL-TAG leaves less time for changes in structure to occur to circulating lipoproteins as there is reduced competition for hydrolysis by lipoprotein lipase into the tissues.

Both skeletal muscle lipoprotein lipase and VLDL can be measured directly from the blood or plasma. However, measurement of these is expensive and requires specialist techniques. Indications of lipoprotein lipase activity in skeletal muscle can be inferred from plasma insulin response as the activity of this enzyme is decreased by insulin (Kiens *et al.*, 1989; Richelsen *et al.*, 1993). Secretion of hepatic VLDL can be indicated from measurements of the ketone body 3-hydroxybutyrate in the plasma. In the liver non-esterified fatty acids (NEFA) can either be oxidised for energy or esterified into VLDL. During hepatic oxidation of fatty acids the metabolites 3-hydroxybutyrate and acetoacetate are released into the blood stream. An increased output of these ketone bodies would indicate an increase in hepatic NEFA oxidation and therefore a decrease in VLDL output. Previous researchers (Koutsari *et al.*, 2000;

Miyashita *et al.*, 2006) have used 3-hydroxybutyrate as a method of inferring VLDL secretion in postprandial studies.

The major determinant of the exercised induced reduction in postprandial TAG concentrations appears to be energy expenditure. Tsetsonis and Hardman (1996a) found that when subjects walked on a treadmill for 90 minutes at 31% or 61% of maximum oxygen uptake only walking at 61% reduced the area under the TAG concentration *versus* time curve in comparison with a control trial. In a second experiment, however, Tsetsonis and Hardman (1996b) matched the total energy expenditure of the exercise trials with subjects either walking for 90 minutes at 63% of maximum oxygen uptake, or 3-h at 32% of maximum oxygen uptake. The total area under the TAG concentration *versus* time curve at both exercise intensities were similar and were significantly lower than the control trial. Gill & co-workers (2002) provided further evidence to support this finding by comparing the TAG response between 1-h and 2-h walks at 50% of maximum oxygen uptake. In comparison to a control trial the 1-h walk reduced postprandial lipaemia by a mean of 9.3%, whereas the 2-h walk reduced it by 22.8%. This demonstrates that the beneficial effects of exercise on postprandial lipaemia are related to the duration and, therefore, the energy expenditure of the exercise session (Gill *et al.*, 2002). Petitt & Cureton (2003) examined the effect of prior exercise on postprandial lipaemia using meta-analytic methods to quantify effect sizes. They found no effect of exercise intensity, duration or timing of exercise on the postprandial response. However, they observed a significant relationship between effect size and energy expenditure suggesting this may play the dominant role in the magnitude of the postprandial response.

The energy expenditure of exercise may also influence which mechanism (increased skeletal muscle lipoprotein lipase activity or reduced hepatic VLDL output) is primarily responsible for a reduction in TAG after exercise. A number of studies provide support that changes in postprandial TAG responses may be related more to changes in VLDL-TAG output than lipoprotein lipase activity (Malkova *et al.*, 2000; Gill *et al.*, 2001; Gill *et al.*, 2003). However, Ferguson and colleagues (1998) compared the effect of four randomly assigned treadmill exercise sessions on lipoprotein lipase activity. On each session participants ran at 70% of maximal oxygen uptake until they had expended 3.4 MJ (800 kcal), 4.6 MJ (1100 kcal), 5.5 MJ (1300 kcal) or 6.3 MJ (1500 kcal). Compared with values 24 hours before exercise lipoprotein lipase activity was greater 24 hours post exercise only after the 4.6 MJ, 5.5 MJ and 6.3 MJ exercise sessions. This suggests that a minimum of 4.6 MJ needs to be expended through exercise to induce significant increases in lipoprotein lipase activity 24 hours post-exercise. Moreover, it suggests that this mechanism only makes a measurable contribution to the post exercise reduction in TAG only after exercise with high energy expenditure.

Exercise appears to have an effect on postprandial lipaemia which is over and above that brought on by energy deficit alone. Gill and Hardman (2000) reported that, in comparison with a control trial, an energy deficit incurred by exercise produced a 20% significant reduction in postprandial TAG concentrations compared with a 7% non-significant reduction for a similar energy deficit incurred by dietary restriction. Therefore, exercise appears to have an effect on postprandial TAG concentrations which is greater than an energy deficit incurred by diet alone. However, whether the greater reduction with exercise is from up-regulation of lipoprotein lipase activity in

muscle or reduced VLDL secretion was not determined. One note from this study is that technical difficulties produced a slightly greater energy deficit on the exercise trial (1.73 MJ) compared with the dietary restriction trial (1.44 MJ). This small difference seems unlikely, though, to account for the much larger reduction of TAG in response to the test meal the following day.

The timing of exercise in relation to ingestion of a meal may affect the reduction in postprandial lipaemia. Skeletal muscle lipoprotein lipase activity is thought to peak >8 hours post exercise (Seip *et al.*, 1997). Therefore, in the majority of studies investigating this topic, the test meal has been given to participants to correspond approximately with this time frame. However, a number of studies have examined the effect of feeding much earlier in the post exercise period (Zhang *et al.*, 1998; Murphy *et al.*, 2000; Katsanos *et al.*, 2004; Katsanos & Moffatt, 2004; Petridou *et al.*, 2004; Pfeiffer *et al.*, 2005). Most of these studies terminated exercise immediately, or at most an hour, prior to consumption of the test meal. The majority found a reduction in postprandial lipaemia in comparison to a control trial (Zhang *et al.*, 1998; Murphy *et al.*, 2000; Katsanos *et al.*, 2004; Katsanos & Moffatt, 2004). It needs to be noted, though, that Katsanos *et al.* only observed a reduction when moderate (65% peak oxygen consumption) and not low (25% peak oxygen consumption) intensity exercise was performed. This lends more weight to the fact that total energy expenditure is the prime determinant of the exercise induced reduction in postprandial lipaemia.

However, in two studies (Petridou *et al.*, 2004; Pfeiffer *et al.*, 2005), although a reduction in the total lipaemic response after exercise was found, compared to a control trial, this difference was not statistically significant. Petridou and colleagues

had participants cycle for 45 minutes at 62% of their maximal heart rate. A test meal of 35% fat content was given immediately afterwards and postprandial responses measured for 8 hours. The total TAG reduction on the exercise trial was 17% lower than on the control trial. Pfeiffer and co-workers had volunteers complete either 30, 60 or 90 minutes of treadmill walking at 50% of their maximum oxygen uptake. Participants were then fed two meals, served 3-h apart, the first of which was given immediately after exercise. Both meals fat content was 33% of total energy. The lack of a statistical difference observed in these two studies is probably because the test meal employed contained only a moderate fat content, as opposed to the higher fat load used in other studies (Zhang *et al.*, 1998; Katsanos *et al.*, 2004; Katsanos & Moffatt, 2004: all >60% fat total energy). Interestingly, though, Murphy and coworkers (2000) fed participants breakfast, lunch and an early evening meal of only moderate fat content (35% total energy from fat). Participants either exercised for half an hour before breakfast or ten minutes before each meal. Plasma TAG responses were observed for 11 hours after breakfast and the reduction in the lipaemic response was the same on both trials in comparison to the control trial. The reduction in TAG concentrations in this study became apparent only in the afternoon and early evening, more than five hours after breakfast. It seems likely that when test meals with only a moderate fat content are employed significant reductions in TAG will be observed much later in the day and furthermore, only when multiple meals are consumed which reinforce the pattern of TAG storage (Murphy *et al.* 2000).

Several studies have also examined the effect of exercising during the postprandial period (Schlierf *et al.*, 1987; Hardman & Aldred, 1995; Zhang *et al.*, 1998; Katsanos & Moffatt; 2004). The majority of these studies found a reduction in the total TAG

response (Schlierf *et al.*, 1987; Hardman & Aldred, 1995; Katsanos & Moffatt; 2004). Interestingly Zhang and colleagues did not observe any difference in TAG with postprandial exercise. Theoretically exercise in the postprandial period may effect gastric emptying, intestinal absorption or splanchnic blood flow (Hardman & Aldred, 1995). The exercise performed by participants in the study by Zhang and coworkers was of a higher intensity than that in the other studies cited and may have had a different effect on digestion. However, one observation of the study by Zhang and colleagues is that they make no mention of correcting for free glycerol in the TAG analysis in their study. When measuring TAG from plasma samples it is the glycerol portion of the TAG molecule that is measured in the analysis. However, after exercise, glycerol from adipose tissue breakdown remains higher for a short period than typically observed, as the energy supply to the working muscle is still high. Therefore, free glycerol should ideally be corrected for. The TAG peak on the postprandial exercise trial was observed at the end of exercise in the study by Zhang *et al.* which is when free glycerol is highest. A failure to account for this may result in a falsely high TAG area under the curve. One final note is despite differences observed in some of these studies, Petitt & Cureton (2003) in their meta-analytic study found no effect of timing of exercise on the postprandial response.

These observations of the effect of aerobic exercise on postprandial TAG concentrations provide a strong body of evidence on which to base studies of resistance exercise and TAG metabolism. However, the nature of resistance exercise is fundamentally different from aerobic exercise. The energy systems and fuel utilised, the amount of muscle fibre recruitment, the effect on mitochondria and capillaries are all examples of potential differences between the two types of exercise.

These and other differences all need to be considered when designing studies or drawing conclusions from studies on the effects of resistance exercise on postprandial TAG concentrations.

2.8 Resistance exercise and cardiovascular disease

There is good evidence from epidemiological studies to suggest that resistance exercise has the ability to reduce cardiovascular disease and in particular the incidence of coronary heart disease. Paffenbarger and colleagues (1975) examined work activity in relation to coronary mortality in 6,351 dock workers, aged 35 to 74 years upon entry, in the San Francisco Bay Area. These dock workers were followed for 22 years, or to death, or to the age of 75 years. Work activity was computed in terms of work-years according to categories of heavy (21.8 to 31.5 kJ per minute), moderate (10.1 to 21.0 kJ per minute) and light (6.3 to 8.4 kJ per minute) energy output and work assignments were reclassified to allow for the effect of job transfers. On average, jobs classified as heavy work required 7.9 MJ over basal output per eight hour work day, moderate 6.2 MJ and light 3.6 MJ. The adjusted relative risk of coronary heart disease for those longshoremen in the moderate and light work categories was 1.7 and 1.8 respectively compared to those longshoremen involved in heavy work (relative risk 1.0). Taking the sudden death rate from coronary heart disease for heavy working men as 1.0, the relative risk among those in moderate work was 3.5 and those in lighter work was 2.8. It should be noted that a full description of the activities involved in each of the three work categories is not provided by Paffenbarger and colleagues. However, they do state that, "The most active longshoremen worked in repeated bursts of peak effort rather than a steady lower pace of energy output." Repeated bouts of short effort also characterise resistance exercise,

although, the work performed by the longshoremen was likely more a combination of aerobic and resistance activity than resistance exercise per se. Whether the reduction in coronary heart disease was because of the total energy expended, the intensity of the work or both in the Paffenbarger *et al.* study is difficult to conclude. Paffenbarger and colleagues do suggest a critical-threshold concept though, where peak energy output may be more meaningful than overall energy output at a lesser intensity of effort.

Further evidence for a role of resistance exercise in the prevention of coronary heart disease is provided by Tanasescu and colleagues (2002). Tanasescu *et al.* followed a cohort of 44,452 male health professionals in the United States for 12 years. Those men who performed resistance training for less than 30 minutes or for more than 30 minutes per week had a relative risk for coronary heart disease of 0.83 and 0.65 compared with those men who did no resistance training. Moreover, in multivariate analyses that also controlled for other types of physical activity those health professionals who trained with weights for more than 30 minutes each week had a 23% reduction in risk for coronary heart disease. These findings are interesting as 30 or even 60 minutes a week is a relatively short period given the intermittent nature of resistance exercise. Moreover, in the same cohort of subjects, men who ran for between for 30 to 60 minutes a week demonstrated a 21% reduction in risk for coronary heart disease. This suggests that short bouts of high intensity resistance exercise may confer the same protection against coronary heart disease as longer, more sustained exercise which has higher total energy expenditure.

Jurca and co-workers (2004) tested 8,570 men in the United States for cardiorespiratory fitness and muscular strength. Cardiorespiratory fitness was quantified by an age-specific maximal treadmill exercise test time. Age specific muscular strength was computed by combining the body weight adjusted one repetition maximum measures for leg press and bench press. After adjustment for age and smoking, an inverse association was seen between metabolic syndrome (characterised by abdominal obesity and disorders of lipid and carbohydrate metabolism) prevalence and quartiles of muscular strength, which remained even after adjustment for maximal treadmill time. Furthermore, significant inverse associations were found between quartiles of muscular strength and the prevalence of the metabolic syndrome amongst men with low and moderate cardiorespiratory fitness. This association did not hold for men with high cardiorespiratory fitness.

In summary these epidemiological findings highlight that resistance exercise has the potential to reduce coronary heart disease risk. The total energy expenditure and intensity of exercise needed for a protective effect still needs to be clarified. Furthermore, these findings do not provide information on the ability of resistance exercise to modify individual cardiovascular disease risk factors. The findings do, however, provide a base for which randomised control trials that establish causality can be conducted.

2.9 Resistance exercise and fasting TAG

A number of studies have found a reduction in fasting TAG concentrations with weight training (Goldberg *et al.*, 1984; Behall *et al.*, 2003). In an early study Goldberg and co-workers (1984) showed a 28.3% lowering of fasting TAG after 16

weeks of training in women. However, despite favourable changes to HDL-cholesterol no reduction in fasting TAG was observed in men over the same time period. In a more recent study Behall and colleagues (2003) showed significant reductions in fasting TAG in both pre- and post-menopausal women after 12 weeks of resistance training. Conversely, other studies (Prabhakaran *et al.*, 1999; LeMura *et al.*, 2000; Elliot *et al.*, 2002; Banz *et al.*, 2003) found no changes in fasting TAG after 10, 14, 16 and 8 weeks of resistance training respectively. Interestingly, most of these studies examined women. Differences in whether the women were pre- or post-menopausal, or which stage of the menstrual cycle measurements were made could account for some of the different findings between studies. The volume, frequency and duration of training completed present further difficulties when assessing the evidence.

2.10 Resistance exercise and postprandial TAG

With the exception of some of the work presented in this thesis only two published studies to date have investigated the effect of resistance exercise on postprandial TAG concentrations (Pettit *et al.*, 2003; Shannon *et al.*, 2005). Pettit and coworkers compared the effect of a single bout of resistance exercise with aerobic exercise and a non-exercise control trial on postprandial TAG concentrations in ten men and four women. All participants were resistance trained. The resistance exercise consisted of an 88 minute bout of weight training; three sets of 10 repetitions of 10 exercises performed at participants' 10 repetition maximum. There were 2 minutes between each set and exercise. The aerobic exercise bout consisted of walking for the same duration as the resistance exercise at an intensity designed to elicit the same energy expenditure as the resistance exercise. Exercise was performed 15 hours (the day

before) prior to consumption of a high fat test meal. Mean gross energy expenditure of the exercise bouts was estimated at 1.7 MJ. Serum TAG concentrations were significantly lower at baseline and for the first three hours postprandially on the resistance exercise trial compared with the aerobic exercise and control trial. The total lipaemic response adjusted for baseline differences was also lower on the resistance exercise trial compared with the aerobic exercise and control trials. There was no significant difference in TAG concentrations after the aerobic exercise bout compared with the control trial.

The results of the study by Petitt and colleagues are surprising. The studies by Tsetsonis and Hardman (1996a; 1996b) suggest that the total energy expenditure of exercise is the primary determinant of an exercise induced reduction in postprandial TAG concentrations. Therefore, one might have expected that the reduction in TAG concentrations, in the study by Petitt and coworkers, would be similar after the two exercise bouts, regardless of the mode of exercise. Petitt and colleagues consider this. They state that the energy expenditure of exercise in their study is at the low end of that typically seen in studies of aerobic exercise (mean 3.4 MJ; range 1.6-7.2 MJ) which have found a reduction in postprandial TAG concentrations. They suggest that although TAG reductions after aerobic exercise may be related to the energy expenditure of the exercise, with resistance exercise this may not be the case. Instead, possibly some other factor linked to strenuous muscle contraction associated with weight lifting may be the cause of the reduction. Among these factors they suggest a greater excess post-exercise oxygen consumption with resistance exercise and a greater increase in skeletal muscle lipoprotein lipase activity.

The study by Shannon and colleagues (2005) determined whether prior resistance exercise exhibited a dose response effect on postprandial TAG concentrations while controlling for energy balance. Four men and six women, all resistance trained, participated in four different randomised treatments the day before a high fat test meal. These treatments were either a resistance exercise session consisting of one, three or five sets of eight different resistance exercises or a non-exercise control day. Resistance exercise was performed in the early evening. Each set of resistance exercises consisted of 10 repetitions performed at 75% of participants one repetition maximum, with 60 seconds rest between exercises and sets. Each participant was given a standardised post-exercise meal within 60 minutes of completion of exercise. The energy content of the post-exercise meal was calculated as 33% of 24-hour energy expenditure plus the energy cost of the resistance exercise. A high fat test meal was then consumed after an overnight fast approximately 13 hours post-exercise. No differences were observed in fasting or postprandial TAG concentrations between treatments.

These findings of Shannon conflict with those of Petitt and colleagues. One major difference between these two studies is in the nature of the post-exercise food consumption. Petitt and colleagues had participants record their diet on the day before their first test meal and then repeat their diet the day before successive test meals. This approach to dietary control can be criticised as a participant completing an exercise trial first would be in energy surplus on the control trial whereas a participant completing the control trial first would be in energy deficit on the exercise trial. In contrast Shannon and colleagues made sure there was no difference in energy stores post-exercise by controlling for the energy cost of the resistance exercise in the post-

exercise meal. Certainly, the findings of these two studies present an unclear picture of the effect of resistance exercise on postprandial TAG concentrations.

The approach by Shannon and colleagues of negating the energy deficit of exercise, by controlling for the energy cost of the weights sessions in the post-exercise meal, failed to reduce postprandial TAG concentrations. This is perhaps surprising if, as suggested by Gill & Hardman (2000), exercise reduces TAG concentrations through mechanisms over and above an energy deficit created by diet alone. Certainly the estimated energy expenditure in the three (1.72 MJ) and five set trials (2.58 MJ) in the study by Shannon *et al.* was similar to, or greater than, the energy expenditure of the walking (1.73 MJ) in the Gill & Hardman study. The different methodologies between the two studies may explain the different findings. Gill & Hardman produced an energy deficit by exercise in one trial and compared its effects on postprandial TAG concentrations with an energy deficit produced by diet alone in another trial. Shannon and colleagues however produced an energy deficit through exercise and then negated this deficit by feeding a post-exercise meal equivalent in energy to the bout of exercise. An interesting future study may be to repeat the Gill & Hardman study and add a fourth trial where participants are re-fed the energy deficit of exercise. It should be noted that one problem with these comparisons are that different modes of exercise were employed in the two studies. As the evidence for resistance exercise on postprandial TAG concentrations is unclear the study by Shannon makes it difficult to make comparisons and draw conclusions.

2.11 Physical activity and CRP

The effect of physical activity on CRP will not be extensively reviewed in this thesis as the majority of studies have examined aerobic exercise. Instead the reader is directed to a recent review paper by Kasapis & Thompson (2005). However, some of the observations of the effect of aerobic exercise on CRP may be relevant when considering resistance exercise. As noted in the introduction to the present work the majority of cross-sectional studies have noted an inverse relationship between physical activity or physical fitness and serum CRP (Dufaux *et al.*, 1984; Albert *et al.*, 2004; Aronson *et al.*, 2004; Katja *et al.*, 2005; LaMonte *et al.*, 2005). Training studies have failed to agree on the effect of aerobic exercise on CRP levels after training (Liesen *et al.* 1977; Tisi *et al.*, 1997; Smith *et al.*, 1999; Mattusch *et al.*, 2000; Marcell *et al.*, 2005; Hammett *et al.*, 2004; Hammett *et al.*, 2006). The failure to randomise participants, or include a control group in some of these prospective studies (Mattusch *et al.*, 2000; Smith *et al.*, 1999) makes it difficult to draw conclusions.

Several studies have demonstrated that acute strenuous exercise produces a transient increase in CRP levels (Taylor *et al.*, 1987; Weight *et al.*, 1991; Fallon *et al.*, 2001; Siegel *et al.*, 2001). However, a single bout of moderate exercise has been shown to have no effect on CRP (Murtagh *et al.*, 2005). These studies suggest that the increase in CRP post-exercise seems to be proportional to the volume/intensity of activity and the extent of muscle injury (Strachan *et al.*, 1984). One possible mechanism for the post-exercise increase in CRP with acute exercise, is the interleukin-6 increase that occurs during exercise (Febbraio & Pedersen, 2002). Interleukin-6 is the main stimulant for hepatic production of CRP and increases up to 100-fold during and after

strenuous exercise. The observation that interleukin-6 increases greatly after strenuous aerobic exercise, and thus increases CRP production, may be relevant when considering differences between acute studies of resistance exercise and CRP.

2.12 Resistance exercise and CRP

To date, only one study has examined the effect of resistance training on CRP concentrations (Castaneda *et al.*, 2004). Castaneda and colleagues in a randomised, controlled trial examined the effect of 12 weeks resistance training in combination with a low-protein diet on CRP in adults with chronic kidney disease but not on dialysis therapy. The control group were patients with chronic kidney disease on a low-protein diet only. They observed a significant reduction in serum CRP concentrations after 12 weeks in the group performing resistance training compared to controls. In addition interleukin-6 was significantly reduced in the resistance training group.

Two studies have examined the effect of a single session of resistance exercise on CRP concentrations (Nosaka & Clarkson, 1996; Paulsen *et al.*, 2005). Both studies employed maximal eccentric contractions in isolated muscle groups to examine inflammatory markers after exercise. Nosaka and Clarkson recruited 14 male participants who had not previously been involved in resistance training. Each participant performed a single bout of 24 maximal eccentric actions of the elbow flexors in their non-dominant arm on a modified arm curl machine. Each repetition lasted three seconds with 15 seconds recovery. No change from baseline values was observed in plasma CRP over five days post-exercise, despite up to 100-fold increases in creatine kinase, a post exercise marker of muscle damage, over this period.

Paulsen and coworkers recruited 11 male volunteers, none of whom had previously been involved in resistance training, to a study where each participant performed 300 maximal eccentric contractions of the quadriceps muscle. The exercise was randomly divided between the dominant and non-dominant leg. Serum CRP concentration peaked 47 hours post-exercise and normalised 1 week post-exercise. Creatine kinase peaked 95 hours after exercise and loss of force-generating capacity correlated with peak concentrations of both CRP and creatine kinase. It is possible that differences in the volume of exercise performed and the muscle mass involved may explain the different findings between the Nosaka & Clarkson and Paulsen *et al.* studies.

It is interesting to note that in the Nosaka & Clarkson study CRP was unchanged despite a large increase in creatine kinase and that in the Paulsen *et al.* CRP and creatine kinase showed a different time course to peak concentrations. It has previously been suggested that systemic levels of inflammatory parameters are related to exercise induced muscle damage (Bruunsgard *et al.*, 1997) – at least for interleukin-6 and creatine kinase. However, other studies have not confirmed an association between interleukin-6 and creatine kinase (Ostrowski *et al.*, 1998; Ostrowski *et al.*, 1999; Croisier *et al.*, 1999). Moreover, an interleukin-6 response is also observed during concentric exercise without any signs of muscle damage (Steensberg *et al.*, 2001). As CRP is primarily regulated by interleukin-6 it seems likely that muscle damage is not the main cause for the rise in CRP post-exercise. Indeed, recently it has been postulated that intra-muscular signalling stimulates the immediate increase in plasma interleukin-6 in response to exercise, independently of muscle damage (Febbraio & Pedersen, 2002). In addition, muscle damage per se

elicits repair mechanisms in the muscle leading to interleukin-6 production, which occurs later and is of a smaller magnitude than the interleukin-6 production related to muscle contractions. The difference in injury versus contraction-induced interleukin-6 may explain the observation that the interleukin-6 response is more pronounced, occurs earlier, and is shorter in duration after concentric compared with eccentric muscle contractions. Alongside muscle contractions, the interleukin-6 response is directly related to exercise intensity, duration and the amount of muscle mass recruited during exercise.

These observations may partly explain some of the differences between the Nosaka & Clarkson and Paulsen *et al.* studies. The muscle mass employed and duration/volume of exercise in the Nosaka & Clarkson study were smaller than in the Paulsen study. This may have failed to increase, or led to a smaller increase, in interleukin-6 and therefore had minimal impact on CRP post-exercise. Also the smaller number of muscle contractions performed in the Nosaka & Clarkson study may have failed to elicit a contraction-induced interleukin-6 response and therefore failed to elevate CRP. These observations may also explain why an increase in CRP is observed in more strenuous but not moderate aerobic activities. As noted earlier the rise in CRP is proportional to the volume/intensity of activity and the extent of muscle injury. Perhaps contractile-induced interleukin-6 concentrations need to reach a set point to stimulate a significant increase in CRP concentrations, regardless of the type (concentric/eccentric) of exercise.

2.13 Summary

The literature presented in this chapter suggests that resistance exercise has the potential to decrease cardiovascular disease risk and in particular coronary heart disease. One mechanism for this may be a decrease in postprandial TAG concentrations but the evidence for this is currently limited. A programme of prolonged resistance training may also modify cardiovascular disease risk by lowering CRP concentrations. The evidence for this is again limited. However, an acute bout of resistance exercise has the potential to lead to a transient increase in CRP concentrations. The studies presented in the remainder of this thesis will examine some of these questions.

Chapter 3

General Methods

3.1 Participants

All studies in this thesis were conducted with the approval of Loughborough University's Ethical Advisory Committee (see Appendix A). Participants gave written informed consent (Appendix B) after receiving an explanation of the nature and purpose of the studies and the procedures and risks involved. All participants were required to complete a health screen questionnaire (Appendix C) and were recruited only if they met the following criteria:

- a) non-smoking
- b) no known history of cardiovascular disease
- c) aged ≥ 18 and ≤ 40
- d) body mass index (BMI) $< 30 \text{ kg}\cdot\text{m}^{-2}$
- e) resting arterial blood pressure $< 140/90 \text{ mm Hg}$
- f) not taking any medication known to affect lipid or carbohydrate metabolism
- g) free from any injury (acute or chronic)

In addition each time participants reported to the laboratory they were required to complete a fitness to participate (Appendix D) form to check that they were healthy and free from illness before undertaking any trials.

3.2 Resting arterial blood pressure

Blood pressure was measured during screening using a sphygmomanometer (Hawksley, England). Participants remained in a seated position for 5 min before measurement.

3.3 Anthropometry

Height was measured using a Holtain fixed wall stadiometer (Seca, Germany). Participants were barefoot with the head held in the Frankfort plane. Height was measured to the nearest 0.1 cm. Body mass was determined using a beam balance (Avery Industrial Ltd., Leicester, U.K.) to the nearest 0.05 kg. Subjects were barefoot and wearing light clothing. Participants BMI was calculated as weight (kg) divided by the square of the height (m). Waist circumference was measured at the narrowest part of the torso - above the umbilicus and below the xiphoid process (American College of Sports Medicine, 2000).

Measurements of subcutaneous fat were made to estimate total body fatness using skinfold callipers (Holtain Ltd., Crymmych, U.K.). Skinfold thickness was measured at four sites (biceps, triceps, subscapular, suprailiac) and body density calculated using the prediction equations of Durnin & Womersley (1974). Body fat percentage was then estimated using the Siri equation (1956). All measures were made in duplicate on the right side of the body with the participant standing. If duplicate measurements were not within 1 to 2 mm skinfold at the site was measured again. Measurements were made by rotating through the test sites to allow the skin time to regain normal texture and thickness (American College of Sports Medicine, 2005).

3.4 Repetition maximum tests

For each of the resistance exercises employed within a study maximal lifting values were determined for each participant. This was either a 1-, 10- or 12-repetition maximal value (please see individual studies). Maximum values were determined by trial and error by adding/removing weights after each attempt as required. Participants

were allowed to take as long as they felt necessary to recover from each attempt. The order in which the maximum lifts were performed was the same for each participant and followed the order the exercises would be performed in on the trial days.

3.5 Control of diet and exercise

On all studies, for two days preceding main trials participants were asked to refrain from physical activity. Only gentle walking for personal transportation over short distances was permitted. Participants weighed and recorded all food and drink consumed during the 48 h immediately preceding their first trial and they undertook to replicate this intake during the 48 h prior to their second trial. Participants also refrained from alcohol during these periods. For all main trials participants were instructed to come to the laboratory after a 12 h overnight fast. They were instructed to drink water both at night and in the morning to prevent dehydration.

3.6 Measurement of expired air

Expired air samples were collected during exercise into 150 or 200 litre Douglas bags (Plysu Protection Systems, Milton Keynes, U.K.). Participants wore a noseclip and breathed through a mouthpiece (Harvard Apparatus, Edenbridge, U.K.) fitted to a lightweight one-way respiratory valve (Jakeman & Davies, 1979). This was connected to a 1.5 m length of 30 mm bore lightweight tubing (Falconia flexible ducting, Baxter, Woodhouse & Taylor Ltd., Macclesfield, U.K.). The tubing terminated at a two-way valve which opened and closed the Douglas bag.

Resting samples of expired air were always taken fasted, first thing in the morning before cannulation for a total of 6 min. During exercise samples were collected for the

full duration of the lifting and recovery period (see specific chapter). Rate of oxygen uptake ($\dot{V}O_2$) and rate of carbon dioxide production ($\dot{V}CO_2$) were determined from expired air samples using a paramagnetic oxygen analyzer and an infra-red carbon dioxide analyzer respectively (Series 1400; Servomex, Crowborough, East Sussex, U.K.). The analysers were calibrated with certified reference gases (Air Products, Plc, Crewe, U.K.) of known concentration – nitrogen (premier grade $99.99\% \pm 0.00\%$); oxygen ($16.01\% \pm 0.2\%$), carbon dioxide ($4.00\% \pm 2\%$) and nitrogen mix ($79.99\% \pm 0.2\%$) (special mixture). Expired gas volumes were measured using a dry gas meter (Harvard Apparatus, Edenbridge, Kent, U.K.). Temperature of the expired air was measured during evacuation using a thermistor (Edale, type 2984, Model C, Cambridge, UK). Barometric pressure was measured using a Fortin barometer (F.D. and Company, Watford, U.K.). Expired gas volumes were corrected to standard temperature and pressure (dry).

3.7 Estimation of energy expenditure during weight lifting

The short duration, intermittent nature of weight lifting invalidates the typical assumptions of indirect calorimetry because the respiratory exchange ratio is equal to or greater than 1.0. Therefore, energy expenditure was calculated as being 5.047 kcal (21.1 kJ) per litre of oxygen (McArdle, Katch & Katch, 1991). This reflects the assumption that energy was derived from carbohydrate rather than fat oxidation and assumes no protein contribution to energy provision during the exercise. This assumption may not be entirely valid, in which case calculated energy expenditure estimations may be on the high side. Conversely, no attempt was made to quantify the energy contribution from anaerobic sources and this would lead to underestimations of the energy expended during exercise.

3.8 The test meal

Two test meals were employed in these studies. Both meals were mixed but contained a high percentage of energy from fat. The meal given in Study 1 (Chapter 4) was a modification of that used by Schlierf *et al.* (1987) and was identical to that used in the reproducibility study by Gill *et al.* (2005), with the addition of sugar. Sugar was added to the test meal as carbohydrates exaggerate postprandial TAG metabolism (Parks, 2001). With tight control of previous diet, exercise and alcohol intake, the reproducibility of postprandial lipaemic responses within males to this test meal is high (Pearson product-moment correlation of 0.96, within subject co-efficient of variation 10.1%; both for TAG) (Gill *et al.*, 2005). Ingredients of the test meal used in Study 1 are given in Table 3.1. The macronutrient composition and energy content of the meal in Study 1 is given in Table 3.3.

The reproducibility of the test meal used in Studies 2 to 5 (Chapters 5-8) has not been examined. A number of participants in Study 1 (Chapter 4) reported difficulty with consumption of the test meal. Therefore, the meal was changed to make it more palatable to participants. The ingredients of the test meal used in Studies 2 to 5 is given in Table 3.2. The macronutrient composition and energy content of the meal are given in Table 3.3. The test meal in Studies 2 to 5 contains less total fat and less energy from fat than that used in Study 1. However, as noted above, the addition of extra carbohydrate to this meal should exaggerate the fasting and postprandial lipaemic response (Parks, 2001).

Table 3.1. Ingredients of the test meal used in Study 1 (Chapter 4), administered per kg body mass.

Ingredient	Quantity (per kg body mass)	Quantity for a 70 kg subject
Whipping cream (g)	2.53	177
Bananas (g)	1.14	79
Apples (g)	0.67	47
Chocolate – milk (g)	0.13	9
Sultanas (g)	0.13	9
Brazil nuts (g)	0.15	10
Oatmeal – raw (g)	0.75	53
Coconut – desiccated (g)	0.07	5
Sugar, white (g)	0.07	5

Table 3.2. Ingredients of the test meal used in Studies 2-5 (Chapters 5-8), administered per kg body mass.

Ingredient	Quantity (per kg body mass)	Quantity for a 70 kg subject
White bread (g)	1.43	100
Cheddar cheese (g)	0.61	43
Butter (g)	0.22	16
Potato crisps (g)	0.56	39
Whole milk (g)	2.77	194
Mayonnaise (g)	0.22	16
Milkshake powder (g)	0.13	9

Table 3.3. Macronutrient content of the two test meals used in this thesis.

	Study 1 (Chapter 4)	Studies 2-5 (Chapters 5-8)
Energy (kJ)	68	60
Energy (kcal)	16	14
Fat (% energy)	69	56
Carbohydrate (% energy)	26	33
Protein (% energy)	5	11
Fat (g)	1.25	0.89
Carbohydrate (g)	1.13	1.23
Protein (g)	0.19	0.40

3.9 Blood sampling

Blood samples were collected into pre-cooled 9-mL potassium-EDTA monovettes (Sarstedt, Leicester, UK) and kept on ice until centrifugation (within 10 minutes of collection). Blood samples for the collection of insulin and glucose only were collected into 4.9-mL potassium-EDTA monovettes (Sarstedt, Leicester, UK). Samples were centrifuged for 10 minutes at 4000 rpm at 4°C (Beckman CS-15 R, High Wycombe, U.K.). Plasma was divided into aliquots, and stored at -80°C. Blood samples collected into serum monovettes (Sarstedt, Leicester, UK) were allowed to clot for one hour at room temperature before centrifugation. Serum samples were taken and left to clot for 60 minutes before being centrifuged (Beckman CS-15 R, High Wycombe, U.K.) and stored in the same way as plasma. All samples were analysed within six months of collection.

3.10 Analytical methods

Haemoglobin concentration and haematocrit were determined in samples so that changes in plasma volume could be estimated (Dill & Costill, 1974). Haemoglobin concentration was determined on a spectrophotometer (Cecil CE 1011, Cambridge, England) using duplicate aliquots of 20 µl EDTA blood by a cyanmethaemoglobin method. Haematocrit was measured in triplicate from aliquots of EDTA blood dispensed into heparinised micro-haematocrit tubes which were centrifuged for 15 minutes (Mikro 20, Hettich, Germany). Haematocrit values were determined using a micro-haematocrit reader (Hawksley, Lancing, U.K.).

Plasma TAG, myoglobin, glucose, 3-hydroxybutyrate (Randox Laboratories Ltd. U.K.) and NEFA (Wako Chemicals GmbH, Germany) were analysed by enzymatic, colorimetric methods with the use of a centrifugal analyser (Cobas-Mira Plus; Roche, U.K.).

Plasma insulin concentration was determined using a solid-phase ¹²⁵Iodine radioimmunoassay available in a commercial kit (MP Biomedicals, Orangeburg, NY, U.S.). Radioactivity was measured using an automated gamma counting system (Cobra II, Packard Instrument, Downers Grove, IL, U.S.).

Measurement of serum CRP was made using a high sensitivity ELISA assay (DRG International, Inc., USA) and measured on a plate reader (Opsys Microplate Reader, Dynex Technologies Inc., U.S.A.).

Accuracy and precision of the assays described were monitored using quality control sera (Randox Lipid Controls, Level II and III, Randox Laboratories, U.K.; Randox Multi-Sera, Randox Laboratories, U.K.; Seronorm Lipid, Sero AS, Billingstad, Norway; RIA Control Serum Set III, MP Biomedicals, Orangeburg, NY, U.S.). From repeated measurements made in the lab by the author of this work the following within batch coefficients of variation for the assays were calculated:

TAG 1.8%

Myoglobin 2.4%

Glucose 0.9%

3-hydroxybutyrate 2.7%

NEFA 0.8%

Insulin 5.7%

CRP 10.7%

To eliminate inter-assay variation, all samples from each participant were always analysed in the same run.

3.11 Data analysis

Results were analysed using statistical software (SPSS 11.0, SPSS Inc., Chicago, IL, U.S.). Total area under the plasma concentration *versus* time curve was calculated using the trapezium rule. The incremental area under the plasma concentration *versus* time curve was calculated using the same method after correcting for baseline concentrations. A 5% level of significance was adopted throughout, and data are expressed as mean \pm standard error (SE) unless stated.

Chapter 4

The effect of a single session of resistance exercise on postprandial lipaemia

4.1 Introduction

Increased postprandial TAG concentrations may promote atherosclerosis due to: a) an accumulation of TAG-rich lipoprotein remnants in the plasma; b) catabolism of HDL; and c) formation of small, dense LDL which have an increased susceptibility to oxidation (Cohn, 1998; Karpe & Hamsten, 1995). Interventions that reduce postprandial TAG concentrations may, therefore, reduce the development of atherosclerosis.

There is good evidence to show that a single bout of aerobic exercise reduces postprandial lipaemia (Aldred *et al.*, 1994; Tsetsonis & Hardman, 1996a; Tsetsonis & Hardman, 1996b; Tsetsonis *et al.*, 1997; Gill *et al.*, 2003). This reduction is transient, however, since detraining leads to a prompt elevation in postprandial lipaemia (Hardman *et al.*, 1998; Herd *et al.*, 1998; Gill *et al.*, 2003). Thus, exercise must be performed frequently for continued benefit. Furthermore, energy expenditure during exercise appears to be the primary determinant of the exercise-induced reduction in postprandial lipaemia (Tsetsonis & Hardman, 1996b; Gill *et al.*, 2002; Petitt & Cureton, 2003).

Although many studies have addressed the effects of aerobic exercise on postprandial lipaemia, only two studies have examined the influence of resistance exercise on postprandial lipaemia (Petitt *et al.*, 2003; Shannon *et al.*, 2005). Petitt and coworkers found that postprandial lipaemia was decreased following resistance exercise in

comparison to a control trial. This is perhaps surprising as the estimated energy expenditure during the resistance exercise was only 1.7 MJ. This is at the lower end of the range of values reported in studies of aerobic exercise and postprandial lipaemia (1.5 to 7.2 MJ) (Petitt & Cureton, 2003). Conversely, Shannon and colleagues failed to show any dose response effect of resistance exercise on postprandial lipaemia with differing volumes of resistance exercise. This occurred despite an estimated mean energy expenditure of 1.72 and 2.58 MJ in two of the exercise conditions in their trial.

Therefore, the first study presented in this thesis attempts to clarify the influence of resistance exercise on postprandial lipaemia in a group of young adult males. The individuals in the present study unlike those in the two previous studies in this area were untrained. Aerobic exercise has been shown to effectively reduce postprandial lipaemia in both trained and untrained individuals after a single bout of exercise (Tsetsonis et al., 1997). The weight lifting exercise used in the present study is based on current exercise recommendations for resistance training (Kraemer *et al.*, 2002; Kraemer *et al.*, 2004). Furthermore, the session was designed to maximise the energy expenditure of the resistance exercise to increase the likelihood of seeing a reduction in the lipaemic response post-exercise. Specifically, we sought to evaluate the hypothesis that a single session of resistance exercise would lower postprandial lipaemia.

4.2 Methods

4.2.1 Participants

Eleven male volunteers aged 19-33 years participated in the study. Some physical characteristics of the participants are shown in Table 4.1. Most participants reported that they were involved in some form of recreational physical activity but none of them performed resistance exercise on a regular basis.

Table 4.1. Physical characteristics of the participants.

Age (y)	23.5 ± 4.6
Height (m)	1.80 ± 0.05
Body Mass (kg)	84.3 ± 9.1
BMI (kg·m ⁻²)	25.9 ± 2.4
Body fat (%)	14.4 ± 5.2

Values are mean ± standard deviation (SD), *n* = 11.

4.2.2 Study design

Prior to the main trials participants visited the laboratory twice. During the first visit anthropometric data were collected and participants performed 10-repetition maximum tests for each of the 11 resistance exercises employed in the study. The order in which the 10-repetition maximum tests were performed was the same for each participant: dead lift, bench press, upright row, squat, shoulder press, bent-over row, lunges, barbell pullover, bicep curls, triceps press and sit ups. On the second visit participants completed a practice

weight-lifting session identical to the one to be used in the main trials. The purpose of this practice session was to ensure that each participant was able to complete the entire exercise session and also to confirm that the weights lifted were producing fatigue from overload by the end of the session. This was confirmed by visual inspection and by verbal feedback from participants.

Following the preliminary visits participants completed two main trials - exercise and control. The order of the trials was randomised and balanced. The interval between each main trial was at least one-week. Participants recorded their diet during the 48 hours preceding their first trial. They then replicated this for the 48 hours prior to their second trial. Participants were also asked to refrain from physical activity 48 hours preceding each main trial (Chapter 3 Pg.37). On both main trials participants consumed a test meal. The first main trial was performed a minimum of one-week after the practice weight lifting-session. The afternoon prior to one test meal participants performed an 88 minute bout of resistance exercise starting at approximately 16.00 h (exercise trial). The afternoon prior to the other test meal participants rested (control trial) (Figure 4.1).

4.2.3 Weight lifting protocol

Each participant performed four sets of 10 repetitions of 11 different weight-lifting exercises at 80% of 10-repetition maximum. Four sets of each exercise were employed in order to maximise the total energy expenditure of the session and hence the likelihood of reducing postprandial lipaemia. A training intensity of 80% of 10-repetition maximum was chosen as a compromise between muscular strength and endurance. Pilot work

revealed that a higher training intensity would have prevented most participants from completing four sets of each exercise and thus would have lowered the total energy expenditure. Participants were given 2 min in which to complete each set. On completion of the 10 repetitions participants rested for the remainder of the 2 min. Therefore, the whole exercise session lasted 88 min (11 exercises \times 4 sets \times 2 min). The order of the exercises was uniform for all participants and matched the order used in the preliminary testing. All sets for one exercise were completed before moving on to the next exercise. The session was structured to avoid local muscular fatigue from exercising similar muscle groups consecutively. It was also designed so that large muscle groups and exercises involving multiple joints were exercised first. Standard free weights were used, including dumbbells, bent bar, an Olympic bar and a mobile bench. Water intake was permitted *ad libitum* throughout the resistance exercise session.

4.2.4 Estimation of energy expenditure during weight lifting

Expired air samples were collected into Douglas bags (Plysu Protection Systems, Milton Keynes, U.K.) during the third set of each weight lifting exercise (pilot work revealed that there was no systematic variation in oxygen consumption values measured during the first, second, third and fourth set of each exercise). Samples were collected for the full duration of the lifting and recovery period i.e. 2 min. Energy expenditure was calculated as being 5.047 kcal (21.1 kJ) per litre of oxygen (McArdle, Katch & Katch, 1991) (see General Methods - Chapter 3, Pg.38).

4.2.5 Test meals

Participants reported to the laboratory at 08.00 h after a 12 h fast. Participants rested quietly, lying supine on a couch, for 10 min before a resting expired air sample was taken. A cannula was inserted into a forearm or antecubital vein and the participant rested quietly for 10 min before a baseline blood sample was obtained. The test meal was then consumed. A clock was started with the first bite of this test meal. Further blood samples were obtained at 0.5, 0.75 and 1 h after the start of the meal and then hourly for a total of 6 h. The cannula was kept patent by flushing with nonheparinised saline ($9 \text{ g}\cdot\text{L}^{-1}$, B.Braun Medical Ltd., Buckinghamshire, UK). The first 2 mL of blood withdrawn was always discarded to avoid dilution of the sample. Every participant consumed all of the prescribed meal. Only water was consumed during the 6 h postprandial observation period. Water was available *ad libitum* during the first trial; the volume ingested was recorded and replicated in the second trial. Participants rested (reading, working quietly, watching television) throughout each observation period and were always lying in a supine position for at least 5 min before blood samples were taken. A schematic representation of the main trial protocol is given in Figure 4.1.

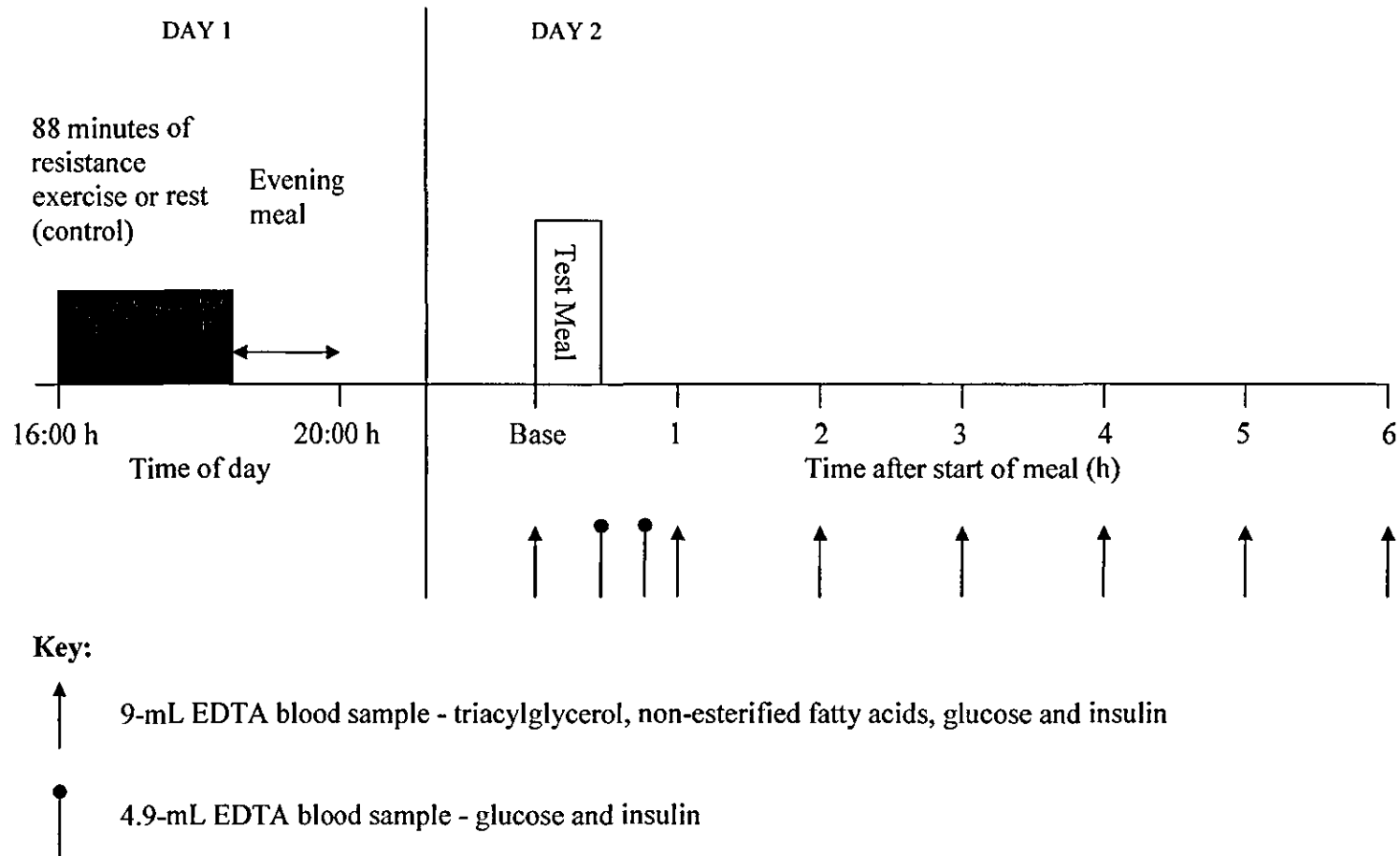
4.2.6 Haematocrit and haemoglobin

Haematocrit and haemoglobin concentration were determined in samples collected at baseline and 6 h to estimate changes in plasma volume (Dill & Costill, 1974) (see General Methods - Chapter 3, Pg.42).

4.2.7 Data analysis

Total and incremental postprandial responses for TAG, NEFA, glucose and insulin were calculated using the six hour areas under the plasma concentration *versus* time curves. Fasting, area under the curve values, peak TAG concentration and time to peak TAG concentration were compared between trials using Student's t-tests for correlated means. Two-way analysis of variance (ANOVA) (repeated measures) was used to determine differences between trials and over time for fasting and postprandial plasma concentrations of TAG, NEFA, glucose and insulin. Where appropriate, post-hoc pair wise comparisons were made using the Bonferroni method. Relationships between variables were evaluated using Pearson's product-moment correlation coefficient.

Figure 4.1. Schematic representation of the main trial protocol.



4.3 Results

4.3.1 Responses during weight lifting

The mean weight lifted during the 88 minute resistance exercise session was 14,214 ± 613 kg. The gross energy expenditure from the exercise was estimated to be 2.3 ± 0.3 MJ and the net (gross minus resting) energy expenditure was estimated at 1.8 ± 0.1 MJ.

4.3.2 Plasma concentrations in the fasted state

Plasma concentrations in the fasted state are shown in Table 4.2. No significant differences were seen in fasting plasma TAG, NEFA, glucose or insulin concentrations between control and exercise trials, although plasma insulin showed a tendency to be higher in the control trial ($P < 0.10$).

Table 4.2. Plasma concentrations in the fasted state.

	Control			Exercise			<i>P</i>
Plasma TAG (mmol·L ⁻¹)	1.03	±	0.13	0.94	±	0.09	0.34
NEFA (mmol·L ⁻¹)	0.51	±	0.05	0.48	±	0.05	0.57
Glucose (mmol·L ⁻¹)	5.11	±	0.27	4.86	±	0.44	0.46
Insulin (pmol·L ⁻¹)	204	±	14	168	±	22	0.08

Values are mean ± SE, $n = 11$. TAG, triacylglycerol; NEFA, non-esterified fatty acids.

4.3.3 Postprandial plasma responses to the test meals

Changes in plasma volume over the period of observation were small and did not differ significantly between control ($0.2 \pm 2.7 \%$) and exercise ($-2.5 \pm 2.0 \%$) trials. No adjustments were made, therefore, to measured concentrations of plasma constituents.

Total and incremental plasma TAG concentrations following the test meals are shown in Figure 4.2, with summary measures (areas under the curve) of these responses in Table 4.3. No difference was seen in either the total or the incremental area under the curve values between the control and exercise conditions. There were no differences between trials, differences over time or in the pattern of TAG response to the test meals. No difference was observed in peak TAG concentration (control $2.15 \pm 0.26 \text{ mmol}\cdot\text{L}^{-1}$, exercise $2.16 \pm 0.26 \text{ mmol}\cdot\text{L}^{-1}$) or time-to-peak TAG concentration (control $3.45 \pm 0.43 \text{ h}$, exercise $3.45 \pm 0.49 \text{ h}$). Positive relationships were seen in both trials between the total area under the curve and the fasting TAG concentrations (control $r=0.95$, $P<0.0005$; exercise $r=0.93$, $P<0.0005$) (Figure 4.3).

Table 4.3. Six-hour areas under the plasma concentration *versus* time curves after consumption of a test meal in the control and resistance exercise trials.

	Control			Exercise			<i>P</i>
Total TAG (mmol·L ⁻¹ ·6h)	9.84	±	1.40	9.38	±	1.12	0.47
Incremental TAG (mmol·L ⁻¹ ·6h)	3.66	±	0.67	3.81	±	0.64	0.63
NEFA (mmol·L ⁻¹ ·6h)	2.75	±	0.20	2.95	±	0.20	0.32
Glucose (mmol·L ⁻¹ ·6h)	30.21	±	0.92	28.93	±	0.83	0.30
Insulin (pmol·L ⁻¹ ·6h)	2051	±	272	1901	±	364	0.36

Values are mean ± SE, *n* = 11. TAG, triacylglycerol; NEFA, non-esterified fatty acids.

Postprandial plasma NEFA, glucose and insulin responses are shown in Figure 4.4 and area under the curve values are presented in Table 4.3. There were no significant differences in area under the curve values between trials for any of these variables. No main effects were observed for any of these variables. Neither were there any significant differences between trials for peak insulin concentration (control 829 ± 108 pmol·L⁻¹, exercise 763 ± 202 pmol·L⁻¹) or time-to-peak insulin concentration (control 0.64 ± 0.05 h, exercise 0.70 ± 0.07 h).

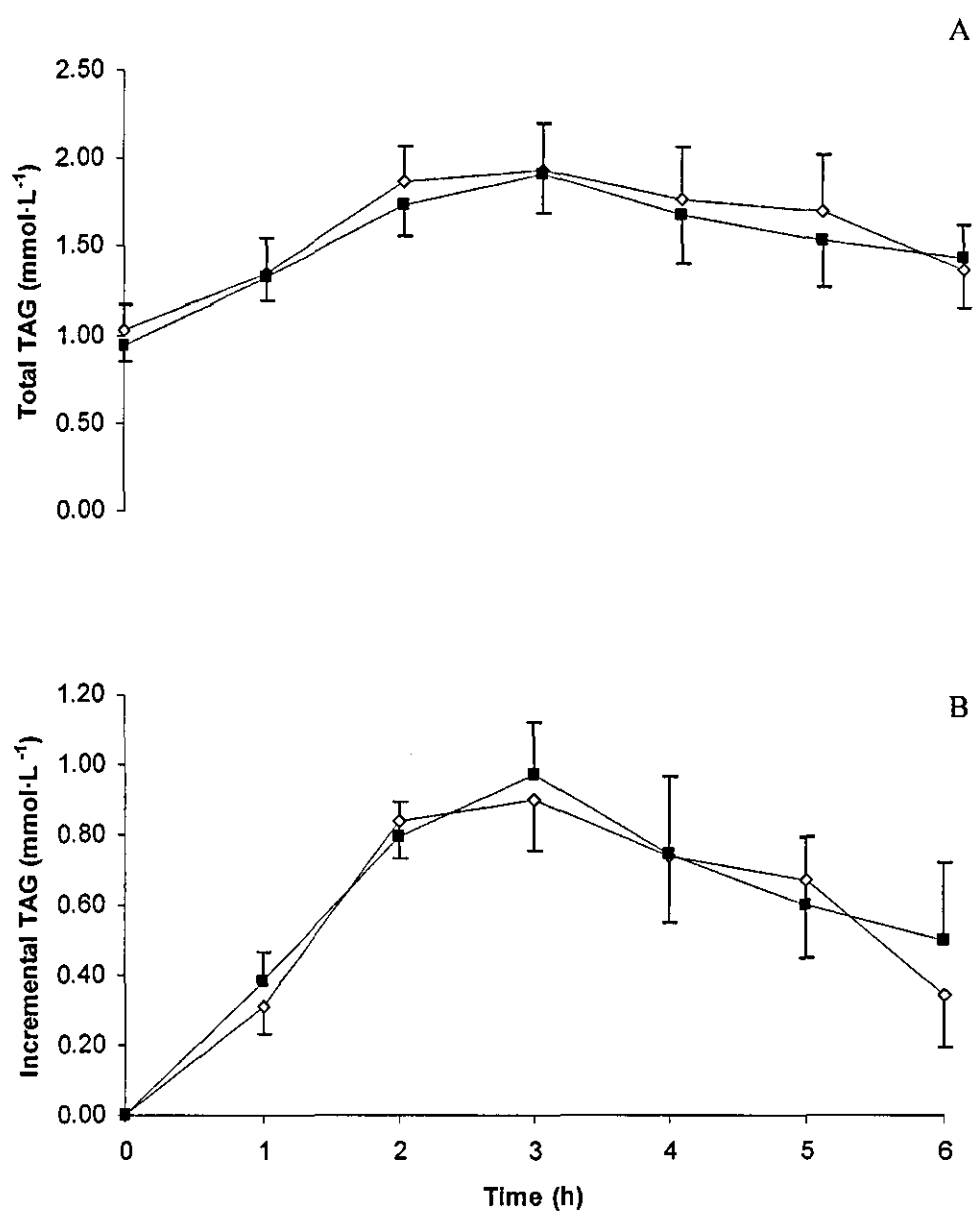


Figure 4.2. Total (A) and incremental (B) plasma triacylglycerol (TAG) concentrations in the fasted state (0 h) and for 6 h following consumption of a test meal in the control (◇) and resistance exercise (■) trials. Values are mean \pm SE, $n = 11$.

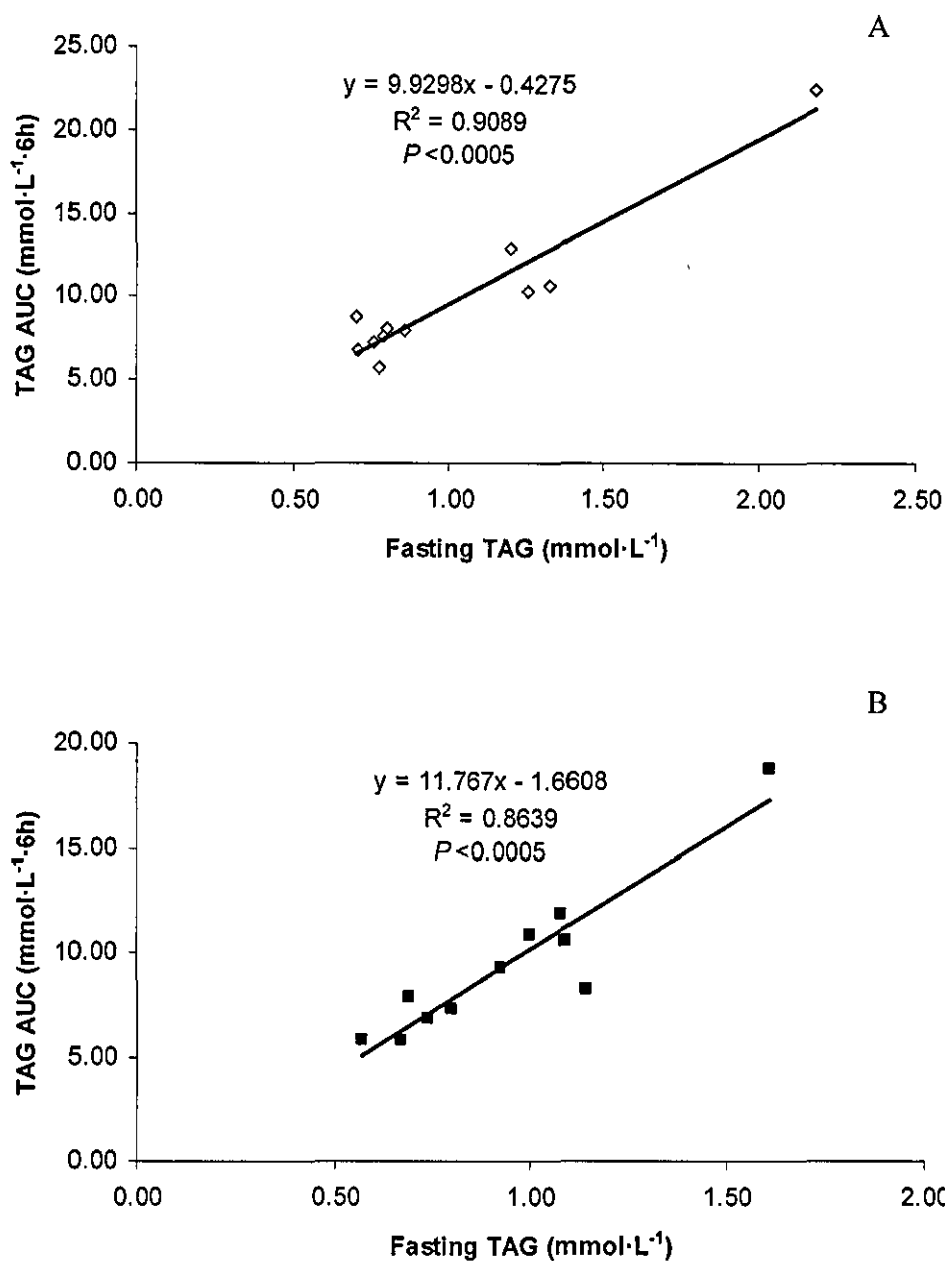


Figure 4.3. Relationship between fasting triacylglycerol (TAG) and total area under the TAG concentration *versus* time curve (AUC) for the control ($r=0.95$, $P<0.0005$) (A) and resistance exercise ($r=0.93$, $P<0.0005$) (B) trials. $n = 11$.

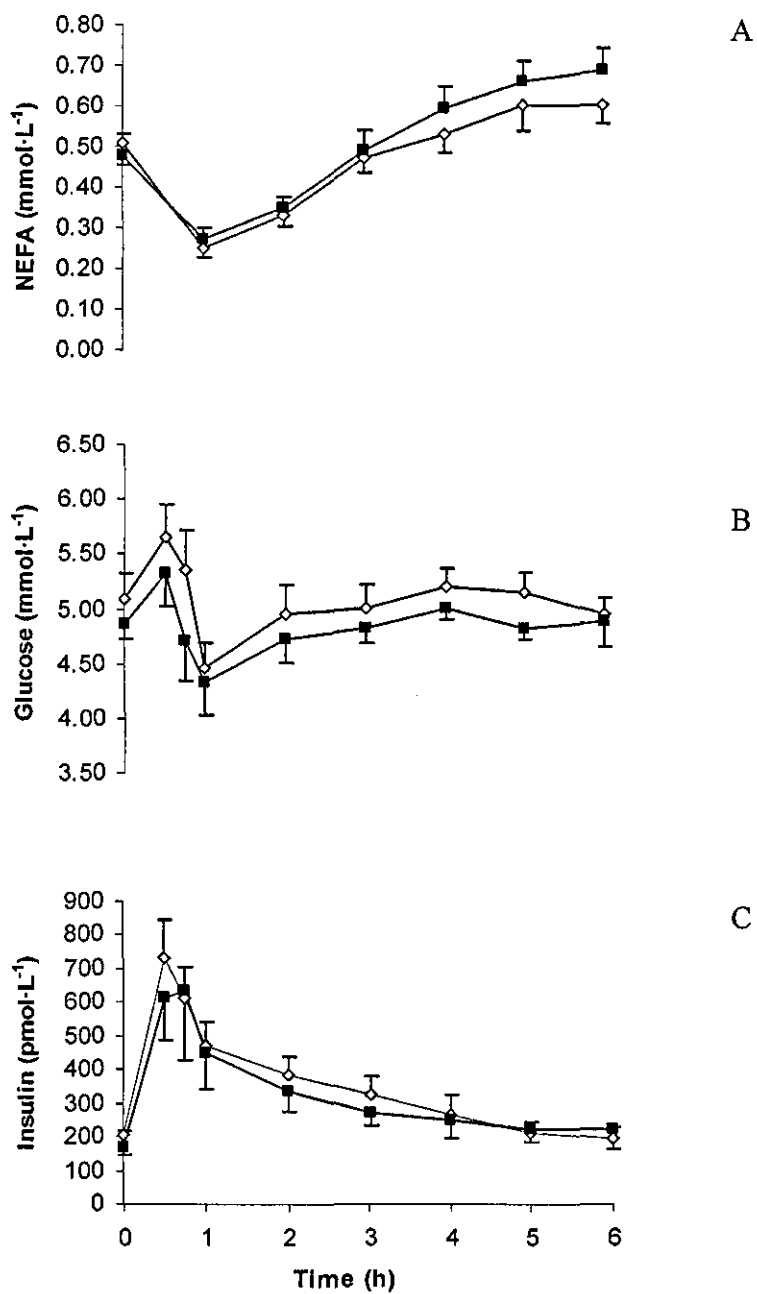


Figure 4.4. Plasma non-esterified fatty acids (NEFA) (A), glucose (B) and insulin (C) concentrations in the fasted state (0 h) and for 6 h following consumption of a test meal in the control (◇) and resistance exercise (■) trials. Values are mean \pm SE, $n = 11$.

4.4 Discussion

The main finding in the present study is that a single session of resistance exercise, performed 16 hours prior to consumption of a test meal, did not influence postprandial lipaemia. No indicators (peak TAG concentration, time to peak TAG concentration, total area under the TAG concentration *versus* time curve, incremental area under the TAG concentration *versus* time curve) of improved TAG metabolism were observed in the resistance exercise trial. This is in contrast with findings from studies of aerobic exercise which demonstrate a lowering of postprandial lipaemia the day after a single session of exercise (Aldred *et al.*, 1994; Tsetsonis & Hardman, 1996a; Tsetsonis & Hardman, 1996b; Tsetsonis *et al.*, 1997; Gill *et al.*, 2003). The findings of the present study also conflict with those of Petitt and colleagues (2003) who found a significant reduction in postprandial lipaemia 16 hours after a bout of resistance exercise. However, they concur with the findings of Shannon and co-workers (2005) who observed no effect of different volumes of resistance exercise on postprandial TAG responses.

A possible explanation for the failure of resistance exercise to influence postprandial lipaemia in the present study is that insufficient energy was expended in the exercise session. The estimated gross energy expended during the resistance exercise in our study was 2.3 MJ (range = 2.0-2.6 MJ). In previous studies examining the acute effects of aerobic exercise on postprandial lipaemia, the average gross energy expenditure found to attenuate the lipaemic response has ranged from 1.5 to 7.2 MJ (Petitt & Cureton, 2003). The energy expenditure elicited in the present study is at the lower end of this spectrum. Although Tsetsonis and colleagues (1997) found an expenditure of 2.3 MJ to be sufficient

to attenuate postprandial lipaemia in untrained participants, their participants were females who were on average 20 years older (43.8 y) and 20 kg lighter (62.2 kg) than the males in the present study. Thus, relative to body mass, 2.3 MJ represents a greater level of energy expenditure for the participants in the Tsetsonis *et al.* (1997) study than for the participants in the present study. Nonetheless, Petitt *et al.* (2003), using resistance exercise, observed a reduction in TAG concentrations with much lower energy expenditure (1.7 MJ) than that of the present study. This, they attribute to some factor linked to strenuous muscle contractions associated with weight lifting. However, similar energy was expended (2.58 MJ) in the treatment with the highest work volume in the study by Shannon and colleagues (2005) to that in the present study. Both these studies failed to find a reduction in postprandial lipaemia.

Another possible explanation for the conflict between the findings of the present study and studies of aerobic exercise relates to the enzyme lipoprotein lipase. This enzyme is located on the capillary endothelium and is particularly abundant in the heart, adipose tissue and skeletal muscle (Seip & Semenkovich, 1998). Lipoprotein lipase is responsible for hydrolysing TAG-rich lipoproteins and directing the liberated fatty acids into the surrounding tissues. Increased skeletal muscle lipoprotein lipase activity following a single bout of prolonged aerobic exercise has previously been demonstrated (Lithell *et al.*, 1979; Lithell *et al.*, 1981) and this is thought to be the major mechanism by which such exercise reduces postprandial lipaemia.

To the author's knowledge no studies have examined the effects of resistance exercise on lipoprotein lipase activity, but an acute bout of knee extension exercise appears to have a less dramatic effect on lipoprotein lipase activity than the changes found following aerobic exercise (Kiens & Lithell, 1989; Kiens *et al.*, 1989). This is possibly because knee-extension exercise, unlike whole-body exercise, evokes little catecholamine response and catecholamines are one of the factors leading to activation of lipoprotein lipase (Hardman, 1998). Thus, it may be that the small muscle mass utilised in some of the exercises in the present study had minimal influence on catecholamines and therefore lipoprotein lipase activity. However, some of the exercises employed in our study did involve major muscle groups and multiple joints. Moreover, the plasma catecholamine response has been elevated in other studies examining acute hormonal responses to heavy resistance exercise in both trained and untrained men (Kraemer *et al.*, 1993; Kraemer *et al.*, 1999). It seems unlikely, therefore, that a reduced catecholamine response would, on its own, explain our findings.

Neither of the previous explanations (insufficient energy expenditure, insufficient activation of lipoprotein lipase) can account for the differences between the findings in the present study and those of Petitt *et al.* (2003). Petitt and colleagues found a 14% reduction in postprandial lipaemia following a bout of resistance exercise involving 3 sets of 10 repetitions of 10 exercises performed at 10 repetition maximum. In the present study a greater volume of exercise was completed (4 sets of 10 repetitions of 11 exercises) although the intensity was lower (80% of 10 repetition maximum). The length of the resistance exercise session was identical in the two studies (88 min) but estimated

energy expenditure was 35% higher in our study (2.3 versus 1.7 MJ). This was probably due to a greater volume of work being completed in our study although inconsistency between the methods used to estimate energy expenditure may also have contributed to the difference (a precise description of how energy expenditure was estimated is not given by Petitt and co-workers). The gap between the resistance exercise and the test meal was also identical between studies (16 hours) and the test meals employed in each study provided very similar energy and macronutrient content. Thus, none of these factors would seem to explain the discrepancy between studies. Furthermore, in the treatment with the highest work volume, in the study by Shannon *et al.* (2005) volume of exercise (5 sets of 10 repetitions of 8 exercises), time to complete the resistance exercise (90 minutes), total energy expenditure (2.58 MJ) and time to consumption of the test meal (approximately 13 hours) were all similar to the present study.

One factor which may explain the differences in findings between these studies is the nature of the participants involved. The study of Petitt and colleagues (2003) involved males ($n=10$) and females ($n=4$) who had six years experience of weight lifting. In contrast, none of the participants in the present study were regularly involved in resistance training. Thus, it is possible that the physiological stress associated with this unfamiliar form of exercise may have caused skeletal muscle damage in the participants in the present study. This may not have occurred in the relatively well-trained participants in the study by Petitt and colleagues. Skeletal muscle damage may elevate concentrations of the cytokine tumor necrosis factor- α which has been associated with transient insulin resistance due to a down regulation of insulin-receptor-signalling in adipocytes,

hepatocytes and skeletal muscle (Kirwan & Jing, 2002). This may have impaired the uptake of TAG into adipose tissue/skeletal muscle (via a reduced stimulation of lipoprotein lipase). It may also have reduced the efficiency of insulin in suppressing fat mobilisation from adipose tissue and the liver. However, there is no firm evidence to support these ideas. Moreover, Shannon and co-workers (2005) used participants who were resistance trained ($\geq 3\text{d/wk}$ for ≥ 1 year) in their study. Also, the familiarisation session completed by participants in the present study should have had a prophylactic effect and therefore muscle damage should have been reduced when participants came to complete the exercise trial (Byrnes *et al.*, 1985). Markers of muscle damage, however, were not measured in the present study.

It is feasible that a reduction in postprandial lipaemia is dependent on the relative substrate contribution to energy metabolism during exercise. If this were the case and if fat was not a major source of fuel during resistance exercise then perhaps there would be limited impact on postprandial lipaemia. However, there is evidence to show that fat does provide a significant source of fuel during resistance exercise since muscle biopsy samples taken from the vastus lateralis muscles of bodybuilders immediately after a 30 minute bout of heavy resistance exercise indicated a 30% reduction in intramuscular TAG in comparison to pre-exercise values (Essen-Gustavsson & Tesch, 1990). Moreover, Malkova and colleagues (1999) found no difference in the postprandial TAG response following 90 min of treadmill running when fat metabolism was inhibited by acipimox during running on one occasion. They concluded that the mechanisms by which

prior exercise attenuates postprandial lipaemia are not influenced by the relative contributions of fat and carbohydrate to energy metabolism during exercise.

In conclusion, the findings in the present study indicate that a single session of resistance exercise does not influence postprandial lipaemia. These findings conflict with those of Petitt and colleagues (2003), but agree with those of Shannon and co-workers (2005). Further research is required to clarify this relationship, given the emphasis on resistance exercise in physical activity recommendations (U.S. Department of Health and Human Services, 1996; Department of Health, 2004). Given that total energy expenditure is the prime determinant of the exercise-induced reduction in postprandial TAG concentrations in studies using aerobic exercise the same question needs addressing for resistance exercise. Whether or not any protective effect is conferred by resistance exercise remains to be seen.

Chapter 5

Resistance exercise and postprandial triacylglycerol concentrations: influence of energy expenditure

5.1 Introduction

A single bout of aerobic exercise has been shown to cause a transient reduction in postprandial TAG concentrations (Aldred *et al.*, 1994; Tsetsonis & Hardman, 1996a; Tsetsonis & Hardman, 1996b; Tsetsonis *et al.*, 1997; Gill *et al.*, 2003). The energy expenditure of exercise appears to be the primary determinant of the exercise-induced reduction in postprandial TAG concentrations (Tsetsonis & Hardman, 1996b; Gill *et al.*, 2002; Petitt & Cureton, 2003). However, the study in the previous chapter (Chapter 4) along with studies by Petitt and colleagues (2003) and Shannon and co-workers (2005) have failed to demonstrate consistent findings for an effect of a single session of resistance exercise on postprandial TAG concentrations. Gross energy expenditure in studies of aerobic exercise that have attenuated postprandial TAG concentrations has ranged from 1.5 to 7.2 MJ (Petitt & Cureton, 2003). Gross energy expenditure for studies on resistance exercise (range 0.57 to 2.58 MJ) are at the lower end of that reported for aerobic exercise. It is possible, therefore, that the lack of an observable difference in TAG concentrations in two of the studies of resistance exercise was because of the low energy expenditure of the exercise. Nonetheless, the study of Petitt *et al.* (2003) demonstrated a significantly decreased lipaemic response after resistance exercise, even with low energy expenditure (1.7 MJ) from exercise.

Epidemiological studies provide some evidence for a potential protective effect of resistance training against coronary heart disease. Studies of longshoremen

(Paffenbarger *et al.*, 1975) found that those who were performing heavy work had a greater protective effect against coronary heart disease than their colleagues performing light or moderate work. The rate of energy expenditure in these workers performing heavy work ranged from 21.8 to 31.5 kJ per minute and on average these jobs required 7.9 MJ over basal output per eight hour work day. This suggests that heavy work may provide protection against coronary heart disease. More recently, a study by Tanasescu and colleagues (2002) found that men who trained with weights for 30 minutes or more per week had a 23% risk reduction in coronary heart disease. Other types of physical activity, smoking, family history and nutrient intake were controlled for in the analysis. Whilst these studies (Paffenbarger *et al.*, 1975; Tanasescu *et al.*, 2002) suggest that weight training provides protection from coronary heart disease the benefits of resistance exercise or resistance training for individual coronary heart disease risk factors remains to be established. Whether part of this protection is conferred through beneficial changes in postprandial lipid metabolism is unknown. However, these studies are consistent with the notion that resistance exercise has the potential to reduce postprandial lipaemia.

The aim of the present study, therefore, was to examine the effect of increasing energy expenditure, by performing multiple light bouts of weight lifting throughout the day, on postprandial TAG concentrations the following day.

5.2 Methods

5.2.1 Participants

Twenty four male volunteers aged 20-32 years participated in the study. Some physical characteristics of the participants are shown in Table 5.1.

Table 5.1. Physical characteristics of the participants.

Age (yrs)	23.5	±	3.4
Height (m)	1.78	±	0.07
Body mass (kg)	75.8	±	10.9
BMI (kg·m ⁻²)	23.8	±	2.7
Body fat (%)	16.7	±	4.8
Waist circumference (m)	0.82	±	0.08
Systolic blood pressure (mm Hg)	116	±	8
Diastolic blood pressure (mm Hg)	72	±	9

Values are mean ± SD, *n*=24

5.2.2 Preliminary tests

Prior to the main trials, participants visited the laboratory once for collection of anthropometric data and to complete one-repetition maximum weight lifting tests for five different resistance exercises. The order in which the one-repetition maximum tests were performed was the same for each participant: squat, bench press, lunges, bicep curl and shoulder press (seated).

5.2.3 Main Trials

Following the preliminary tests, participants undertook two, 2 d trials – exercise and control. On day one of the exercise trial participants performed multiple bouts of light weight resistance exercise over the course of the day. On day one of the control trial participants rested. On day two of both trials participants came to the lab fasted and consumed a test meal. The order of the trials was randomised and balanced. The interval between the two trials was at least one week. Participants recorded their diet during the 48 hours preceding their first trial. They then replicated this for the 48 hours prior to their second trial. Participants were also asked to refrain from physical activity 48 hours preceding each main trial (Chapter 3 Pg.37).

5.2.4 Weight lifting protocol – day 1

On the exercise trial participants reported to the laboratory at 9.00 a.m. Over the day, each participant completed 20 sets of 15 repetitions of 5 different weight-lifting exercises i.e. 100 sets and 1500 lifts in total for all exercises. These sets were divided into five, 45 min bouts of weight lifting. Each 45 min bout of lifting consisted of 4 sets of each of the 5 different exercises. Participants completed one set of each exercise sequentially in the same order as described for the preliminary tests. Upper body exercises were completed at 30% of one repetition maximum, whereas lower body exercises were completed at 40% of one repetition maximum. Participants were given one minute to recover between each exercise. After the last exercise (shoulder press) participants were given 5 minutes to recover. The sequence was then repeated until 4 sets of each exercise had been completed. A schematic representation for one 45 minute bout of lifting is given in Figure 5.1.

After each 45 minute bout of lifting (4 sets of each exercise) the recovery time was extended. There was a 15 min rest interval after the first two 45 min bouts, a 105 min lunch break after the third 45 min bout and a 15 min rest interval between the fourth and fifth 45 min bout. The fifth bout of exercise finished at 3.15 p.m. The high volume of work over the day was designed in order to maximise the total energy expenditure of the session and hence increase the likelihood of reducing postprandial TAG concentrations. A schematic representation of the entire weight lifting protocol for day one is given in Figure 5.2.

5.2.5 Estimation of energy expenditure during weight lifting

Expired air samples were collected into Douglas bags (Plysu Protection Systems, Milton Keynes, U.K.). Samples were collected during the 1st and 4th sets of each exercise in the first and fifth 45 min bouts of exercise. The samples were collected into separate Douglas Bags for each exercise. Samples were collected for the full duration of the lifting and recovery period i.e. the exercise time plus 1 min recovery. For the shoulder press the sample was collected for the exercise time plus 5 min recovery.

A two-way ANOVA (see section 5.2.8, Pg.70) was used to determine if there were any differences in oxygen uptake between expired air samples. Oxygen uptake per minute was calculated as the mean of all five exercises over the four sets collected. Energy expenditure was calculated as being 5.047 kcal (21.1 kJ) per litre of oxygen (McArdle, Katch & Katch, 2001) (see General Methods - Chapter 3, Pg.38). The duration of the exercise was calculated as the mean time over the four sets of collections multiplied by twenty (the total number of sets completed). Gross energy

expenditure was calculated as mean oxygen uptake per minute multiplied by 21.1 kJ multiplied by the duration of exercise. Net energy expenditure was calculated as gross minus resting energy expenditure over the same duration.

5.2.6 Main trial protocol - day 2

Participants reported to the laboratory at 08.00 h after a 12 h fast. A cannula was inserted into an antecubital or forearm vein and a baseline blood sample taken. Participants then consumed the test meal. Further blood samples were obtained 0.5, 0.75 and 1 h after the start of the meal and then hourly for a total of 6 h. The cannula was kept patent by flushing with nonheparinised saline ($9 \text{ g}\cdot\text{L}^{-1}$, B.Braun Medical Ltd, Buckinghamshire, UK). The first 2-mL of blood withdrawn was always discarded to avoid dilution of the sample. Water was available *ad libitum* during the first trial; the volume ingested was recorded and replicated in the second trial. Participants rested (reading, working, watching television) throughout the observation period and were always lying in a supine position for at least 5 min before each blood sample was taken. A schematic representation of the main trial protocol (day 2) is given in Figure 5.3.

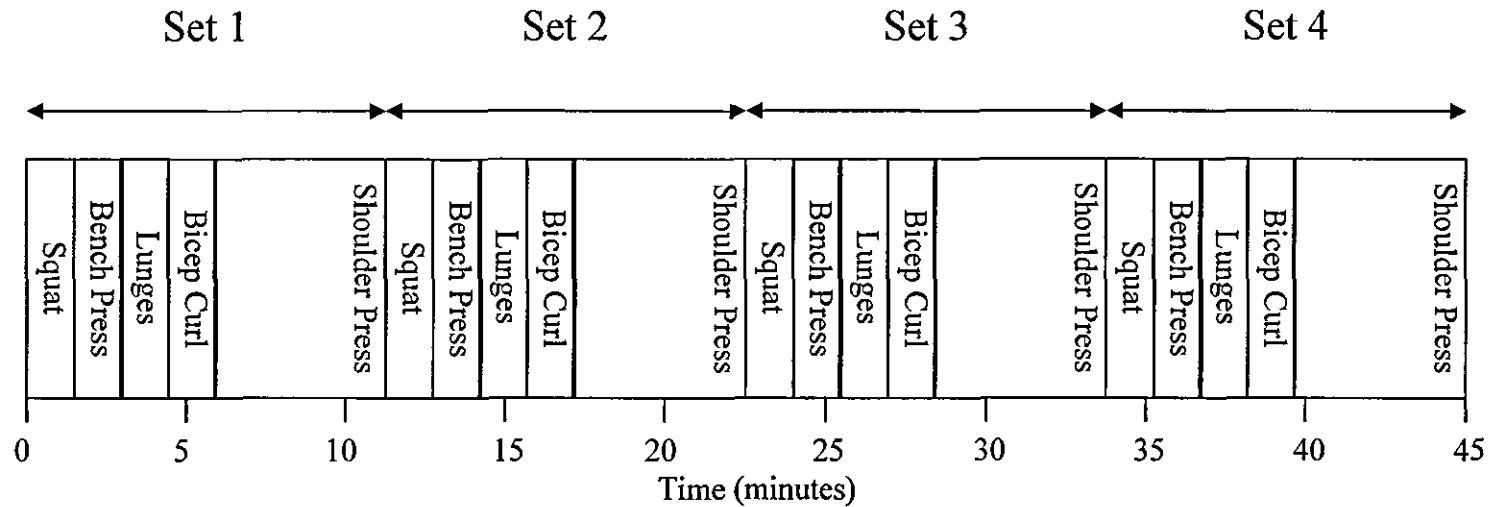
5.2.7 Analytical Methods

Plasma samples were analysed for TAG, glucose, insulin, NEFA and 3-hydroxybutyrate by enzymatic colorimetric methods (see General Methods - Chapter 3, Pg.42). Haemoglobin concentration and haematocrit were determined in samples collected at baseline and at the end of the day so that changes in plasma volume could be estimated (Dill & Costill, 1974) (see General Methods - Chapter 3, Pg.42).

5.2.8 Data Analysis

Two-way ANOVA (repeated measures) was used to determine differences between oxygen uptake at each expired air sample measurement point (i.e. 1st and 4th sets of the first and fifth 45 min bouts of exercise) and the exercise being performed (i.e. squat, bench press, lunges, bicep curl, shoulder press). Six hour total and incremental area under the plasma concentration *versus* time curve was calculated for TAG. Fasting and area under the curve values were compared between trials using Student's *t*-tests for correlated means. Two-way ANOVA (repeated measures) was used to determine differences between trials and over time for concentrations of TAG, glucose, insulin, NEFA and 3-hydroxybutyrate. Where appropriate, post-hoc pair wise comparisons were made using the Bonferroni method. Relationships between variables were evaluated using Pearson's product-moment correlation coefficient.

Figure 5.1. Schematic representation of one 45 minute bout of weight lifting.



- One forty five minute bout of weight lifting divided into four sets.
- Each set consists of 15 repetitions of each exercise plus 1 min recovery. 5 min recovery after shoulder press
- Squat and lunges at 40% of one repetition maximum.
- Bench press, bicep curl and shoulder press at 30 % of one repetition maximum

Figure 5.2. Schematic representation of weight lifting protocol – day 1.

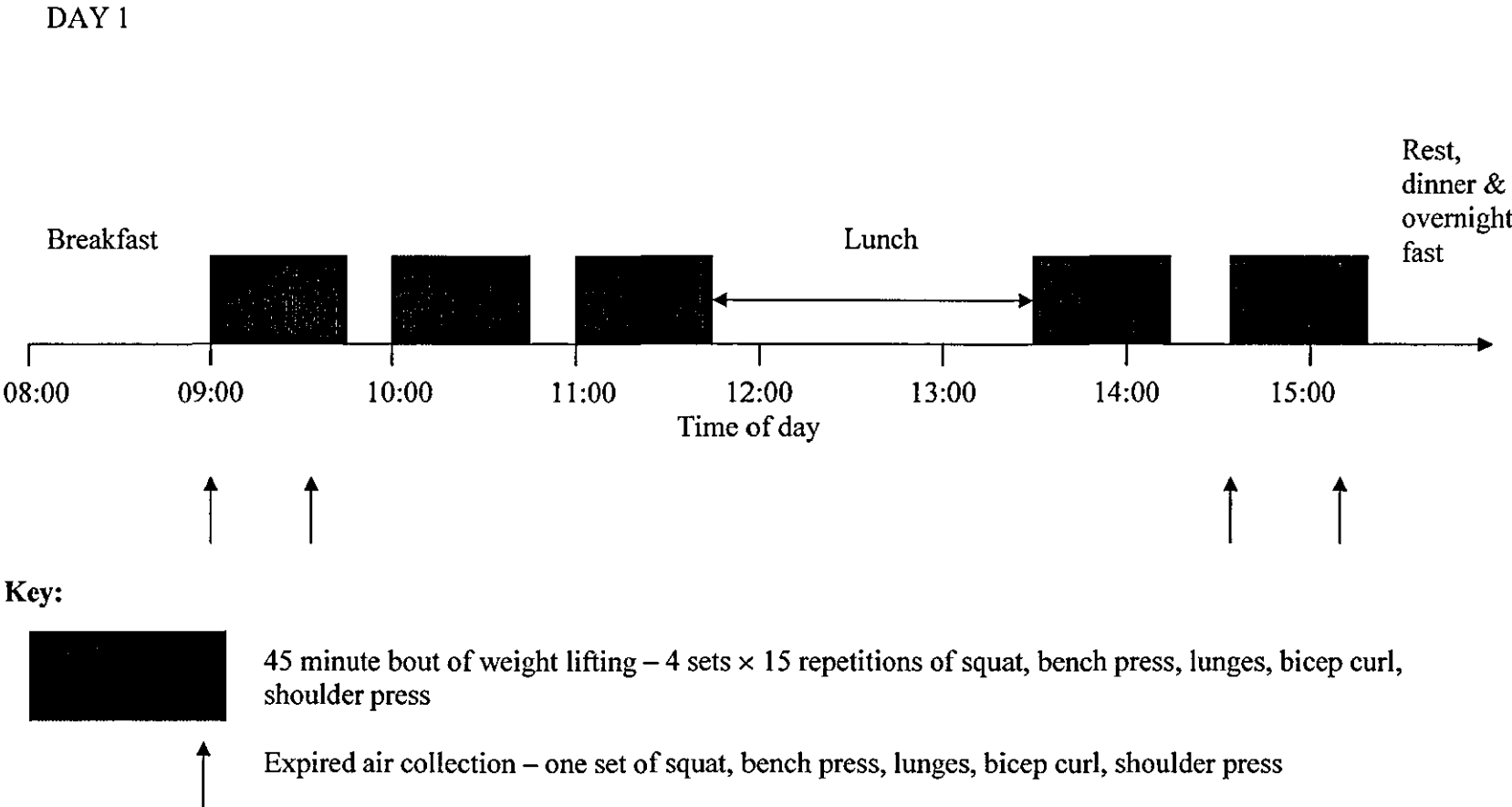
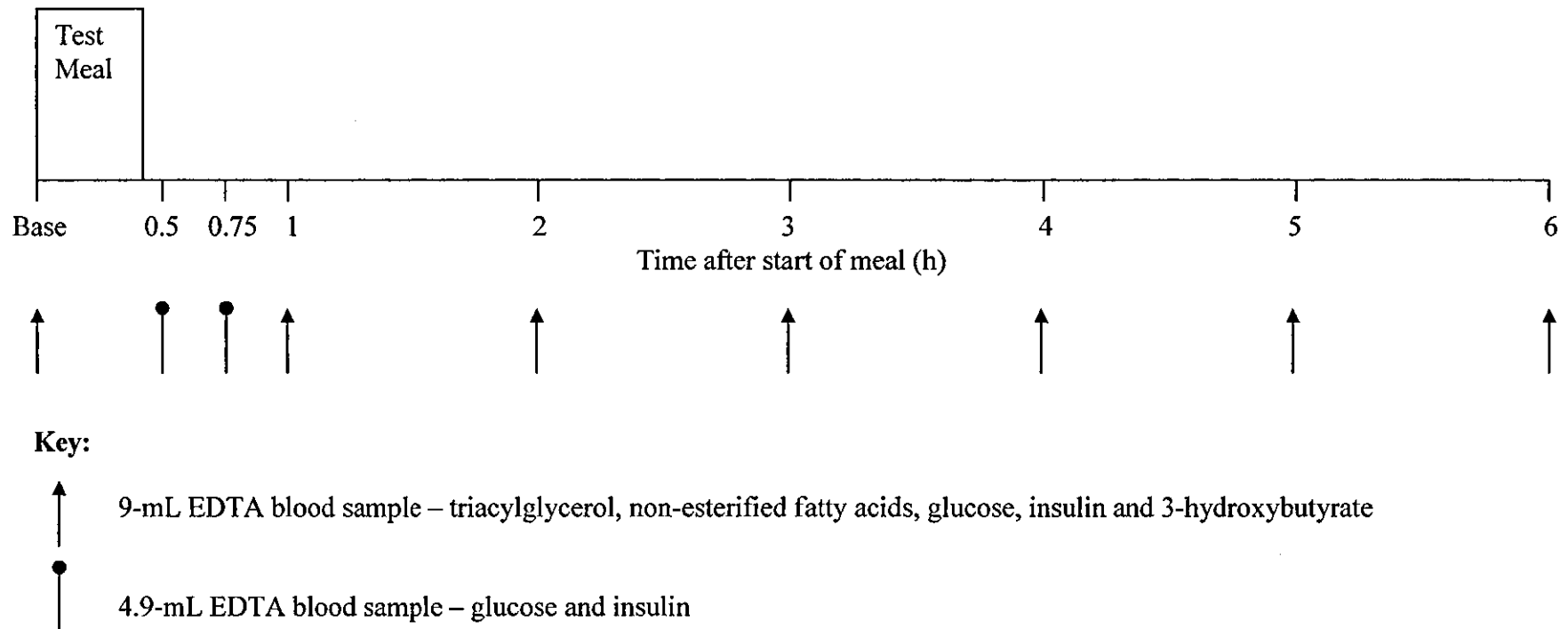


Figure 5.3. Schematic representation of the main trial protocol – day 2.

DAY 2



5.3 Results

5.3.1 Resistance exercise session

The mean weight lifted over the course of the day was 36997 ± 1268 kg. The average weight lifted on each exercise was 25 ± 1 kg. There was no difference in oxygen uptake at each expired air sample measurement point over time i.e. 1st versus 5th bout ($P=0.170$). Oxygen uptake differed between exercises ($P<0.0005$) but did not show a different pattern of response at measurement points ($P=0.088$). The mean gross energy expenditure from the exercise was estimated to be 5.1 ± 0.2 MJ over the day. Mean net energy expenditure from exercise was estimated to be 3.5 ± 0.1 MJ.

5.3.2 Plasma concentrations in the fasted state

Plasma concentrations in the fasted state on day 2 are shown in Table 5.2. There were no between trial differences for TAG, glucose, NEFA or 3-hydroxybutyrate concentrations. Plasma insulin concentrations were higher on the control trial than the exercise trial.

Table 5.2. Plasma concentrations in the fasted state on day 2.

	Control			Exercise			P
TAG (mmol·L ⁻¹)	1.05	±	0.09	0.97	±	0.07	0.275
Insulin (pmol·L ⁻¹)	71	±	6	57	±	3	0.015
Glucose (mmol·L ⁻¹)	5.03	±	0.07	5.00	±	0.06	0.329
NEFA (mmol·L ⁻¹)	0.45	±	0.05	0.49	±	0.05	0.365
3-Hydroxybutyrate (mmol·L ⁻¹)	0.10	±	0.03	0.13	±	0.03	0.282

Values are mean ± SE, *n*=24. For NEFA and 3-hydroxybutyrate *n*=19. TAG, triacylglycerol; NEFA, non-esterified fatty acids.

5.3.3 Postprandial plasma responses

Changes in plasma volume over the period of observation did not differ between trials (*P*=0.728). No adjustments were made, therefore, to measured concentrations of plasma constituents.

Plasma TAG concentrations were higher on the control than the exercise trial throughout the postprandial period (Figure 5.4): main effect of trial (*P*=0.044), main effect of time (*P*<0.0005). There was no difference in the pattern of response for plasma TAG concentrations between trials. The total area under the TAG concentration *versus* time curve during the postprandial period was 12% lower on the exercise compared with the control trial (8.76 ± 0.72 *versus* 9.94 ± 0.88 mmol·L⁻¹·6h respectively; *P*=0.037). The incremental area under the TAG concentration *versus* time curve was 18% lower on the exercise compared with the control trial (2.99 ± 0.38 *versus* 3.64 ± 0.39 mmol·L⁻¹·6h respectively; *P*=0.043). There was no difference

in peak TAG concentration between trials or time to peak TAG concentration between trials. Fasting TAG concentrations were positively related on both trials with total area under the TAG concentration versus time curve (control $r=0.96$, $P<0.0005$; exercise $r=0.90$, $P<0.0005$) and incremental area under the TAG concentration versus time curve (control $r=0.78$, $P<0.0005$; exercise $r=0.57$, $P=0.004$) (Figure 5.5).

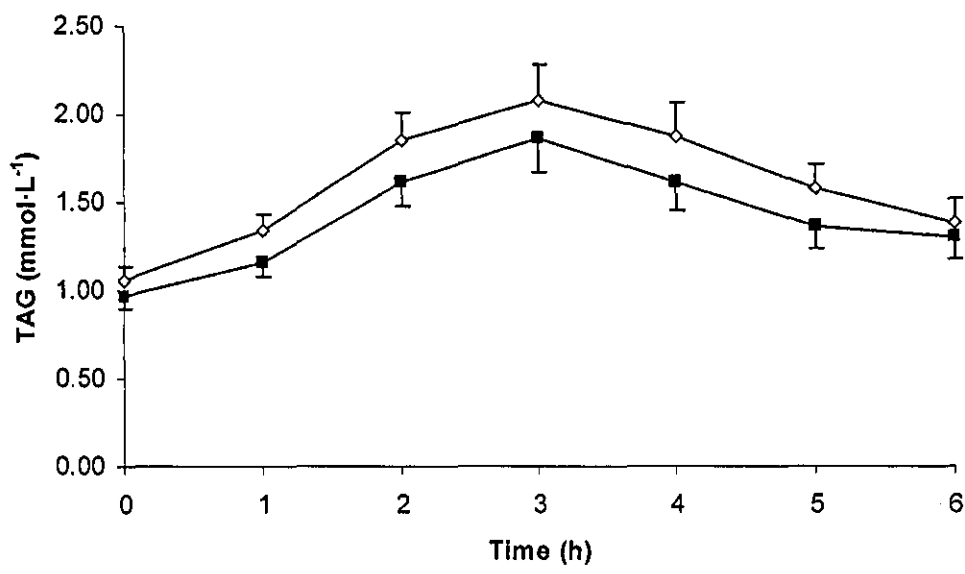


Figure 5.4. Postprandial total plasma triacylglycerol (TAG) concentrations for 6 h following consumption of a test meal in the control (◇) and resistance exercise (■) trials. Values are mean \pm SE, $n = 24$. Main effect of trial ($P=0.044$), main effect of time ($P<0.0005$).

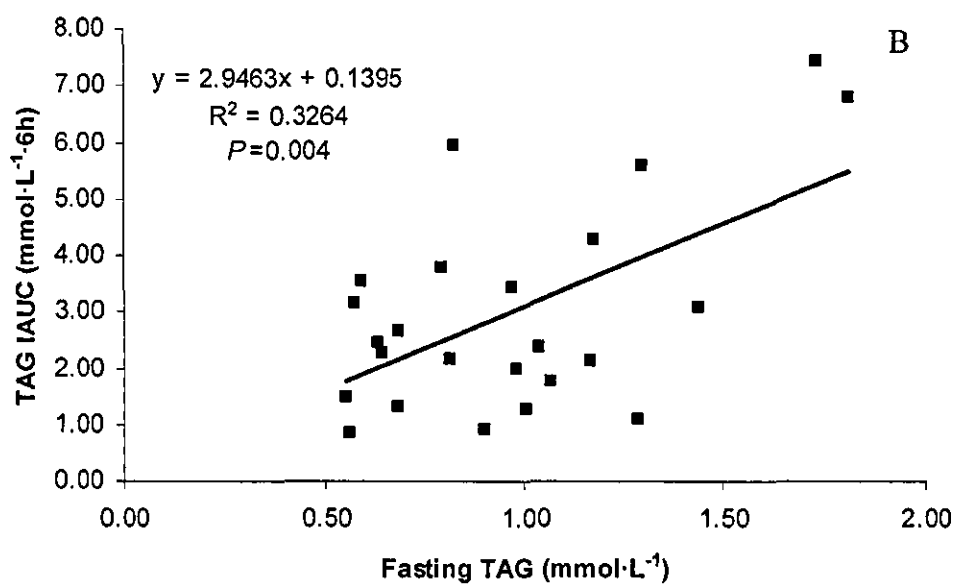
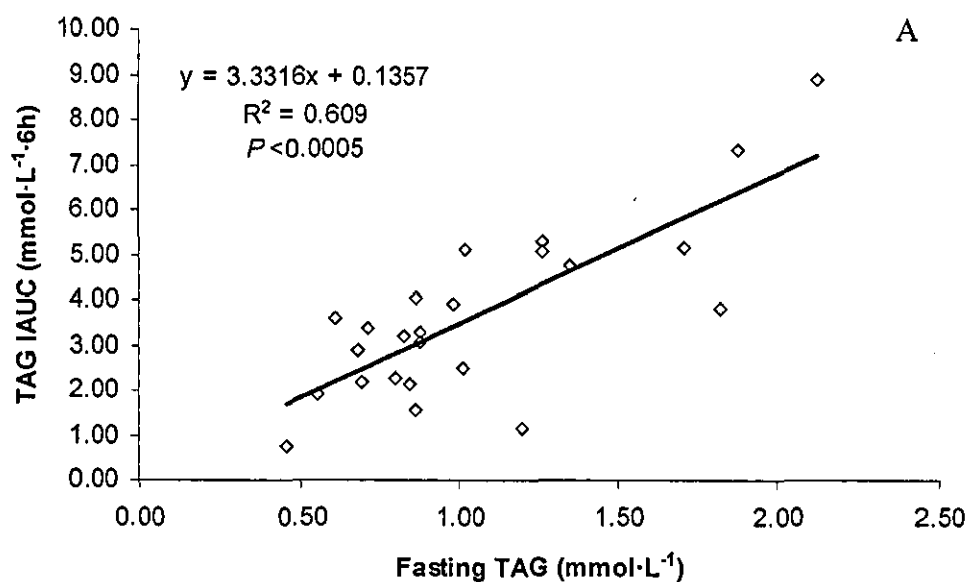


Figure 5.5. Relationship between fasting triacylglycerol (TAG) concentration and incremental area under the TAG concentration *versus* time curve (IAUC) for the control ($r=0.78$, $P<0.0005$) (A) and resistance exercise ($r=0.57$, $P=0.004$) (B) trials. $n = 24$.

Plasma concentrations for insulin and glucose are shown in Figure 5.6. Insulin concentrations were significantly higher on the control than the exercise trial: main effect of trial ($P=0.043$). Insulin concentrations rose steeply and then fell after the test meal on both the control and exercise trials: main effect of time ($P<0.0005$). There was no difference in the pattern of insulin concentrations between trials. Peak insulin concentrations were significantly higher on the control than the exercise trial ($P=0.041$). Adjusted peak insulin response (peak minus fasting concentrations) also tended to be higher on the control than exercise trial ($P=0.052$). Time to peak TAG on the control trial was significantly related to time to peak insulin ($r=0.40$, $P=0.050$).

Plasma glucose concentrations did not differ between trials. On both trials plasma glucose concentrations rose steeply in the early postprandial period and then fell back to baseline concentrations within the hour: main effect of time ($P<0.0005$). There was no difference in the pattern of glucose concentrations between trials.

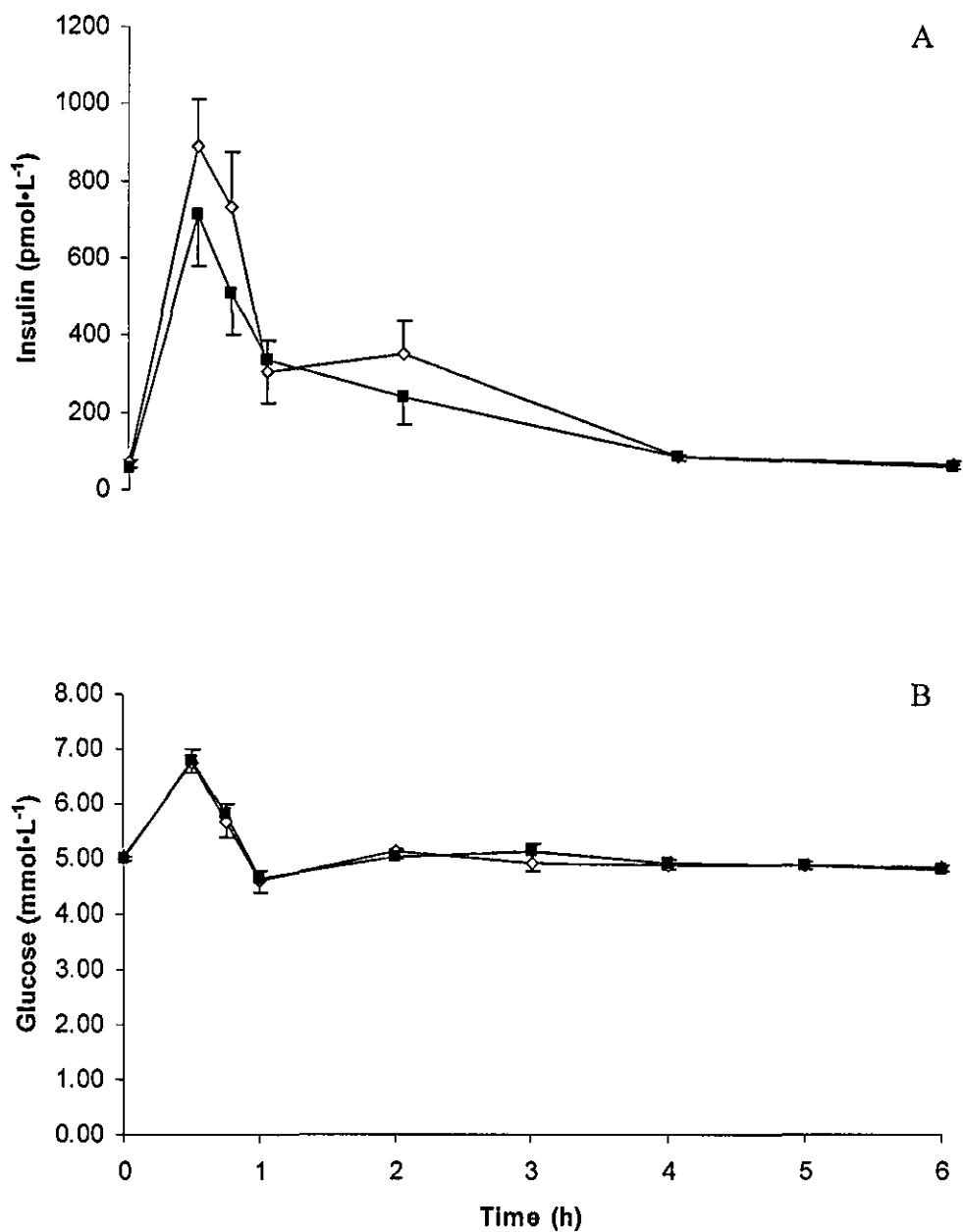


Figure 5.6. Postprandial plasma insulin (A) and glucose (B) concentrations for 6 h following consumption of a test meal in the control (◇) and resistance exercise (■) trials. Values are mean \pm SE, $n=24$. Main effect of trial ($P=0.043$), main effect of time ($P<0.0005$) for insulin. Main effect of time ($P<0.0005$) for glucose.

Plasma NEFA concentrations tended to be higher on the exercise than the control trial although this did not reach significance: main effect of trial ($P=0.058$). Plasma NEFA concentrations fell on both trials during the first hour after the test meal (Figure 5.7). On both trials NEFA concentrations then rose steeply until the end of the trial: main effect of time ($P<0.0005$). Plasma 3-hydroxybutyrate concentrations were higher on the exercise than control trial: main effect of trial ($P=0.001$). Plasma 3-hydroxybutyrate dropped for the first two hours in the postprandial period on both trials. Thereafter, 3-hydroxybutyrate rose until the end of both trials, with a steeper increase on the exercise than control trial (Figure 5.7): main effect of time ($P<0.0005$), trial \times time interaction ($P=0.028$). Post-hoc tests revealed that the difference in 3-hydroxybutyrate concentrations occurred at 6 h into the trial ($P=0.012$).

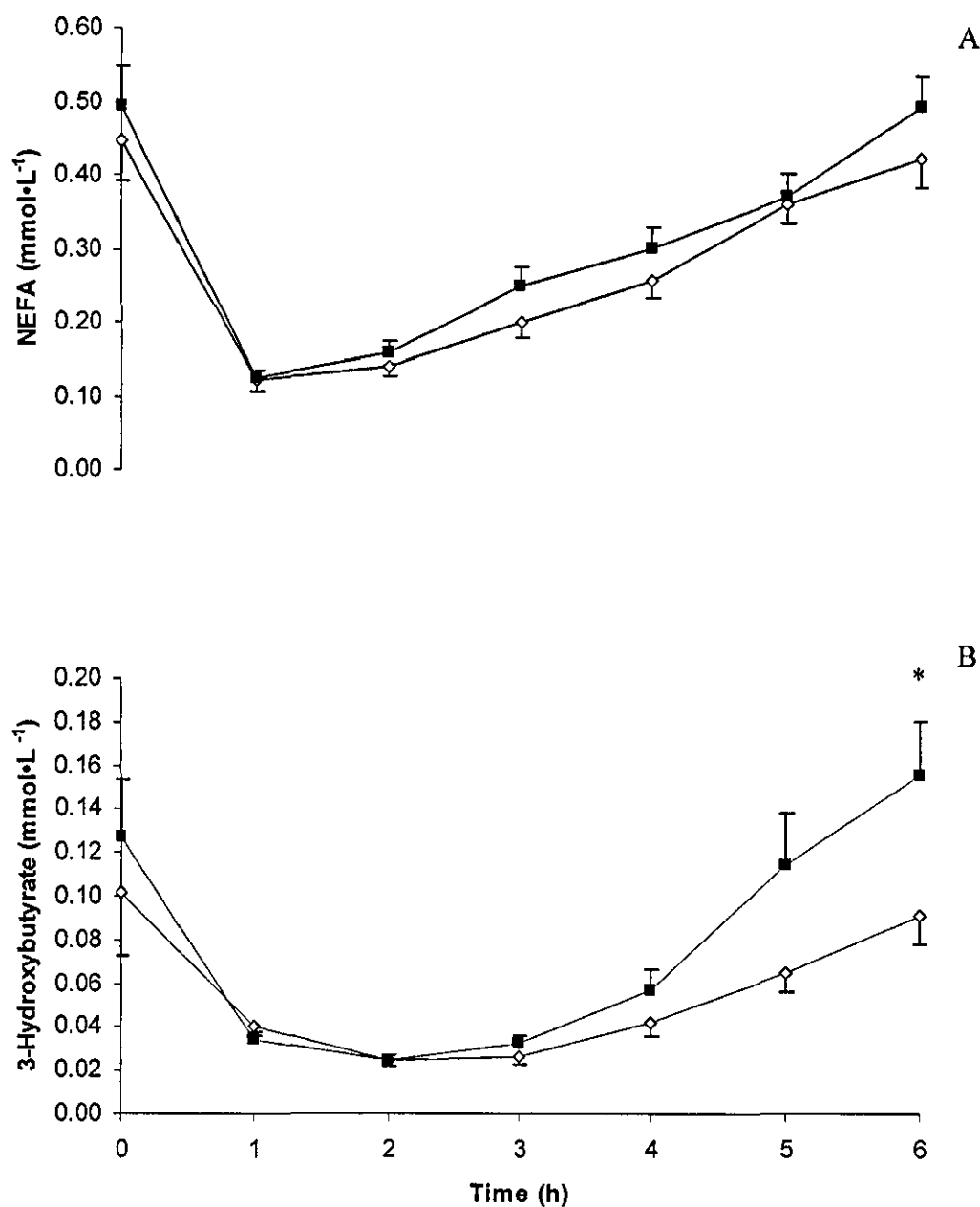


Figure 5.7. Postprandial plasma non-esterified fatty acid (NEFA) (A) and 3-hydroxybutyrate (B) concentrations for 6 h following consumption of a test meal in the control (◇) and resistance exercise (■) trials. Values are mean \pm SE, $n=19$. Main effect of time ($P<0.0005$) for NEFA. Main effect of trial ($P=0.001$), main effect of time ($P<0.0005$), trial \times time interaction ($P=0.028$) for 3-hydroxybutyrate. *Significantly different ($P<0.05$) between trials using a Bonferroni post hoc test.

5.4 Discussion

The main finding in the present study is that performing multiple bouts of resistance exercise the day before a test meal causes a significant reduction in postprandial TAG concentrations. This finding extends those in study one (Chapter 4) of the present thesis and the findings of Shannon and colleagues (2005) by suggesting that if energy expenditure with resistance exercise is high enough then a reduction in postprandial lipaemia will follow. The findings in the present study do not explain why Petitt *et al.* (2003) found a decrease in postprandial lipaemia when energy expenditure during exercise was low.

Tsetsonis & Hardman (1996b) demonstrated that walking for 90 minutes at 63% of maximum oxygen uptake, or 3-h at 32% of maximum oxygen uptake produced similar reductions in lipaemia. Gill & co-workers (2002) provide further support for this argument by showing that a one hour walk reduced postprandial lipaemia by a mean of 9.3%, whereas a 2-h walk reduced it by 22.8%. These studies demonstrate that the beneficial effects of aerobic exercise on postprandial lipaemia are related to the duration and, therefore, the energy expenditure of the exercise session. The reduced TAG concentrations on the exercise trial in the present study seem to support the fact that energy expenditure is also a major determinant of the reduction in lipaemia after resistance exercise.

The estimated total energy expenditure in the present study (5.1 MJ) is much greater than that seen in previous studies (Petitt *et al.*, 2003; Shannon *et al.*, 2005; Chapter 4) on resistance exercise and postprandial lipaemia. It seems likely that as for aerobic exercise a certain 'threshold' of energy expenditure must be reached before the

lipaemic reduction becomes significant. Whether this 'threshold' is the same in all individuals remains to be determined, but in young healthy males, such as those examined in the present study, it is likely to be high. Study one (Chapter 4) of the present thesis did not find a reduction in lipaemia with a total energy expenditure of 2.3 MJ. Shannon and colleagues (2005) did not find a reduction in the postprandial TAG curve over six hours when participants performed five sets of eight different resistance exercises. Total energy expenditure in that trial was estimated to be 2.58 MJ. Energy expenditure in these two studies was approximately half the total energy expended by participants in the present study, although it needs to be noted that in the study by Shannon *et al.* the post-exercise meal replaced the energy debt created. It does seem, however, that a single session of resistance exercise would have to be very strenuous and of fairly long duration in order to achieve the energy expenditure needed to reduce lipaemia. Furthermore, it is important to remember that weight lifting invalidates the typical assumptions of indirect calorimetry because the respiratory exchange ratio is consistently equal to or greater than 1.0. Therefore the energy expenditure cited in the present study, study one (Chapter 4) and the studies by Petitt *et al.* and Shannon *et al.* may be over/under estimations of the actual energy expended. This makes comparisons between these studies difficult and determining the amount of energy expenditure needed to reduce postprandial lipaemia problematic.

In the present study the energy debt created by exercise was not replaced. In comparison, Shannon and colleagues (2005) replaced the energy used in exercise in the post-exercise meal which may have reduced the extent of the TAG reduction. Shannon and co-workers may have found a significant reduction in the lipaemic

response if they had not corrected for the energy deficit produced by exercise. It is possible that any significant reduction in lipaemia after resistance exercise is only a product of the energy debt produced by exercise. However, as Gill & Hardman (2000) showed, aerobic exercise reduces TAG concentrations through mechanisms over and above an energy deficit created by diet alone. Further investigation is needed to see if this is the case with resistance exercise.

It is unlikely that many individuals would choose a pattern of exercise similar to that in the present study. The estimated rate of energy expenditure (22.5 kJ per minute) in the present study, however, is similar to that seen in the epidemiological studies on longshoremen involved in heavy work (21.8 to 31.5 kJ per minute) (Paffenbarger *et al.*, 1975). The findings in the present study, therefore, in conjunction with the study on longshoremen provides evidence that individuals involved in heavy manual labour will have an increased protection against coronary heart disease. The present study also suggests that some of the benefits from this type of work will be from the acute rather than chronic effects of exercise.

Estimated total energy expenditure from the multiple bouts of resistance exercise (5.1 MJ) in the present study is lower than the energy expended by longshoremen over a days work (7.9 MJ) (Paffenbarger *et al.*, 1975). This is because longshoremen worked an eight hour day, whereas the multiple bouts of resistance exercise were over five hours. However, for longshoremen involved in moderate and light work, both total energy expenditure per eight hour work day (moderate 6.2 MJ; light 3.6 MJ) and the rate of energy expenditure (work intensity) (moderate 10.1 to 21 kJ per minute; light 6.3 to 8.4 kJ per minute) was much lower than their colleagues involved in

heavy work. The adjusted relative risk of coronary heart disease for those longshoremen in the moderate and light work categories was 1.7 and 1.8 respectively compared to those longshoremen involved in heavy work (relative risk 1.0). Therefore, only those in the heavy work category had a reduced risk of coronary heart disease. Whether the reduction in coronary heart disease was because of the total energy expended, the intensity of the work or both in the Paffenbarger *et al.* study is difficult to conclude. The present study found a reduction in postprandial TAG concentrations with a total energy expenditure equivalent to the moderate workers in the Paffenbarger *et al.* study and a rate of energy expenditure which would put them in heavy work group. Certainly, previous studies on aerobic exercise (Tsetsonis & Hardman, 1996b; Gill *et al.*, 2002) suggest that total energy expenditure is more important than intensity of exercise for postprandial lipaemia. The present study in conjunction with the study by Shannon *et al.* (2005) and the study in Chapter 4 of this thesis would also seem to support this being the case for resistance exercise and postprandial lipaemia. The study by Petitt and colleagues would suggest that exercise intensity was more important, although the exercise intensity in their study was similar to the other studies in this area. Despite the differences noted here Paffenbarger and colleagues were examining relative risk of coronary heart disease with work related activity, whereas the present study was examining a single risk factor for heart disease.

The relationship between fasting TAG and total and incremental area under the TAG concentration *versus* time curve is interesting. Previous studies have demonstrated a positive relationship between fasting TAG concentrations and postprandial lipaemia (Patsch *et al.*, 1983; Cohn *et al.*, 1988; Potts *et al.*, 1994). However, despite the close

relationship between fasting and postprandial TAG concentrations, the postprandial TAG response is often independent of basal TAG concentrations. This has been demonstrated in studies where some participants exhibited higher postprandial lipaemia than others with similar fasting TAG concentrations (Merrill *et al.*, 1989; Cohen *et al.*, 1989). In the present study fasting TAG may have explained a large amount of the variance in the total area under the TAG concentration versus time curve (control $r^2=0.92$; exercise $r^2=0.81$). However, for the incremental area under the TAG concentration versus time curve, fasting TAG did not explain so much of the variance (control $r^2=0.61$, exercise $r^2=0.33$), particularly for the exercise trial. This means that only a small part of the increase in TAG concentrations on the exercise trial is to be explained by the post-absorptive concentrations.

It is difficult to determine whether the reduction in TAG concentrations seen the day after the resistance exercise in the present study was because of an increase in skeletal muscle lipoprotein lipase activity or a decrease in hepatic VLDL output. There was a strong tendency toward increased NEFA concentrations on the exercise trial. An increased output of NEFA in the postprandial period from adipose tissue could increase delivery of this substrate to the liver. In the liver NEFA can be esterified into VLDL particles. This would increase the output of hepatic VLDL. However, the ketone body 3-hydroxybutyrate is a product of NEFA oxidation in the liver and the significant increase seen on the exercise trial in this study suggests that hepatic NEFA oxidation was increased. Increased hepatic NEFA oxidation would be in response to the energy debt created by exercise. This would lead to decreased VLDL output. Furthermore, an increased supply of NEFA on the exercise trial may have been directed toward skeletal muscle for oxidation in response to the energy debt created

by the resistance exercise. There is some evidence, therefore, for a decreased VLDL output on the exercise trial.

It is difficult to provide firm evidence for an increased removal of TAG particles by skeletal muscle lipoprotein lipase in the present study. As noted in study one (Chapter 4, Pg.60) the effect of resistance exercise on lipoprotein lipase in skeletal muscle, to the author's knowledge, is unknown. A single session of prolonged and vigorous aerobic exercise has been shown to increase lipoprotein lipase activity and its gene expression in skeletal muscle (Sady *et al.*, 1986; Ferguson *et al.*, 1998; Kiens & Richter, 1998; Greiwe *et al.*, 2000). Ferguson and colleagues (1998) suggest that a minimum of 4.6 MJ needs to be expended through exercise to induce significant increases in lipoprotein lipase activity 24 hours post-exercise. The total energy expenditure in the present study is above the value suggested by Ferguson *et al.* This could be one reason why a reduction in TAG concentrations was seen in the present study and not in some of the previous studies (Shannon *et al.*, 2005; Chapter 4 in this thesis) on resistance exercise and postprandial lipaemia. Lipoprotein lipase activity was not measured in the present study, though, and so support for this hypothesis cannot be provided.

Skeletal muscle lipoprotein lipase activity may also have been increased because of the reduced insulin concentrations observed in the exercise trial of the present study. The activity of lipoprotein lipase in muscle is decreased by insulin (Kiens *et al.*, 1989; Richelsen *et al.*, 1993). However, despite the decreased insulin concentrations with exercise in the present study no indices of insulin and TAG showed a significant relationship. This agrees with the findings of Gill and co-workers (2002) who found

no relationship between the exercise-induced changes in fasting or postprandial TAG and insulin after aerobic exercise. Interestingly, in the present study time to peak TAG was correlated with time to peak insulin on the control trial. Perhaps insulin may affect TAG concentrations more in the absence of exercise than in the presence of exercise. Nonetheless, no other indices of insulin and TAG were correlated on the control trial and so too much speculation should be avoided.

Despite the improved insulin concentrations observed with resistance exercise in the present study the effect of resistance exercise on insulin is debatable. Some previous studies have found no change in fasting insulin concentrations 22-24 hours after the last bout of resistance exercise in trained persons (Kraemer *et al.*, 1998; Pratley *et al.*, 1994). Other studies have reported that resistance training decreases insulin concentrations (Poehlman *et al.*, 2000), that resistance trained persons have lower absolute insulin concentrations than untrained persons (Poehlman *et al.*, 1992) or that resistance training improves insulin signalling (Yaspelkis III, 2006). It is possible that as with aerobic exercise (King *et al.*, 1988) improvements in insulin sensitivity with resistance exercise are not seen in the absence of acute exercise.

In summary, the present study demonstrated that if energy expenditure from resistance exercise is high enough then a reduction in postprandial lipaemia is achieved. This suggests that, as with aerobic exercise, energy expenditure appears to be the main determinant of the exercise-induced reduction in postprandial TAG concentrations. Despite the reduction observed in TAG concentrations in the present study it seems unlikely that most individuals would undertake, or have time to complete, a pattern of exercise similar to that used in the protocol here. Moreover,

current recommendations for resistance exercise do not suggest performing multiple bouts daily.

Chapter 6

The effect of a single session of resistance exercise on postprandial triacylglycerol responses in a group of resistance trained males

6.1 Introduction

The results of the previous chapter suggest that if energy expenditure from resistance exercise is high enough then a reduction in postprandial lipaemia, similar to that seen in studies of aerobic exercise, can be achieved. This is supported by evidence from epidemiological studies which suggest that people who regularly perform heavy work have a reduced risk of cardiovascular disease (more precisely coronary heart disease in these studies) in comparison with similar workers involved in more sedentary work (Paffenbarger *et al.*, 1975).

Expending energy by performing heavy work throughout the day is less common in society than previously. Labour saving devices and sedentary jobs prevent many people in society from accumulating energy expenditure in this way. Moreover, current exercise recommendations for resistance training do not suggest performing multiple bouts daily (Kraemer *et al.*, 2002; Kraemer *et al.*, 2004). To date only three studies, including one in this thesis (Study 1, Chapter 4), have examined the effect of a single session of resistance exercise on postprandial lipaemia (Pettit *et al.*, 2003; Shannon *et al.*, 2005). The findings by Pettitt and colleagues that a single bout of resistance exercise reduces postprandial lipaemia conflict with those in study one of this thesis and require clarification. A possible explanation for these discrepant findings is the nature of the participants involved in the respective studies i.e. resistance trained (Pettit *et al.*, 2003) *versus* untrained (Study 1, Chapter 4). Skeletal

muscle soreness/damage may occur in untrained individuals who are unaccustomed to resistance exercise. It is possible this interfered in some way with TAG transport across the muscle membrane and hence the clearance of TAG from plasma into skeletal muscle. However, the recent study by Shannon and co-workers (2005) involved resistance trained males and females and failed to detect any influence of resistance exercise on postprandial TAG concentrations assessed the following day.

To date, no studies have examined postprandial TAG responses immediately after resistance exercise. The present study examined the effect of resistance exercise one-hour before a test meal on postprandial TAG concentrations in a group of resistance trained young adult males. One-hour post-exercise was chosen as the time to give the test meal to participants as free glycerol from adipose tissue breakdown may still be high in the immediate post-exercise period. This may falsely elevate postprandial TAG concentrations when plasma samples were analysed (see Chapter 2, Pg.23). The present study employed a one-day model i.e. exercise and postprandial testing were performed on the same day. This contrasts with the previous studies which have used a two-day model i.e. exercise on day one and postprandial testing on day 2 (Petitt *et al.*, 2003; Shannon *et al.*, 2005; Chapter 4). A one-day model was employed to negate any differences in physical activity and diet that may occur post-exercise in a two-day model. Another reason was to examine postprandial TAG responses during an earlier portion of the post-exercise period. Skeletal muscle lipoprotein lipase activity has a delayed increase post-exercise and peak muscle lipoprotein lipase activity is thought to occur >8 hours after exercise (Seip *et al.*, 1997). However, this post-exercise change in skeletal muscle lipoprotein lipase activity has only been demonstrated with aerobic exercise, whereas resistance exercise has not been examined. Moreover,

changes in postprandial TAG responses may be related more to changes in VLDL-TAG output than lipoprotein lipase activity (Malkova *et al.*, 2000; Gill *et al.*, 2001; Gill *et al.*, 2003). Changes to VLDL-TAG output may take place during the early portion of the post-exercise period. Certainly in the post-absorptive state, VLDL concentration has been found to drop 4.5 hours following moderate aerobic exercise (Borsheim *et al.*, 1999). Indeed, aerobic exercise has been shown to lower postprandial TAG concentrations on the same day as exercise is performed (Zhang *et al.*, 1998; Murphy *et al.*, 2000; Katsanos *et al.*, 2004; Katsanos & Moffatt, 2004) and one meta-analytic study found no effect of timing of exercise on the postprandial response to exercise (Petitt & Cureton, 2003). This demonstrates the importance of examining all time points in the postprandial period.

6.2 Methods

6.2.1 Participants

Ten male volunteers aged 22-31 years participated in the study. In addition to screening criteria, all participants in this study were required to be regularly participating in resistance exercise (\geq two sessions a week \geq 6 months). Some physical characteristics of the participants are shown in Table 6.1.

Table 6.1. Physical characteristics of the participants.

Age (yrs)	25.2	\pm	2.5
Height (m)	1.74	\pm	0.07
Body mass (kg)	78.6	\pm	12.2
BMI (kg·m ⁻²)	25.8	\pm	2.5
Body fat (%)	16.8	\pm	3.9
Waist circumference (m)	0.82	\pm	0.08
Systolic blood pressure (mm Hg)	120	\pm	8
Diastolic blood pressure (mm Hg)	69	\pm	7

Values are mean \pm SD, $n=10$

6.2.2 Preliminary tests

Prior to the main trials participants visited the laboratory twice for preliminary tests. On the first visit anthropometric data were collected and 12-repetition maximum tests completed. Twelve-repetition maximum tests were completed for each of the 10 resistance exercises employed in the study. The order in which the 12-repetition

maximum tests were performed was the same for each participant i.e. squat, dumbbell lateral raise, bench press, upright row, lunges, bicep curl, barbell pullover, shoulder press (seated), triceps extension and bent over row.

On the second visit participants completed a 90 min familiarisation resistance exercise session. The purpose of the familiarisation session was to ensure that each participant was able to complete the entire exercise session and also to confirm that the weights lifted were producing fatigue from overload by the end of the session. This was confirmed by visual inspection and by verbal feedback from participants.

6.2.3 Main trials

Following the preliminary tests participants undertook two trials – exercise and control. The order of the trials was randomised and balanced. The first trial was performed a minimum of one-week after the familiarisation session. The interval between the two trials was at least one-week. Participants recorded their diet during the 48 hours preceding their first trial. They then replicated this for the 48 hours prior to their second trial. Participants were also asked to refrain from physical activity 48 hours preceding each main trial (Chapter 3 Pg.37).

6.2.4 Main trial protocol

Participants reported to the laboratory at 08.00 h after a 12 h overnight fast. A baseline blood sample was obtained from an antecubital or forearm vein by venepuncture. Participants then consumed a confectionary bar (Mars 54 g). A clock was started with the first bite of this bar. Participants rested for the next 30 minutes. On the exercise trial participants then completed a 90 minute weight lifting session.

On the control trial participants continued resting during this period. At the end of this period a cannula was inserted into an antecubital or forearm vein. One hour later (approximately 11.00 h) a blood sample was taken. Participants then consumed the test meal. All participants consumed the meal within 20 minutes. The clock was re-started with the first bite of this meal (time 0). Further blood samples were obtained 0.25, 0.5, 1, 2, 3, 4 and 5 hours after the re-start of the clock. The cannula was kept patent by flushing with nonheparinised saline ($9 \text{ g}\cdot\text{L}^{-1}$, B.Braun Medical Ltd, Buckinghamshire, UK). The first 2 mL of blood withdrawn was always discarded to avoid dilution of the sample. Water was available ad libitum during both trials. Participants rested (reading, working, watching television) throughout the postprandial observation period. A schematic representation of the main trial protocol is given in Figure 6.1.

6.2.5 Weight lifting protocol

Each participant performed three sets of 12 repetitions of 10 different weight-lifting exercises at 80% of 12-repetition maximum. Three sets of 12 for each exercise were employed in order to maximise the total energy expenditure of the session and hence increase the likelihood of reducing postprandial TAG concentrations. A training intensity of 80% of 12-repetition maximum was chosen to represent a typical resistance-training program reflecting a balance between strength training and hypertrophy (Kraemer *et al.*, 2002). Pilot work revealed that a higher intensity would have prevented most participants from completing three sets of each exercise and thus would have lowered the total energy expenditure. Participants were given three minutes in which to complete each set. On completion of the 12 repetitions participants rested for the remainder of the three minutes. Thus, the whole exercise

session lasted for 90 minutes (10 exercises \times 3 sets \times 3 min). Exercises were completed in the order described for the preliminary tests. All sets for one exercise were completed before moving on to the next exercise.

6.2.6 Expired air collections during weight lifting

Expired air samples were collected into Douglas bags (Plysu Protection Systems, Milton Keynes, U.K.) during the third set of each weight lifting exercise. Samples were collected for the full duration of the lifting and recovery period i.e. three minutes. Energy expenditure was calculated as being 5.047 kcal (21.1 kJ) per litre of oxygen (McArdle, Katch & Katch, 1991) (see General Methods - Chapter 3, Pg.38).

6.2.7 Analytical methods

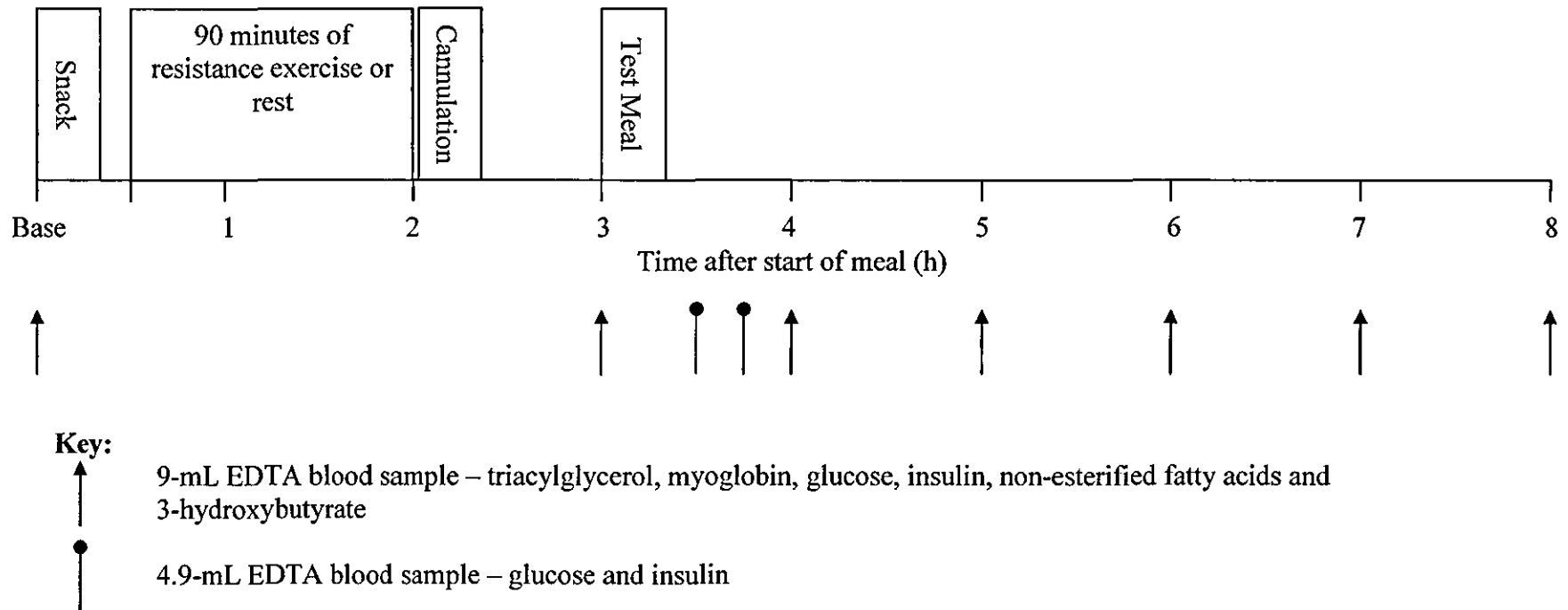
Plasma samples were analysed for TAG, myoglobin (an early marker of muscle damage), glucose, insulin, 3-hydroxybutyrate (a ketone body produced during the oxidation of fatty acids in the liver) and NEFA (see General Methods - Chapter 3, Pg.42). Haemoglobin concentration and haematocrit were determined in samples collected at baseline and at the end of the day so that changes in plasma volume could be estimated (Dill & Costill, 1974) (see General Methods - Chapter 3, Pg.42).

6.2.8 Data analysis

Five-hour area under the plasma concentration *versus* time curve (i.e. during the postprandial period) was calculated for TAG and myoglobin. The incremental area under the plasma concentration *versus* time curve was calculated for TAG. Fasting and area under the curve values were compared between trials using Student's *t*-tests for correlated means. Two-way ANOVA (repeated measures) was used to determine

differences between trials and over time for five-hour plasma concentrations of TAG, myoglobin, insulin, glucose, NEFA and 3-hydroxybutyrate. Where appropriate post-hoc pair wise comparisons were made using the Bonferroni method. Relationships between variables were evaluated using Pearson's product-moment correlation coefficient.

Figure 6.1. Schematic representation of the main trial protocol.



6.3 Results

6.3.1 Resistance exercise session

The mean weight lifted during the 90 minute resistance exercise session was 12951 ± 588 kg. The gross energy expenditure from the exercise was estimated to be 1.63 ± 0.09 MJ and the net (gross minus resting) energy expenditure was estimated at 1.09 ± 0.09 MJ.

6.3.2 Plasma concentrations in the fasted state

Plasma concentrations in the fasted state are shown in Table 6.2. There were no between trial differences for TAG, myoglobin, insulin, glucose or 3-hydroxybutyrate concentrations. Plasma NEFA concentrations were higher on the control trial than the exercise trial.

Table 6.2. Plasma concentrations in the fasted state.

	Control			Exercise			<i>P</i>
TAG (mmol·L ⁻¹)	1.11	±	0.10	1.45	±	0.30	0.197
Myoglobin (nmol·L ⁻¹)	2.06	±	0.21	2.03	±	0.21	0.861
NEFA (mmol·L ⁻¹)	0.42	±	0.05	0.26	±	0.03	0.048
Insulin (pmol·L ⁻¹)	119.4	±	16.0	119.6	±	13.5	0.983
Glucose (mmol·L ⁻¹)	4.44	±	0.10	4.47	±	0.11	0.762
3-Hydroxybutyrate (mmol·L ⁻¹)	0.06	±	0.02	0.04	±	0.01	0.148

Values are mean ± SE, *n*=10. TAG, triacylglycerol; NEFA, non-esterified fatty acids.

6.3.3 Postprandial plasma responses

Changes in plasma volume over the period of observation did not differ between trials ($P=0.849$). No adjustments were made, therefore, to measured concentrations of plasma constituents.

Plasma TAG concentrations were higher on the exercise than the control trial throughout the postprandial period (Figure 6.2): main effect of trial ($P=0.010$), main effect of time ($P<0.0005$). The increase in plasma TAG concentrations was steeper on the exercise than the control trial after the test meal: trial \times time interaction ($P=0.007$). Post-hoc tests revealed that the difference in TAG concentrations between trials occurred two, three and four hours after consumption of the meal. The total area under the TAG concentration *versus* time curve during the postprandial period was 48% (148% of control) higher on the exercise compared to the control trial (11.76 ± 1.64 *versus* 7.94 ± 1.08 mmol \cdot L $^{-1}\cdot$ 5h respectively; $P<0.008$). The incremental area under the TAG concentration *versus* time curve was 89% higher (189% of control) on the exercise compared to the control trial (5.06 ± 0.69 *versus* 2.68 ± 0.40 mmol \cdot L $^{-1}\cdot$ 5h respectively; $P<0.005$).

Myoglobin concentrations were higher on the exercise trial compared to the control trial (Figure 6.3): main effect of trial ($P=0.007$). Myoglobin concentrations declined steadily on the exercise trial but remained constant during the control trial: main effect of time ($P<0.0005$), trial \times time interaction ($P=0.001$). Post-hoc tests revealed that differences in myoglobin concentrations between trials occurred at the 1 h and 2 h time points post-exercise. The total area under the myoglobin concentration *versus* time curve was 145% higher (245% of control) on the exercise compared to the

control trial (16.68 ± 3.34 versus 6.80 ± 0.64 nmol·L⁻¹·5h respectively; $P<0.010$). No relationship was observed between total area under the TAG and myoglobin concentration *versus* time curves for either trial. Moreover, there was no significant relationship between the incremental area under the curve for TAG and myoglobin total area under the curve on the exercise trial, $r=-0.199$, $P=0.582$. Similarly, there was no relationship between the change in the AUC for TAG (exercise values minus control values) and the change in the AUC for myoglobin (exercise values minus control values), $r=-0.134$, $P=0.711$.

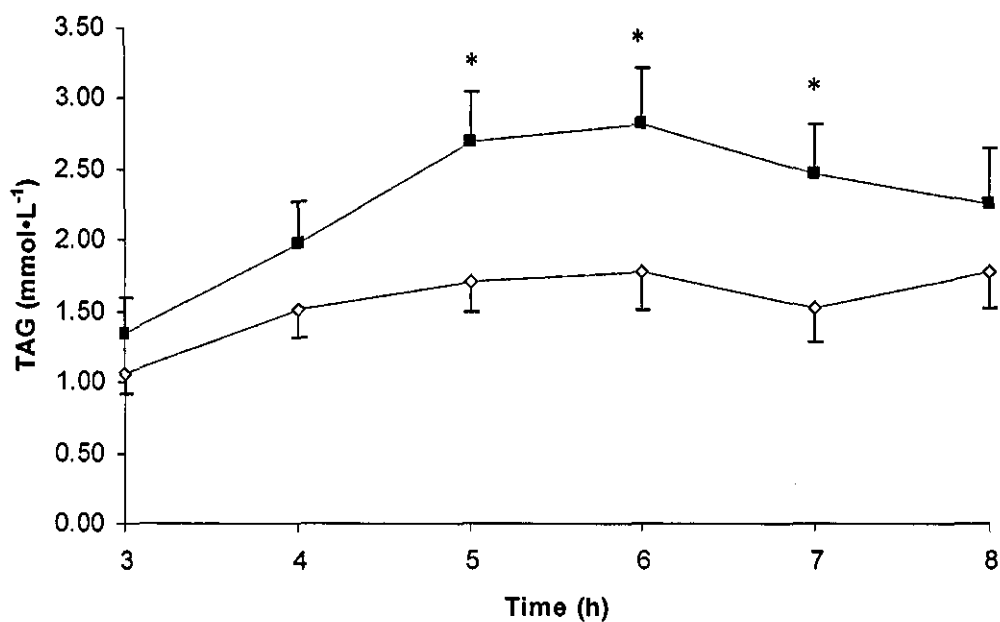


Figure 6.2. Postprandial total plasma triacylglycerol (TAG) concentrations for 5 h following consumption of a test meal in the control (◇) and resistance exercise (■) trials. Values are mean \pm SE, $n = 10$. Main effect of trial ($P=0.010$), main effect of time ($P<0.0005$), trial \times time interaction ($P=0.007$). *Significantly different ($P<0.05$) between trials using a Bonferroni post hoc test.

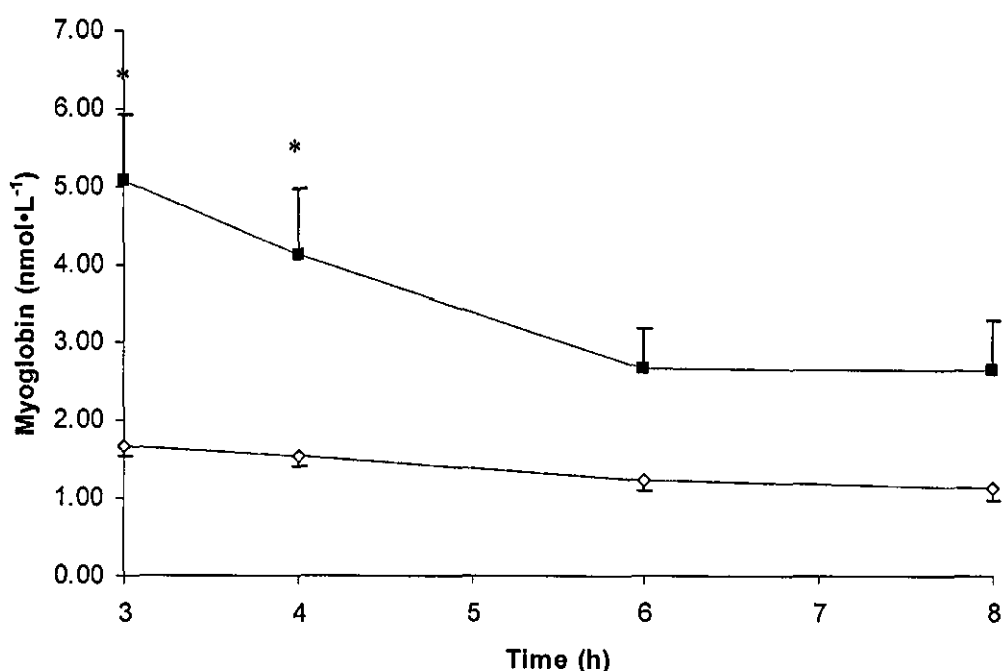


Figure 6.3. Postprandial total plasma myoglobin concentrations for 5 h following consumption of a test meal in the control (◇) and resistance exercise (■) trials. Values are mean \pm SE, $n = 10$. Main effect of trial ($P=0.007$), main effect of time ($P<0.0005$), trial \times time interaction ($P=0.001$). *Significantly different ($P<0.05$) between trials using a Bonferroni post hoc test.

Plasma concentrations during the five-hour postprandial observation period for insulin and glucose are shown in Figure 6.4. Insulin concentrations rose steeply and then fell after the test meal on both the control and exercise trials: main effect of time ($P<0.0005$). Plasma glucose concentrations showed a similar pattern of response to insulin on both trials, rising steeply in the early postprandial period and then falling back to baseline concentrations within the hour: main effect of time ($P=0.003$). There were no between trial differences or trial by time interactions for glucose and insulin.

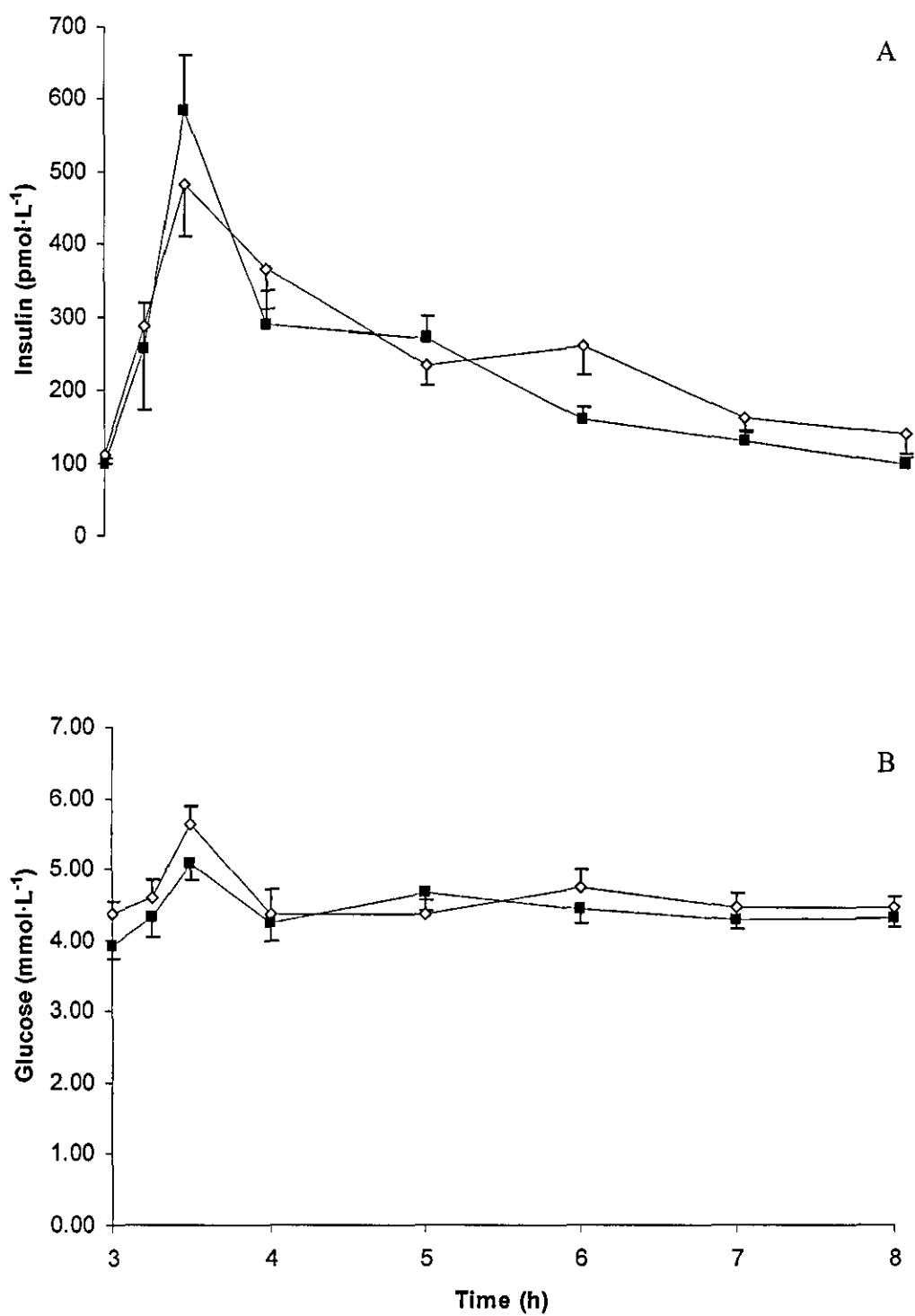


Figure 6.4. Postprandial plasma insulin (A) and glucose (B) concentrations for 5 h following consumption of a test meal in the control (◇) and resistance exercise (■) trials. Values are mean \pm SE, $n=10$. Main effect of time ($P<0.0005$) for insulin. Main effect of time ($P=0.003$) for glucose.

Plasma NEFA concentrations fell on both trials during the first hour after the test meal (Figure 6.5). On the exercise trial NEFA concentrations then rose steeply until the end of the trial. In comparison, on the control trial NEFA concentrations continued falling to the third hour of the postprandial period after which they increased: main effect of trial ($P=0.016$), main effect of time ($P<0.0005$), trial \times time interaction ($P=0.006$). Post-hoc tests revealed that between trial differences occurred during the last three hours of the postprandial period. Plasma 3-hydroxybutyrate concentrations were higher postprandially on the exercise than control trial: main effect of trial ($P=0.030$), main effect of time ($P=0.022$). The pattern of response was similar between trials for 3-hydroxybutyrate (Figure 6.5).

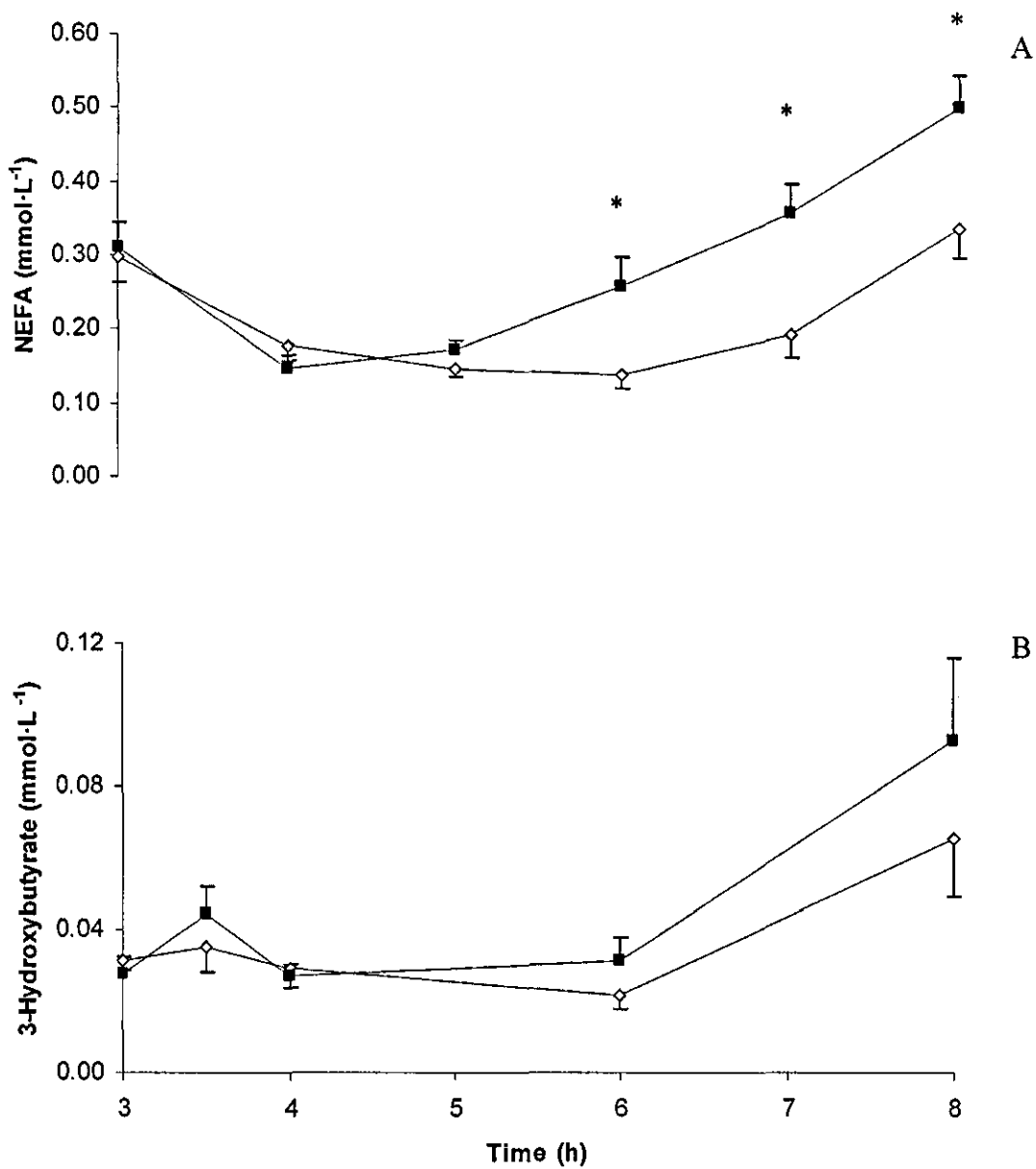


Figure 6.5. Postprandial plasma non-esterified fatty acid (NEFA) (A) and 3-hydroxybutyrate (B) concentrations for 5 h following consumption of a test meal in the control (◇) and resistance exercise (■) trials. Values are mean \pm SE, $n=10$. Main effect of trial ($P=0.016$), main effect of time ($P<0.0005$) and a trial \times time interaction ($P=0.006$) for NEFA. Main effect of trial ($P=0.030$), main effect of time ($P=0.022$) for 3-hydroxybutyrate. *Significantly different ($P<0.05$) between trials using a Bonferroni post hoc test.

6.4 Discussion

The main finding in the present study is that a single session of resistance exercise, performed one-hour prior to a test meal, increases postprandial TAG concentrations. This is in contrast to findings from studies of aerobic exercise, which demonstrate a lowering of postprandial TAG concentrations either on the same day as exercise is performed (Zhang *et al.*, 1998; Murphy *et al.*, 2000; Katsanos *et al.*, 2004; Katsanos & Moffatt, 2004) or on the following day (Aldred *et al.*, 1994; Tsetsonis & Hardman, 1996a; Tsetsonis & Hardman, 1996b; Tsetsonis *et al.*, 1997; Gill *et al.*, 2003). Moreover, the findings in the present study are also in conflict with previous studies (Pettit *et al.*, 2003; Shannon *et al.*, 2005; Chapter 4) that have examined resistance exercise and postprandial TAG concentrations.

It is possible that the gross energy expenditure elicited in the present study (mean = 1.6 MJ; range = 1.3-2.2 MJ) was insufficient to reduce TAG concentrations. In previous studies of aerobic exercise that have attenuated postprandial TAG concentrations gross energy expenditure has ranged from 1.5 to 7.2 MJ (Petitt & Cureton, 2003). The energy expenditure in the present study is at the lower end of this spectrum. However, Petitt and co-workers (2003) observed a reduction in postprandial TAG concentrations following resistance exercise in their study and the gross energy expenditure (1.7 MJ) was estimated to be very similar to that observed in the present study. Moreover, the energy expenditure elicited in the present study does not explain why postprandial TAG concentrations are more elevated following exercise.

In the first study of this thesis (Chapter 4, Pg.61-62) the author speculated that the physiological stress of unfamiliar resistance exercise might cause skeletal muscle

damage in participants who are not resistance trained and that this may inhibit TAG clearance into muscle. It is known that skeletal muscle damage may cause a transient insulin resistance (Del Aguila, 2000; Kirwan & Jing, 2002). It is feasible that this could cause a compensatory increase in insulin secretion from the pancreas. This could reduce the uptake of TAG into skeletal muscle via an inhibition of skeletal muscle lipoprotein lipase activity since the activity of this enzyme in muscle is decreased by insulin (Kiens *et al.*, 1989; Richelsen *et al.*, 1993). However, the participants in the present study were accustomed to resistance exercise and they performed a familiarisation session which should have had a prophylactic effect (Byrnes *et al.*, 1985). Moreover, there is no evidence that insulin concentrations were elevated following exercise in the present study. Certainly the effect of resistance exercise on insulin concentrations is debatable as it has been shown that resistance training improves insulin signalling (Yaspelkis III, 2006), although the acute and chronic effects of exercise do not always match. One last point is that although it has been hypothesised that improved insulin sensitivity reduces TAG clearance, Gill and co-workers (2002) found no relationship between the exercise-induced changes in fasting and postprandial TAG and insulin. This suggests that insulin sensitivity does not mediate TAG disposal.

Some support for a 'muscle damage' hypothesis is provided by the elevated myoglobin concentrations that were observed following resistance exercise in the present study. However, there was no relationship between myoglobin area under the curve values and TAG area under the curve values or between the change in the total area under the curve for TAG (exercise values minus control values) and the change in the total area under the curve for myoglobin. Such relationships might be expected

if muscle damage is a determinant of TAG transport into muscle. However, myoglobin is only a transient marker for muscle damage (Byrnes *et al.*, 1985) and this may explain the lack of association between myoglobin and TAG concentrations.

It is possible that the increase in myoglobin concentrations on the exercise trial in the present study was indicative of an increase in systemic inflammation. Skeletal muscle damage resulting from the exercise could have lead to the release of proinflammatory cells such as tumor necrosis factor- α and interleukin-6 (Del Aguila *et al.*, 2000; Kirwan & Jing, 2002). Inflammation associated with these cells may have resulted in a transient endothelial dysfunction (Van Oostrom *et al.*, 2003) and hence a reduction in muscle blood flow. Such a response is not seen with moderate intensity aerobic exercise, which enhances antioxidant defenses and therefore endothelial function (Di Massimo *et al.*, 2004). However, high intensity exercise, such as that used here, has been shown to cause a temporary increase in free radical formation (Alessio *et al.*, 2000) thereby impairing endothelial function. Whilst the above argument is attractive there is currently little direct data to support it.

Whilst myoglobin has been found to peak early (within hours) after unaccustomed exercise in some studies (Klocke *et al.*, 1982; Byrnes *et al.*, 1985), other studies have seen a much later peak (2 to 4 days) after such exercise (Nosaka *et al.*, 1992; Nosaka & Clarkson, 1996a). If changes in myoglobin had been followed for a more prolonged period a later peak in this marker of muscle damage may have been observed. Furthermore, some of these studies (Nosaka *et al.*, 1992; Nosaka & Clarkson, 1996a) have demonstrated a much larger individual variation in peak myoglobin concentrations (Nosaka *et al.*, 1992 range 4 – 348 nmol·L⁻¹; Nosaka & Clarkson,

1996a range 27 – 171 nmol·L⁻¹) after exercise than that observed in the present study (post exercise range 3 - 10 nmol·L⁻¹). This suggests that in some individuals in the present study myoglobin did not indicate the extent of muscle damage. However, the myoglobin concentrations in the present study agree well with those of Byrnes and colleagues (Byrnes *et al.*, 1985) who observed mean peak myoglobin concentrations of 6.6 nmol·L⁻¹ six hours after a bout of unaccustomed exercise (downhill running) in their study. Nonetheless, using other markers of muscle damage (creatine kinase, heart fatty acid binding protein, muscle function tests, self reported soreness) in the present study alongside myoglobin and following the participants for a more extended period of time would have given a stronger indication of the extent of muscle damage.

Another possible explanation for the increased TAG concentrations on the exercise trial is that hepatic TAG secretion was enhanced. Concentrations of NEFA were higher during the final three hours of the observation period on the exercise compared to the control trial indicating greater endogenous fat mobilisation. It is possible that NEFA were directed to skeletal muscle on the exercise trial to compensate for a reduction in muscle glycogen concentration (Weltan *et al.*, 1998). Alternatively, NEFA supply to the liver may have been increased leading to increased esterification of VLDL-TAG. However, the greater postprandial plasma 3-hydroxybutyrate concentrations on the exercise compared to the control trial indicate that hepatic fatty acid oxidation was increased on the exercise trial. In this case it is likely that VLDL-TAG secretion was reduced (Malkova *et al.*, 2000).

The exercise protocol employed in this study and in previous studies of resistance exercise (Petitt *et al.*, 2003; Shannon *et al.*, 2005; Study 1 Chapter 4) and postprandial

TAG concentrations was not dramatically different. The studies involved a similar number of sets and repetitions, which were performed at similar intensities (the study of Shannon *et al.* involved three different exercise protocols but none exerted an influence on postprandial lipemia). Moreover, the exercise duration in all studies was approximately 90 minutes (one of the exercise protocols in the study of Shannon *et al.* lasted 90 minutes, the others were shorter). The small differences in volume and duration of exercise between these studies seem unlikely to explain the discrepant findings.

More plausible explanations for the different findings can be attributed to the timing of the meals after the exercise. Previous studies (Petitt *et al.*, 2003; Shannon *et al.*, 2005, Study 1 Chapter 4) were conducted over two days and resistance exercise was performed on the afternoon of the first day while postprandial testing was performed on the morning of the following day, approximately 15-16 hours post exercise. Skeletal muscle lipoprotein lipase activity has a delayed increase post-exercise and peak muscle lipoprotein lipase activity occurs >8 hours after exercise (Seip *et al.*, 1997). This may explain why Petitt and colleagues found a reduction in TAG concentrations after exercise and the present study failed to do so. However, this would still not explain why TAG concentrations were elevated following exercise in the present study.

In conclusion, this study has demonstrated that a single bout of resistance exercise increases postprandial TAG concentrations when performed one-hour prior to a test meal. The explanation for this finding requires investigation, but skeletal muscle damage associated with this form of exercise may be responsible.

Chapter 7

A comparison of the effect of resistance exercise and downhill treadmill running on postprandial triacylglycerol concentrations

7.1 Introduction

The results of the previous chapter found that when resistance exercise was performed one-hour prior to a test meal, then a transient increase in postprandial TAG concentrations was observed in comparison to a control (rest) trial. No previous studies have observed an increase in postprandial TAG concentrations after resistance exercise (Petitt *et al.*, 2003; Shannon *et al.*, 2005; Studies 1 & 2 - Chapters 4 & 5). Furthermore, to the author's knowledge, no increase in postprandial TAG concentrations have been observed after a bout of aerobic exercise.

The elevations in plasma myoglobin observed post-exercise in the previous chapter suggest that skeletal muscle damage associated with this form of exercise may have been responsible for the increase in postprandial TAG concentrations by inhibiting TAG clearance into the exercised muscle. Skeletal muscle damage may cause a transient insulin resistance (Del Aguila *et al.*, 2000; Kirwan & Jing, 2002) which could lead to compensatory increases in insulin secretion from the pancreas. This could reduce the uptake of TAG into skeletal muscle via an inhibition of skeletal muscle lipoprotein lipase activity, since the activity of this enzyme in muscle is decreased by insulin (Kiens *et al.*, 1989; Richelsen *et al.*, 1993). However, some studies have found no change in fasting insulin levels 22-24 hours after the last bout of resistance exercise in trained persons (Kraemer *et al.*, 1997; Pratley *et al.*, 1994). Other studies have shown that resistance training decreases insulin concentrations

(Poehlman *et al.*, 2000), or that resistance-trained persons have lower absolute insulin levels than untrained persons (Poehlman *et al.*, 1992). Moreover, no changes in insulin were observed in study three (Chapter 6) of the present thesis. Studies of aerobic exercise do not clarify the evidence as at least one study (Gill *et al.*, 2002) has found no relationship between the exercise-induced changes in fasting and postprandial TAG and insulin.

An alternative explanation may be that skeletal muscle damage from resistance exercise leads to the release of proinflammatory cytokines such as tumor necrosis factor- α and interleukin-6 (Del Aguila *et al.*, 2000; Kirwan & Jing, 2002). Inflammation associated with these cytokines may result in a transient endothelial dysfunction (Van Oostrom *et al.*, 2003) and hence a reduction in muscle blood flow. Furthermore, high intensity exercise, such as resistance exercise, has been shown to cause a temporary increase in free radical formation (Alessio *et al.*, 2000), which may also impair endothelial function.

Whilst the above arguments are attractive there were no direct data from study three to support them. However, to date, no one has examined the effect of a bout of downhill running on postprandial TAG concentrations. Downhill running has been shown to induce post-exercise muscle soreness and markers of muscle damage are increased after downhill running because of the bias towards eccentric contractions (Byrnes *et al.*, 1985; Del Aguila *et al.*, 2000). If muscle damage is a cause of the transient rise in postprandial TAG concentrations following resistance exercise it should follow that this effect may also been seen in downhill running. The present study, therefore, sought to compare the influence of a single session of resistance

exercise and downhill running performed one hour before a test meal on postprandial TAG concentrations. Myoglobin was measured as a marker for post-exercise muscle damage.

In addition to measuring changes in plasma TAG and myoglobin concentrations, serum CRP after resistance exercise was to be measured in this study. Acute strenuous aerobic exercise can produce a transient increase in CRP levels (Taylor *et al.*, 1987; Weight *et al.*, 1991; Fallon *et al.*, 2001; Siegel *et al.*, 2001). Furthermore, at least one study of resistance exercise has demonstrated a 253% significant increase in serum CRP concentrations 47 hours post-exercise in volunteers who completed 300 maximal eccentric contractions of the quadriceps muscle (Paulsen *et al.*, 2005). Therefore, the weight lifting protocol in the present study – which has demonstrated post exercise increases in myoglobin concentrations, one marker of muscle damage (Study 3 Chapter 6) - was deemed ideal to measure CRP in order to see whether weight training would cause changes in CRP concentrations. Moreover, the serum obtained from the participants would be used to pilot test a CRP assay. Traditional assays for CRP have limits of detection between 3-8 mg·L⁻¹ and do not have adequate sensitivity to detect concentrations for healthy individuals (Roberts *et al.* 2001). Therefore, high sensitivity CRP assays have been developed in order to measure CRP in apparently healthy individuals (Roberts *et al.* 2001). These assays have improved sensitivity and precision at low concentrations of CRP and limits of detection are much lower (<0.5 mg·L⁻¹) than seen in traditional assays. However, the author was unsure what values would be obtained before and after weight training. The presence of muscle damage may greatly elevate CRP concentrations and so a traditional CRP assay may be more

appropriate than the high sensitivity version. The secondary aim of this work was to compare serum CRP concentrations between a traditional and high sensitivity assay.

7.2 Methods

7.2.1 Participants

Ten male volunteers aged 21-35 years participated in the study. Some physical characteristics of the participants are shown in Table 7.1.

Table 7.1. Physical characteristics of the participants.

Age (yrs)	25.5	±	4.9
Height (m)	1.79	±	0.05
Body mass (kg)	75.9	±	9.6
BMI (kg·m ⁻²)	23.5	±	2.0
Body fat (%)	17.1	±	4.0
Waist circumference (m)	0.81	±	0.07
Systolic blood pressure (mm Hg)	116	±	13
Diastolic blood pressure (mm Hg)	71	±	7

Values are mean ± SD, *n*=10

7.2.2 Preliminary tests

Prior to the main trials participants visited the laboratory on three separate occasions for preliminary tests. On the first visit anthropometric data were collected. Visit two involved determination of each participants' 12-repetition maximum for 10 different weight lifting exercises. On their third visit to the laboratory participants completed both a submaximal and maximal oxygen uptake ($\dot{V}O_2$ max) test. No familiarisation sessions were performed prior to the main trials as the aim was to maximise the

development of muscle soreness in the two exercise trials and therefore avoid any prophylactic effect of previous exercise.

7.2.3 Twelve-repetition maximum tests

These were completed for each of the 10 resistance exercises employed in the study. The order in which the 12-repetition maximum tests were performed was the same for each participant i.e. squat, dumbbell lateral raise, bench press, upright row, lunges, bicep curl, barbell pullover, shoulder press (seated), triceps extension and bent over row.

7.2.4 Submaximal treadmill test

A 16 min, four-stage, submaximal treadmill test was used to determine the relationship between running speed and $\dot{V}O_2$. Initial running speed was dependent upon participants' running ability and experience. The treadmill was level throughout the test. Speed was increased by between 1 and 1.6 km·h⁻¹ every 4 minutes depending on participants' fitness. Expired air samples, heart rate and ratings of perceived exertion (Borg, 1973) were collected during the final minute of each stage. A linear regression equation was used to calculate the relationship between running speed and $\dot{V}O_2$.

7.2.5 Maximum oxygen uptake test

This was determined using an incremental protocol in three minute stages (Taylor *et al.*, 1955). Treadmill speed remained constant throughout the test. The initial incline of the treadmill was set at 3.5%. Treadmill gradient was increased by 2.5% every three minutes. Expired air samples, heart rate and ratings of perceived exertion were

collected from 1:45 to 2:45 minutes of each stage and throughout the final minute of the test. Participants determined the end point of the test by indicating to the experimenters when they felt they could run for only one further minute. The final expired air collection was started at that point. Strong verbal encouragement was given to participants throughout the test.

7.2.6 Main trials

Following the preliminary tests participants undertook three main trials – resistance exercise, downhill running and control. The order of the trials was randomised and balanced. The interval between each main trial was at least one-week. Participants recorded their diet during the 48 hours preceding their first trial. They then replicated this for the 48 hours prior to their second and third trial. Participants were also asked to refrain from physical activity 48 hours preceding each main trial (Chapter 3 Pg.37).

7.2.7 Main trial protocol

Participants reported to the laboratory at 08.00 h. A baseline blood sample was obtained from an antecubital or forearm vein by venepuncture. Participants then consumed a confectionary bar (Mars 54 g). A clock was started with the first bite of this bar. On the resistance exercise trial participants rested for the next 30 minutes and then completed a 90 minute weight lifting session. The weight lifting session followed the same protocol as that used in study three (Chapter 6). The lifting session aimed to maximise the total energy expenditure of the session and cause muscle damage similar to that seen in study three. The intensity of 80% of 12-repetition maximum represents a typical resistance-training program reflecting a balance between strength training and hypertrophy (Kraemer *et al.*, 2002). In the downhill running trial

participants rested for 85 minutes and then ran for 35 minutes on a treadmill. This protocol has previously been used (Byrnes, 1985) and causes significant increases in muscle soreness, myoglobin and creatine kinase (both markers of muscle damage). On the control trial participants rested for two hours. The resistance exercise and the downhill running were completed so that the interval between cessation of exercise and consumption of the test meal was identical.

At the end of the two hours a cannula was inserted into an antecubital or forearm vein. One hour later (approximately 11.00 h) a blood sample was taken. Participants then consumed the test meal. All participants consumed the meal within 25 minutes. The clock was re-started with the first bite of this meal (time 0). Further blood samples were obtained 0.5, 0.75, 1, 2, 3, 4 and 5 hours after the second start of the clock. The cannula was kept patent by flushing with nonheparinised saline ($9 \text{ g}\cdot\text{L}^{-1}$, B.Braun Medical Ltd, Buckinghamshire, UK). The first 2 mL of blood withdrawn was always discarded to avoid dilution of the sample. Water was available *ad libitum* during all trials. Participants rested (reading, working, watching television) throughout the postprandial observation period and were always lying in a supine position for at least 5 min before each blood sample was taken. A schematic representation of the main trial protocol is given in Figure 7.1.

7.2.8 Blood sampling for CRP

In addition to the blood samples taken as described above (section 7.2.7), blood samples were collected for CRP into 9-mL serum monovettes (see General Methods - Chapter 3, Pg.41). A baseline sample was obtained by venepuncture at 08:00 h when participants reported to the laboratory. A second sample was taken via the venous

cannula one hour after the end of the resistance exercise bout (approximately 11:00 h). The final sample was obtained by venepuncture 24 h post-exercise (approximately 10:00 h the morning following the main trial).

7.2.9 Weight lifting protocol

All parts of this protocol were as described in study 3 (Chapter 6, Pg.95). In brief, each participant performed three sets of 12 repetitions of 10 different weight-lifting exercises at 80% of 12-repetition maximum. Each set, including recovery time, took three minutes to complete. The whole exercise session lasted for 90 minutes (10 exercises \times 3 sets \times 3 min). Exercises were completed in the order described for the preliminary tests. All sets for one exercise were completed before moving on to the next exercise.

7.2.10 Estimation of energy expenditure during weight lifting

Expired air samples were collected into Douglas bags (Plysu Protection Systems, Milton Keynes, U.K.) during the third set of each weight lifting exercise. Samples were collected for the full duration of the lifting and recovery period i.e. three minutes. Energy expenditure was calculated as being 5.047 kcal (21.1 kJ) per litre of oxygen (McArdle, Katch & Katch, 1991) (see General Methods - Chapter 3, Pg.38).

7.2.11 Downhill treadmill running

Participants were initially set running at a treadmill speed calculated to elicit 70% of their $\dot{V}O_2$ max. Participants ran for 5 minutes at this speed at a grade of 0%. The slope of the treadmill was then changed to -10% (5.8°) and the subjects continued to run for a further 30 minutes. All subjects therefore exercised at approximately the

same relative work load. A similar protocol to this has previously been used by Byrnes and co-workers and has been found to produce muscle soreness (Byrnes *et al.*, 1985). Expired air samples were collected into Douglas bags (Plysu Protection Systems, Milton Keynes, U.K.) at 4-5, 19-20 and 34-35 mins during the run. Heart rate was measured using short-range telemetry (Polar Electro, OV), and ratings of perceived exertion were recorded during collections of expired air. Oxygen consumption and $\dot{V}CO_2$ production were determined from expired air samples using a paramagnetic oxygen analyzer and an infrared carbon dioxide analyzer (Servomex Analyser Series 1400; Servomex, Crowborough, East Sussex, U.K.). Expired air volumes were measured using a dry gas meter (Harvard Apparatus, Edenbridge, Kent, U.K.) and corrected to standard temperature and pressure (dry). Energy expenditure during exercise, substrate utilization, carbohydrate oxidation rate ($g \cdot min^{-1}$) and fat oxidation rate ($g \cdot min^{-1}$), were calculated using equations for energy expenditure assuming no protein oxidation (Frayn, 1983).

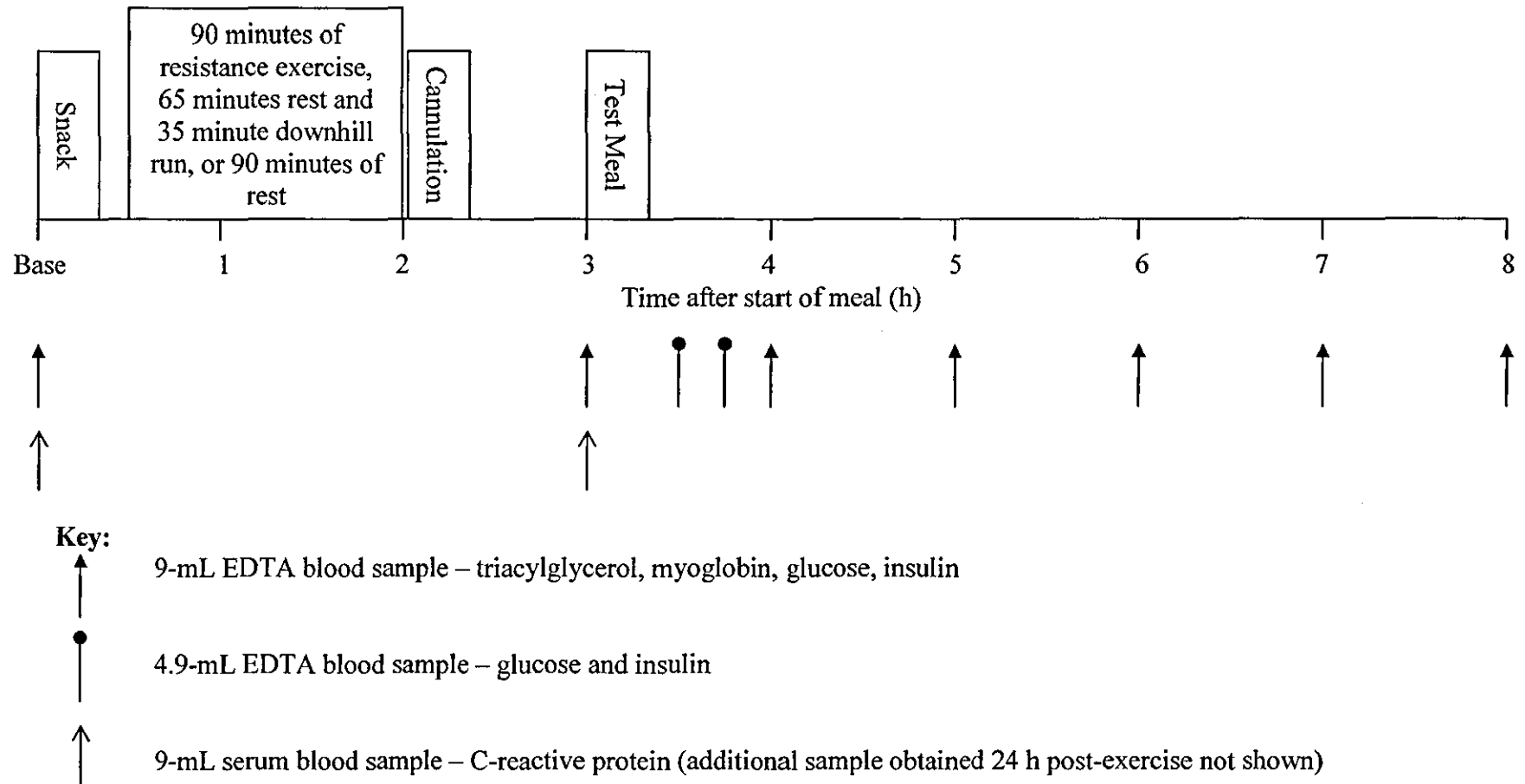
7.2.12 Analytical methods

Plasma samples were analysed for TAG, myoglobin, glucose and insulin (see General Methods – Chapter 3, Pg.42). Serum CRP samples were analysed using a traditional CRP assay (DRG International, Inc., USA) and a high sensitivity CRP assay (DRG International, Inc., USA) (see General Methods - Chapter 3, Pg.42). Haemoglobin concentration and haematocrit were determined in all samples so that changes in plasma volume could be estimated (Dill & Costill, 1974) (see General Methods - Chapter 3, Pg.42).

7.2.13 Data analysis

Five-hour area under the plasma concentration *versus* time curve (i.e. during the postprandial period) was calculated for TAG and myoglobin. The incremental area under the plasma concentration *versus* time curve was calculated for TAG. Fasting and area under the curve values were compared between trials using a one-way ANOVA. Two-way ANOVA (repeated measures) was used to determine differences between trials and over time for five-hour plasma concentrations of TAG, myoglobin, insulin and glucose. Where appropriate post-hoc pair wise comparisons were made using the Bonferroni method. Relationships between variables were evaluated using Pearson's product-moment correlation coefficient.

Figure 7.1. Schematic representation of the main trial protocol.



7.3 Results

7.3.1 Resistance exercise session

The mean weight lifted during the 90 minute resistance exercise session was 12031 ± 510 kg. The gross energy expenditure from the exercise was estimated to be 1.6 ± 0.1 MJ and the net (gross minus resting) energy expenditure was estimated at 1.0 ± 0.0 MJ.

7.3.2 Responses to treadmill running

On a level treadmill average heart rate during running was 153 ± 4 b·min⁻¹ and mean % $\dot{V}O_2$ max elicited was 64 ± 2 %. The median rating of perceived exertion on a level treadmill was 12 (range 9 - 13). In downhill running average heart rate was 152 ± 3 b·min⁻¹ and mean % $\dot{V}O_2$ max elicited was 48 ± 2 %. The median rating of perceived exertion during downhill running was 12 (range 11 - 14). Gross energy expenditure from treadmill running was calculated as 1.7 ± 0.1 MJ with $27 \pm 8\%$ of energy provided from fat and 73 ± 8 % of energy provided from carbohydrate. Net energy expenditure was calculated as 1.5 ± 0.1 MJ.

7.3.3 Plasma concentrations in the fasted state

Plasma concentrations in the fasted state are shown in Table 7.2. There were no between trial differences for TAG, myoglobin, insulin or glucose concentrations.

Table 7.2. Plasma concentrations in the fasted state.

	Control	Weights	Run	<i>P</i>
TAG (mmol·L ⁻¹)	1.34 ± 0.28	1.23 ± 0.21	1.39 ± 0.36	0.509
Myoglobin (nmol·L ⁻¹)	3.45 ± 0.55	2.80 ± 0.23	2.92 ± 0.23	0.454
Insulin (pmol·L ⁻¹)	118 ± 11	145 ± 21	206 ± 64	0.282
Glucose (mmol·L ⁻¹)	5.14 ± 0.13	5.12 ± 0.11	5.46 ± 0.23	0.270

Values are mean ± SE, *n*=10. For myoglobin *n*=8. TAG, triacylglycerol.

7.3.4 Postprandial plasma responses

There was no difference in plasma volume between trials (*P*=0.713). Plasma volume changed over time (*P*=0.038) but did not differ in the pattern of response between trials (*P*=0.668). The change in plasma response over time in each trial meant that concentrations of plasma constituents were adjusted accordingly at each time point.

Plasma TAG concentrations changed over time (*P*<0.0005) but did not differ between trials or in the pattern of TAG response between trials (Figure 7.2). The total area under the TAG concentration *versus* time curve did not differ between control, weights and run trials (10.29 ± 1.63 *versus* 10.60 ± 1.33 *versus* 9.75 ± 1.96 mmol·5h·L⁻¹ respectively; *P*=N.S.). However, the incremental area under the TAG concentration *versus* time curve was significantly different between trials (control

3.51 ± 0.39, weights 4.28 ± 0.58, run 2.62 ± 0.50 mmol·5h·L⁻¹; *P*=0.047). Post-hoc tests could not identify where this difference occurred: weights *versus* control (*P*=0.17); weights *versus* run (*P*=0.14); control *versus* run trial (*P*=0.269). There was no significant difference in peak TAG response (*P*=0.383) or time to peak TAG response (*P*=0.696). Adjusted peak TAG (peak minus fasting concentrations), however, showed a tendency to be different between trials (control 1.28 ± 0.18, weights 1.48 ± 0.23, run 0.94 ± 0.22 mmol·L⁻¹; *P*=0.064).

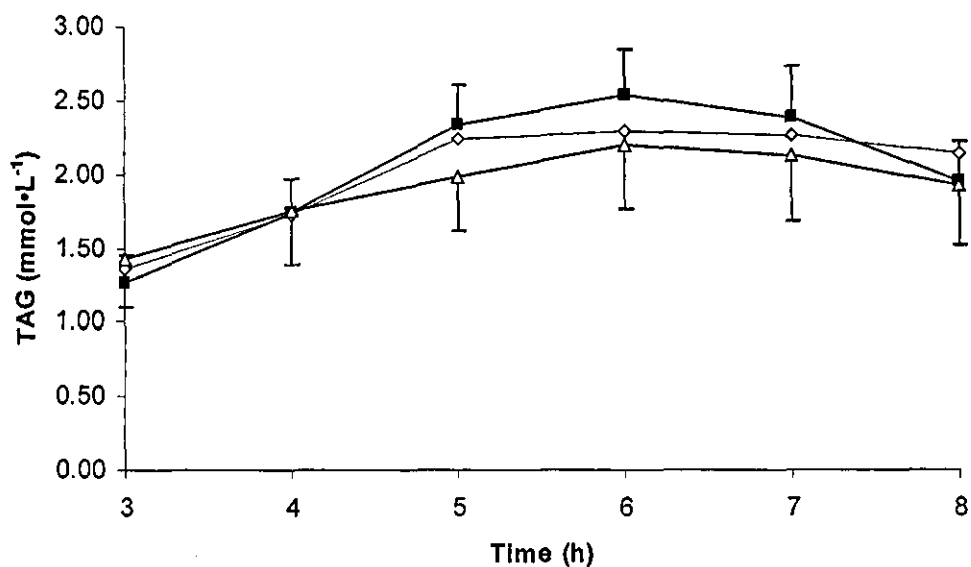


Figure 7.2. Postprandial total plasma triacylglycerol (TAG) concentrations for 5 h following consumption of a test meal in the control (◇), resistance exercise (■) and run (Δ) trials. Values are mean ± SE, *n* = 10. Main effect of time (*P*<0.0005).

Myoglobin concentrations showed a tendency to be higher on the exercise trials compared to the control trial (Figure 7.3): main effect of trial ($P=0.071$). Myoglobin concentrations declined steadily on the exercise trials after the pre-meal sample but remained constant throughout the control trial: main effect of time ($P=0.006$), trial \times time interaction ($P=0.037$). Post-hoc tests could not reveal where the difference in myoglobin concentrations occurred. The total area under the myoglobin concentration *versus* time curve was 117% higher (217% of control) on the weights trial and 115% higher (215% of control) on the run trial compared to the control trial (31.01 ± 5.95 *versus* 30.74 ± 3.54 *versus* 14.27 ± 4.41 $\text{nmol}\cdot\text{5h}\cdot\text{L}^{-1}$ respectively), although these differences were not significant ($P=0.085$).

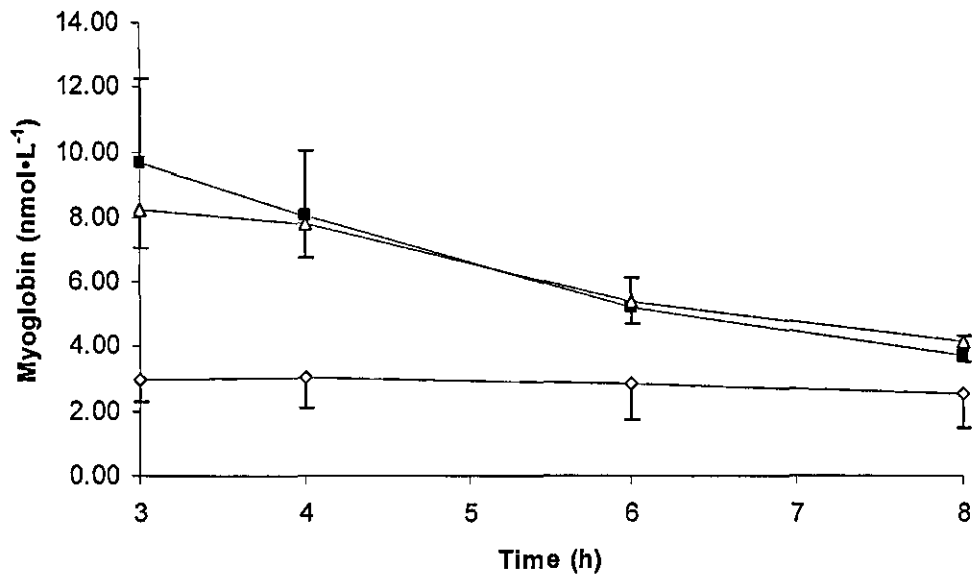


Figure 7.3. Postprandial total plasma myoglobin concentrations for 5 h following consumption of a test meal in the control (\diamond), resistance exercise (\blacksquare) and run (Δ) trials. Values are mean \pm SE, $n = 8$. Main effect of time ($P=0.006$), trial \times time interaction ($P=0.037$).

No relationship was observed in any of the trials between total area under the TAG and myoglobin concentration *versus* time curves. Moreover, there was no significant relationship between the incremental area under the curve for TAG and the total area under the curve for myoglobin on either the weights or run trial. Similarly, there was no relationship between the change in the total area under the curve for TAG (exercise values minus control values) and the change in total area under the curve for myoglobin. No relationship was observed between peak TAG and peak myoglobin values on either exercise trial. Adjusted peak TAG (peak minus fasting concentrations) and adjusted peak myoglobin were not significantly correlated on the weights trial, but a significant correlation was observed on the run trial ($r=0.814$, $P=0.014$) (Figure 7.4). Moreover, on the run trial time to peak TAG and time to peak myoglobin were significantly correlated ($r=0.709$, $P=0.049$) (Figure 7.4).

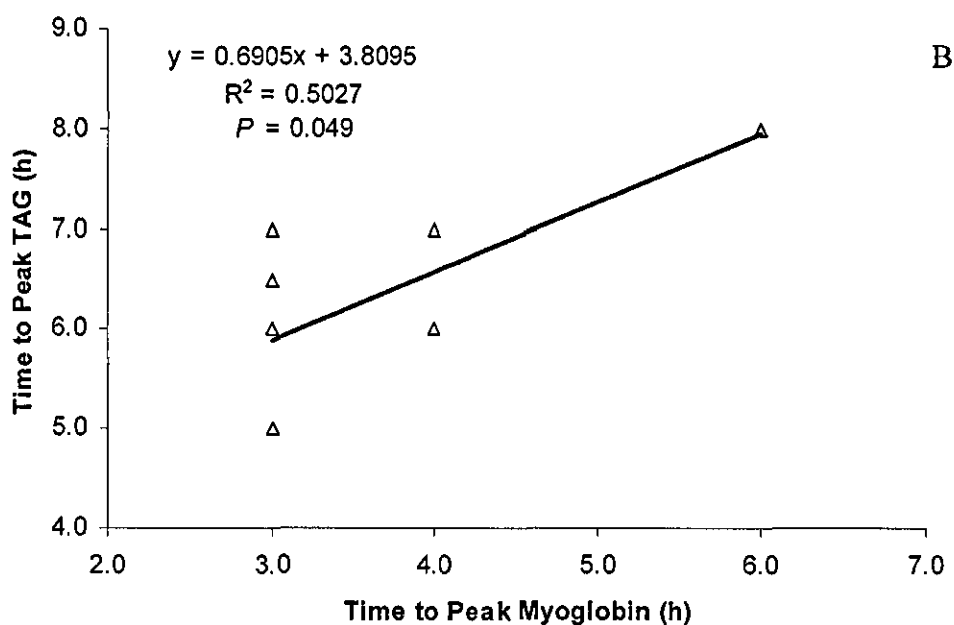
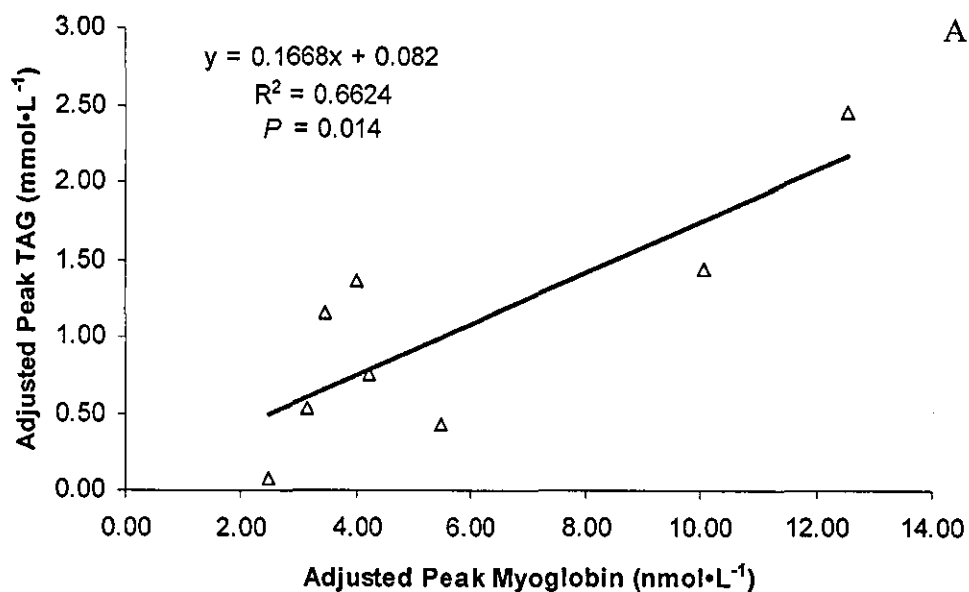


Figure 7.4. (A) Relationship between adjusted peak triacylglycerol (TAG) (peak minus fasting concentrations) and adjusted peak myoglobin on the run trial ($r = 0.814$, $P=0.014$). $n = 8$. (B) Relationship between adjusted peak TAG (peak minus fasting concentrations) and adjusted peak myoglobin on the run trial ($r = 0.709$, $P=0.049$). $n = 8$.

Plasma concentrations during the five-hour postprandial observation period for insulin and glucose are shown in Figure 7.5. Insulin concentrations rose steeply and then fell after the test meal on both the control and exercise trials: main effect of time ($P<0.0005$). Plasma glucose concentrations showed a similar pattern of response to insulin, in all trials, rising in the early postprandial period and then falling back to baseline concentrations within the hour: main effect of time ($P<0.0005$). There were no between trial differences or trial by time interactions for glucose and insulin. No difference was observed in peak insulin response, adjusted peak insulin response or time to peak insulin response between trials. A number of significant (or near significant) relationships were observed between TAG and insulin (Table 7.3) on the run trial. None of these relationships were significant between TAG and insulin in either the control or the weights trial.

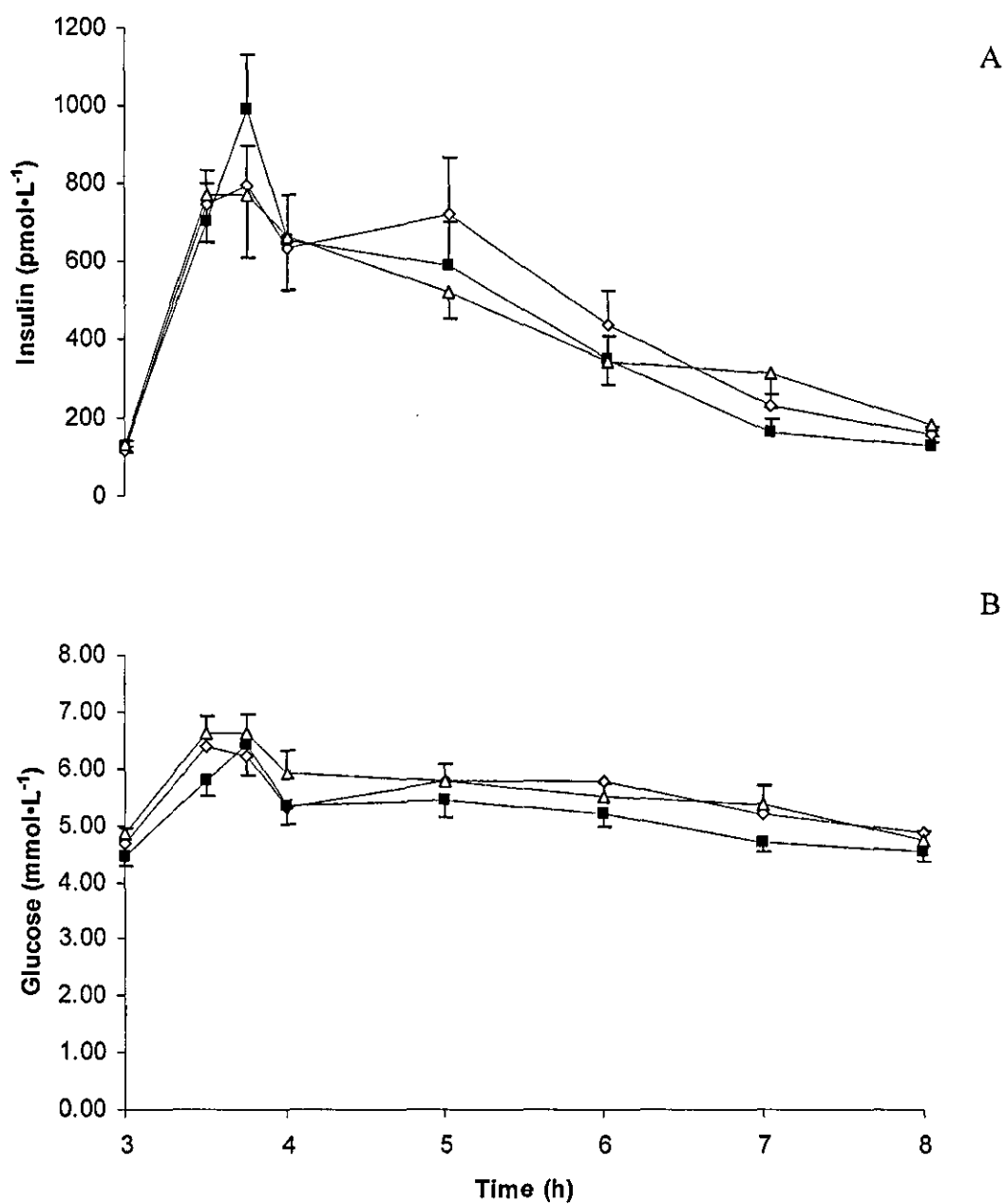


Figure 7.5. Postprandial plasma insulin (A) and glucose (B) concentrations for 5 h following consumption of a test meal in the control (\diamond), resistance exercise (\blacksquare) and run (Δ) trials. Values are mean \pm SE, $n=10$. Main effect of time ($P<0.0005$) for insulin. Main effect of time ($P<0.0005$) for glucose.

Table 7.3. Relationships between indices of TAG and insulin on the run trial.

	Fasting insulin	Insulin total AUC	Peak insulin
Fasting TAG	$r = 0.858$	$r = 0.858$	$r = 0.929$
	$P = 0.002$	$P = 0.001$	$P < 0.0005$
TAG total AUC	$r = 0.534$	$r = 0.728$	$r = 0.798$
	$P = \text{NS}$	$P = 0.017$	$P = 0.006$
TAG IAUC	$r = 0.638$	$r = 0.467$	$r = 0.486$
	$P = 0.047$	$P = \text{NS}$	$P = \text{NS}$
Peak TAG	$r = 0.498$	$r = 0.712$	$r = 0.779$
	$P = \text{NS}$	$P = 0.021$	$P = 0.008$

$n=10$. TAG, triacylglycerol; AUC, area under the concentration *versus* time curve; IAUC, incremental area under the concentration *versus* time curve; NS, non-significant.

7.3.5 Serum CRP concentrations

Complete data sets for serum CRP were collected in six participants only. Two reasons for this were difficulty with venepuncture sampling at baseline or participants failing to return for the 24 hour sample. Nonetheless, the sera were still assayed to compare the concentrations obtained using the traditional CRP assay and the high sensitivity CRP assay.

The traditional CRP assay failed to detect CRP concentrations in over half the samples. The limit of detection for this assay was $1 \text{ mg}\cdot\text{L}^{-1}$. Data from the traditional CRP assay for the six participants who had complete samples is therefore not displayed.

The high sensitivity CRP assay detected CRP concentrations in all assayed samples. The limit of detection for this assay was $0.1 \text{ mg}\cdot\text{L}^{-1}$. There was no significant difference between trials for serum CRP. Serum CRP did not change over time. Although the pattern of CRP response differed between trials (Figure 7.6) this was not significant ($P=0.174$). On the exercise trial CRP dropped one hour post-exercise (11:00 h) from the baseline sample and then climbed 24 hours post-exercise (10:00 h Day2) to above the baseline concentration. On the control trial the pattern of response was the reverse. CRP showed a rise after baseline at the 11:00 h sample and then dropped the following morning (10:00 h Day 2). Individual CRP data from the high sensitivity assay for the six participants who had complete samples is displayed in Table 7.4. There was a large range of values between individuals at baseline on both the control ($0.20\text{-}2.40 \text{ mg}\cdot\text{L}^{-1}$) and exercise ($0.20\text{-}2.20 \text{ mg}\cdot\text{L}^{-1}$) trials. Baseline serum CRP concentrations were not consistent for all participants between trials i.e. a

low/high baseline concentration on one trial was not necessarily matched by a similar value on the other trial. However, concentrations for an individual within a trial were consistent i.e. a low/high concentration at the start of a trial was followed by similar concentrations throughout.

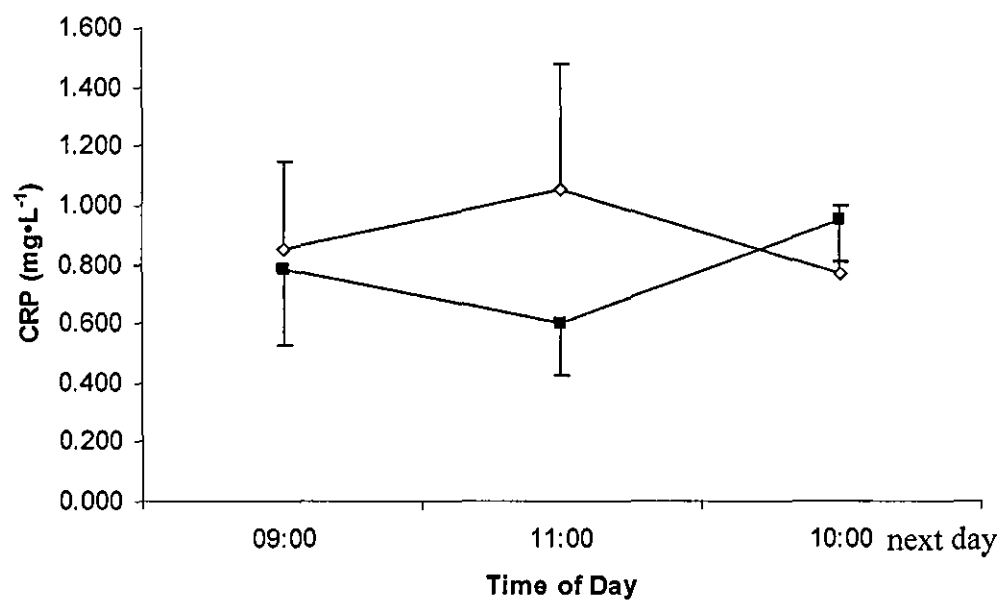


Figure 7.6. Serum CRP concentrations over 26 hours on the control (◇) and resistance exercise (■) trials. Values are mean ± SE, *n*=6.

Table 7.4. Serum C-reactive protein (CRP) concentrations using a high sensitivity assay on the control and resistance exercise trials.

	Day 1		Day 2	
Control	08:00 h	11:00 h	10:00 h	Mean
1	0.50	0.40	0.30	0.40
2	2.40	3.60	2.00	2.67
3	1.60	1.60	1.30	1.50
4	0.20	0.20	0.20	0.20
5	0.10	0.10	0.30	0.17
6	0.30	0.40	0.50	0.40
Mean	0.85	1.05	0.77	
Exercise				
1	0.30	0.10	0.60	0.33
2	0.40	0.40	1.30	0.70
3	2.20	1.30	1.30	1.60
4	0.30	0.20	0.40	0.30
5	1.30	1.30	1.40	1.33
6	0.20	0.30	0.70	0.40
Mean	0.78	0.60	0.95	

All serum CRP concentrations are $\text{mg}\cdot\text{L}^{-1}$.

7.4 Discussion

The main findings in the present study are that a single bout of resistance exercise, performed one-hour prior to a test meal, causes a greater increase in incremental TAG concentrations than a bout of downhill running or resting. Conversely, a single bout of downhill running appears to have no effect on postprandial TAG concentrations in comparison with a control trial. The secondary finding in the present study is that a high sensitivity CRP assay is more appropriate than a traditional CRP assay for detecting changes in CRP in young healthy males after exercise.

Statistical support for increased TAG concentrations after weight training in the present study is not as strong as in study three (Chapter 6). Only incremental TAG concentrations were significantly different between trials in the present study. Post-hoc tests could not identify where this difference occurred. However, the statistical difference between the weights and the control ($P=0.17$) or run trial ($P=0.14$) was higher than the control *versus* run trial ($P=0.269$). Examination of Figure 7.2 (Pg.124) shows that the pre-meal TAG concentration is lowest on the weights trial. Two to four hours into the postprandial period, however, the TAG concentration is highest on the weights trial. This provides supporting evidence for a steeper increase in postprandial TAG concentrations after resistance exercise. Furthermore, adjusted peak TAG concentration was also highest on the weights trial, although again, this did not reach significance ($P=0.064$). Overall, however, the finding of an increased incremental area after resistance exercise provides additional support to the findings in chapter 6. It seems unlikely that the findings in study three (Chapter 6) occurred by chance and therefore the finding that weight lifting produces a transient increase in postprandial TAG concentrations appears to be genuine.

The main hypothesis in the present study was that skeletal muscle damage may inhibit TAG clearance after resistance exercise. Support for this hypothesis is not provided by the findings in the present study. Myoglobin was higher on the exercise trials than the control trials - although this did not reach statistical significance - and decreased on both exercise trials from the pre-meal sample until the end of the trial. However, total and incremental TAG concentrations were lowest on the run trial. If muscle damage was the cause of increased TAG concentrations then a similar increase in incremental TAG between the weights and the downhill run trial should have been seen.

No support for a relationship between skeletal muscle damage and TAG clearance on the weights trial is provided in the present study. No relationship was observed between total myoglobin area under the curve values and TAG area under the curve values (total or incremental) on either exercise trial. The change in the total area under the curve for TAG (exercise values minus control values) and the change in the total area under the curve for myoglobin also showed no relationship on either exercise trial. Neither peak TAG and peak myoglobin, or adjusted peak TAG (peak minus fasting concentrations) and adjusted peak myoglobin, were related on the weights trial. Such relationships might be expected if muscle damage is preventing TAG transport into muscle.

One interesting outcome is that adjusted peak TAG and adjusted peak myoglobin were significantly correlated on the run trial ($r=0.814$, $P=0.014$), as was time to peak TAG and time to peak myoglobin ($r=0.709$, $P=0.049$). This would suggest that on the

run trial muscle damage may be a determinant of TAG transport into the muscles. Certainly, several studies have shown that aerobic exercise lowers postprandial TAG concentrations on the same day as exercise is performed (Zhang *et al.*, 1998; Murphy *et al.*, 2000; Katsanos *et al.*, 2004; Katsanos & Moffatt, 2004) – at least if the test meal contains substantial amounts of fat. It also seems likely, however, that greater energy expenditure during exercise in the studies by Zhang *et al.*, Katsanos *et al.* and Katsanos & Moffat (3.0 MJ – see note at end of chapter -, 4.6MJ and 3.6MJ; respectively) may also explain why the run trial (energy expenditure 1.7 MJ) in the present study failed to decrease postprandial lipaemia. It should be noted, however, that Katsanos and colleagues only found a decrease in postprandial lipaemia with moderate (65% $\dot{V}O_2$ max) and not low (25% $\dot{V}O_2$ max) intensity exercise, in their study, despite total energy expenditure being equal between the two trials. To confuse the evidence further, Murphy and colleagues (2000) found a decrease in the day long lipaemic response with only 30 minutes of walking. This would suggest that the run in the present study should have been enough to reduce the lipaemic response and that possibly muscle damage did interfere with TAG disposal. Certainly the evidence is confusing.

Differences in energy expenditure do not appear to explain why TAG concentrations were higher after resistance exercise than downhill running. Estimated total energy expenditure was similar on the weights and run trial (although this was not planned) and so the postprandial TAG response should have been similar. Net energy expenditure was higher on the run than weights trial because of the shorter duration of exercise. However, differences in the net energy expenditure of exercise may not cause differences in the postprandial TAG concentrations. Tsetsonis & Hardman

(1996b) demonstrated that a 3 hour walk at 32% of maximum oxygen uptake produced a similar reduction in the postprandial response to a 90 minute walk at 63% of maximum oxygen uptake. Differences in net energy expenditure do not, therefore, seem to influence postprandial lipaemia. However, as noted above Katsanos and colleagues (2004) found that exercise intensity and, therefore, net energy expenditure did appear to influence the lipaemic response. Perhaps the timing of exercise before the test meal may affect the extent of a reduction in postprandial lipaemia, although the meta-analytic study by Petitt & Cureton (2003) found no effect of timing of exercise on the postprandial response. Regardless, of whether exercise timing affects the extent of reduction in postprandial lipaemia, the original issue of why TAG concentrations should be raised after a bout of weight training remains unexplained, although it now seems unlikely that muscle damage is the cause.

It has been previously stated (Chapter 6, Pg.107) that the activity of the enzyme skeletal muscle lipoprotein lipase is decreased by insulin (Kiens *et al.*, 1989; Richelsen *et al.*, 1993). This could lead to reduced TAG uptake into skeletal muscle post-exercise. The fact that insulin was not different between the run and weights trial, or the weights and control trial, suggests that a change in insulin sensitivity is not the mechanism responsible for causing a post-exercise increase in TAG on the weights trial. This would concur with the findings in study three (Chapter 6). As noted in study 3 (Chapter 6, Pg.107) although resistance training has been thought to improve insulin signalling (Yaspelkis III, 2006), the acute and chronic effects of exercise do not always match.

On the run trial several indicators of TAG concentration were strongly related to indicators of insulin concentration. This would suggest that insulin was a determinant of TAG concentration on the run trial. This was not the case for either the weights or control trial. Despite the relationships between insulin and TAG on the run trial neither insulin concentrations, nor glucose concentrations, were significantly lowered after running. Gill and co-workers (2002) have previously found no relationship between exercise-induced changes in TAG and insulin after 90 minutes of exercise, at approximately 62% of maximal oxygen uptake, despite decreased TAG concentrations and improved indices of insulin sensitivity. In agreement with other studies (Abbott *et al.*, 1987; Manolio *et al.*, 1990; Jeppesen *et al.*, 1995), however, Gill and colleagues did demonstrate correlations between indices of insulin sensitivity and TAG metabolism in the unexercised state. It is possible that energy expenditure was not high enough on the run in the present study to reduce TAG and induce significant changes to insulin sensitivity. This may explain why TAG and insulin were correlated on the run trial in the present study. It is interesting to note, however, that no correlations were observed between TAG and insulin on the control trial in the present study. No explanation can be offered for this finding.

The finding that a high sensitivity CRP assay is more appropriate than a traditional CRP assay for detecting changes in serum CRP concentrations post-exercise in a group of healthy young males is perhaps not surprising. The majority of serum CRP concentrations at baseline were $<1 \text{ mg}\cdot\text{L}^{-1}$ on both the control and exercise trial which is considered low-risk for a future cardiovascular event (Ridker *et al.*, 2003; Pearson *et al.*, 2003) and reflects the population being tested. However, participants two and three on the control trial and two and five on the exercise trial demonstrated serum

CRP concentrations between 1 and 3 mg·L⁻¹ which is considered a moderate risk for a future cardiovascular event (Ridker *et al.*, 2003; Pearson *et al.*, 2003). Therefore, it seems that serum CRP concentrations can vary quite widely over a short period of time even in healthy young individuals. It should be noted that infection and trauma can effect serum CRP concentrations quite dramatically (Ridker *et al.*, 2003; Pearson *et al.*, 2003) and so it is possible that the change seen in some of these individuals between trials could have been for this reason. Despite the individual variation observed in baseline serum CRP concentrations between the control and exercise trial, serum CRP concentrations did not change dramatically after exercise. It seems therefore that a high sensitivity assay is most appropriate for measuring post-exercise changes in serum CRP.

It may have been expected that an interaction (trial × time) for serum CRP concentrations would have been seen in the present study as the pattern of CRP responses differs between the control and exercise trials. Furthermore, the two CRP curves cross one another (Figure 7.6, Pg.132) which normally indicates an interaction. However, the reduced participant number on the present study probably failed to detect a difference. Nonetheless, these data indicate that CRP response post-exercise may differ from the pattern of response in a non-exercised state and therefore the subject is worth further study. In addition, at least one study of resistance exercise has demonstrated a significant increase in serum CRP concentrations after resistance exercise (Paulsen *et al.*, 2005) which adds support to study the topic further.

In summary, the findings in the present study confirm those in the previous chapter (Chapter 6) of an elevation in postprandial TAG concentrations following resistance

exercise. The increase in TAG concentrations after weight training does not appear to be related to either muscle damage (as indicated by changes in myoglobin concentration) or changes in insulin concentrations. Further investigation is needed to establish the cause of the postprandial increase in TAG concentrations after weight training. A secondary finding is that 30 minutes of downhill running does not lower postprandial TAG concentrations in comparison to a non-exercise control trial. Postprandial TAG concentrations after downhill running may be related to muscle damage or insulin concentrations. Finally, a high sensitivity CRP assay is the most appropriate method for studying changes in CRP with exercise.

Note: The energy expenditure of exercise in the study by Zhang *et al.* (1998) was not stated in the paper. Energy expenditure was estimated from mean $\dot{V}O_2$ max (48 ml·kg⁻¹·min⁻¹), mean body weight (82kg), intensity of the exercise trial (60% of $\dot{V}O_2$ max), duration of exercise (60 minutes of treadmill exercise) and 21.1 kJ per litre of oxygen (McArdle, Katch & Katch, 1991). Energy expenditure was thus calculated from these estimates as:

$$[(48 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1} \times 82 \text{ kg})/1000] \times 0.6 \times 60 \text{ min} \times 21.1 \text{ kJ} = 3.0 \text{ MJ}$$

There may, therefore, be some error between this estimate and the actual energy expenditure of participants in the study by Zhang *et al.* (1998).

Chapter 8

The effect of resistance exercise on serum C-reactive protein concentrations

8.1 INTRODUCTION

Atherosclerosis bears many of the hallmarks of a chronic inflammatory disease (Ross, 1999). C-reactive protein is a marker of inflammation in the circulation and may also play an aetiological role in atherosclerosis (Tracy, 1998; Yeh & Willerson, 2003). Moreover a number of prospective studies have shown a link between CRP and coronary heart disease (Ridker *et al.*, 1998; Ridker *et al.*, 2000). Interventions that reduce CRP concentrations may, therefore, reduce the development of atherosclerosis.

The evidence for physical activity reducing CRP concentrations is confusing. Whilst the majority of cross-sectional studies have noted an inverse relationship between physical activity or physical fitness and serum CRP (Dufaux *et al.*, 1984; Albert *et al.*, 2004; Aronson *et al.*, 2004; Katja *et al.*, 2005; LaMonte *et al.*, 2005), training studies have failed to agree on the effect of aerobic exercise on CRP concentrations (Liesen *et al.* 1977; Tisi *et al.*, 1997; Smith *et al.*, 1999; Mattusch *et al.*, 2000; Marcell *et al.*, 2005; Hammett *et al.*, 2004; Hammett *et al.*, 2006). Moreover, several studies have demonstrated that acute strenuous exercise produces a transient increase in CRP concentrations (Taylor *et al.*, 1987; Weight *et al.*, 1991; Fallon *et al.*, 2001; Siegel *et al.*, 2001). These studies suggest that the increase in CRP post-exercise seems to be proportional to the volume/intensity of activity and the extent of muscle injury (Strachan *et al.*, 1984). One possible mechanism for the post-exercise increase in CRP is the rise in interleukin-6 that occurs during exercise (Febbraio & Pedersen, 2002).

Interleukin-6 is the main stimulant for hepatic production of CRP and increases up to 100-fold during and after strenuous exercise.

To the author's knowledge, only three studies have examined the effect of resistance training/exercise on serum CRP concentrations. Castaneda and colleagues (2004) found a reduction in serum CRP concentrations after 12 weeks of resistance training in kidney patients on a low-protein diet. The effect of an acute bout of resistance exercise on serum CRP concentrations is unclear. Nosaka and Clarkson (1996) found no change in plasma CRP concentrations up to five days post-exercise in participants who completed a single bout of 24 maximal eccentric contractions of the elbow flexors in their non-dominant arm. This was despite up to 100-fold increases in creatine kinase, a post exercise marker of muscle damage, over this period. Conversely Paulsen and coworkers (2005) saw a peak 253% significant increase in serum CRP concentrations 47 hours post-exercise in volunteers who had completed 300 maximal eccentric contractions of the quadriceps muscle. The exercise was randomly divided between the dominant and non-dominant leg. Creatine kinase peaked 95 hours after exercise in this study and loss of force-generating capacity correlated with peak concentrations of both CRP and creatine kinase. As with aerobic exercise, any increase in CRP with resistance exercise may be proportional to the volume/intensity of activity and the extent of muscle injury.

The aim of the present study was to examine the effect of multiple bouts of resistance exercise on serum CRP concentrations. It was expected that the high volume of resistance exercise completed in the present study would increase CRP concentrations in the 24 hour post-exercise period.

8.2 METHODS

8.2.1 Participants

Ten male volunteers aged 20-21 years participated in the study. Some physical characteristics of the participants are shown in Table 8.1.

Table 8.1. Physical characteristics of the participants.

Age (yrs)	20.8 ± 0.5
Height (m)	1.81 ± 0.06
Body mass (kg)	79.4 ± 11.5
BMI (kg·m ⁻²)	24.1 ± 2.7
Body fat (%)	17.3 ± 4.7
Waist circumference (m)	0.87 ± 0.08
Systolic blood pressure (mm Hg)	117 ± 7
Diastolic blood pressure (mm Hg)	75 ± 7

Values are mean ± SD, *n*=10

8.2.2 Preliminary tests

Prior to the main trials participants visited the laboratory once for collection of anthropometric data and to complete one-repetition maximum weight lifting tests for five different resistance exercises. The order in which the one-repetition maximum tests were performed was the same for each participant: squat, bench press, lunges, bicep curl and shoulder press (seated).

8.2.3 Main trials

All parts of the main trial for the present study were identical to those previously described in study two (Chapter 5, Pg.67-73). Only a shortened version of the methods will be described here to remind the reader of the protocol. In brief participants undertook two, 2 d trials – exercise and control. On day one of the exercise trial participants lifted multiple bouts of light weights over the course of the day. On day one of the control trial participants rested. On day two of both trials participants came to the lab fasted and consumed a test meal. The order of the trials was randomised and balanced. The interval between the two trials was at least one week. Participants recorded their diet during the 48 hours preceding their first trial. They then replicated this for the 48 hours prior to their second trial. Participants were also asked to refrain from physical activity 48 hours preceding each main trial (Chapter 3 Pg.37).

8.2.4 Weight lifting protocol – day 1

On the exercise trial participants reported to the laboratory at 9.00 a.m. Over the day, each participant completed 20 sets of 15 repetitions of 5 different weight-lifting exercises. These sets were divided into five, 45 min bouts of weight lifting (Figure 8.1). Each 45 min bout of lifting consisted of 4 sets of each of the 5 different exercises. Participants completed one set of each exercise sequentially in the same order as described for the preliminary tests. Participants were given one minute to recover between each exercise. After the last exercise (shoulder press) participants were given 5 minutes to recover. The sequence was then repeated until 4 sets of each exercise had been completed. After 4 sets of each exercise had been completed an

extended break was given until the end of the hour when the sequence of lifting would resume (Figure 8.2).

8.2.5 Estimation of energy expenditure during weight lifting

Expired air samples were collected during the 1st and 4th sets of the first and fifth 45 min bouts of exercise. The samples were collected into separate Douglas Bags for each exercise. Samples were collected for the full duration of the lifting and recovery period i.e. the exercise time plus 1 min recovery. For the shoulder press the sample was collected for the exercise time plus 5 min recovery.

A two-way ANOVA (see Data Analysis) was used to determine if there were any differences in oxygen uptake between expired air samples. Oxygen uptake was calculated as the mean of all five exercises over the four sets collected. Energy expenditure was calculated as being 5.047 kcal (21.1 kJ) per litre of oxygen (McArdle, Katch & Katch, 1991) (see General Methods - Chapter 3, Pg.38). The duration of the exercise was calculated as the mean collection time over the four sample collections multiplied by twenty (the total number of sets completed). Gross energy expenditure was calculated as mean oxygen uptake multiplied by 21.1 kJ multiplied by duration of exercise. Net energy expenditure was calculated as gross minus resting energy expenditure over the same duration.

8.2.6 Main trial protocol - day 2

Participants reported to the laboratory at 08.00 h after a 12 h fast. A cannula was inserted into an antecubital vein or forearm. Participants then consumed the test meal. Water was available *ad libitum* during the first trial; the volume ingested was

recorded and replicated in the second trial. Participants rested (reading, working, watching television) throughout the observation period (Figure 8.3).

8.2.7 Blood sampling for CRP

The following blood samples were collected for the analysis of serum CRP (see Figures 8.2 and 8.3). On day one of the exercise trial (approximately 9.00a.m.) a 9-mL blood sample was taken by venepuncture into a serum monovette before participants began weight lifting. A second blood sample was collected in the same way at the end of the day after the multiple bouts of weight lifting (approximately 3.30 p.m.). On day two of the exercise trial additional blood samples for the collection of serum were taken at baseline and at three and six hours into the postprandial period. On the control trial blood samples for the analysis of serum were collected at identical time points to those just described.

8.2.8 Analytical methods

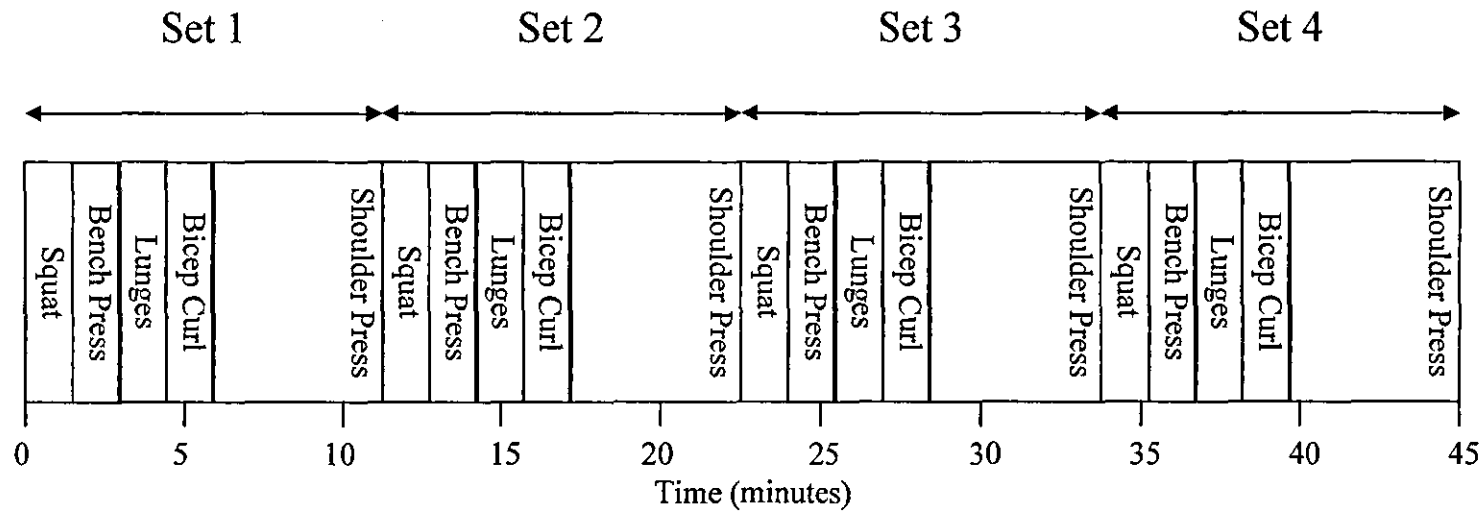
Samples were analysed for serum CRP concentration using a high sensitivity CRP assay (see General Methods - Chapter 3, Pg.42).

8.2.9 Data analysis

Two-way ANOVA (repeated measures) was used to determine differences between oxygen uptake at each expired air sample measurement point (i.e. 1st and 4th sets of the first and fifth 45 min bouts of exercise) and the exercise being performed (i.e. squat, bench press, lunges, bicep curl, shoulder press). Baseline values on day one of each trial were compared between trials using Student's *t*-tests for correlated means. Two-way ANOVA (repeated measures) was used to determine differences between

trials and over time for CRP. Where appropriate post-hoc pair wise comparisons were made using the Bonferroni method. Relationships between variables were evaluated using Pearson's product-moment correlation coefficient.

Figure 8.1. Schematic representation of one 45 minute bout of weight lifting.



- One forty five minute bout of weight lifting divided into four sets.
- Each set consists of 15 repetitions of each exercise plus 1 min recovery. 5 min recovery after shoulder press
- Squat and lunges at 40% of one repetition maximum.
- Bench press, bicep curl and shoulder press at 30 % of one repetition maximum

Figure 8.2. Schematic representation of weight lifting protocol – day 1.

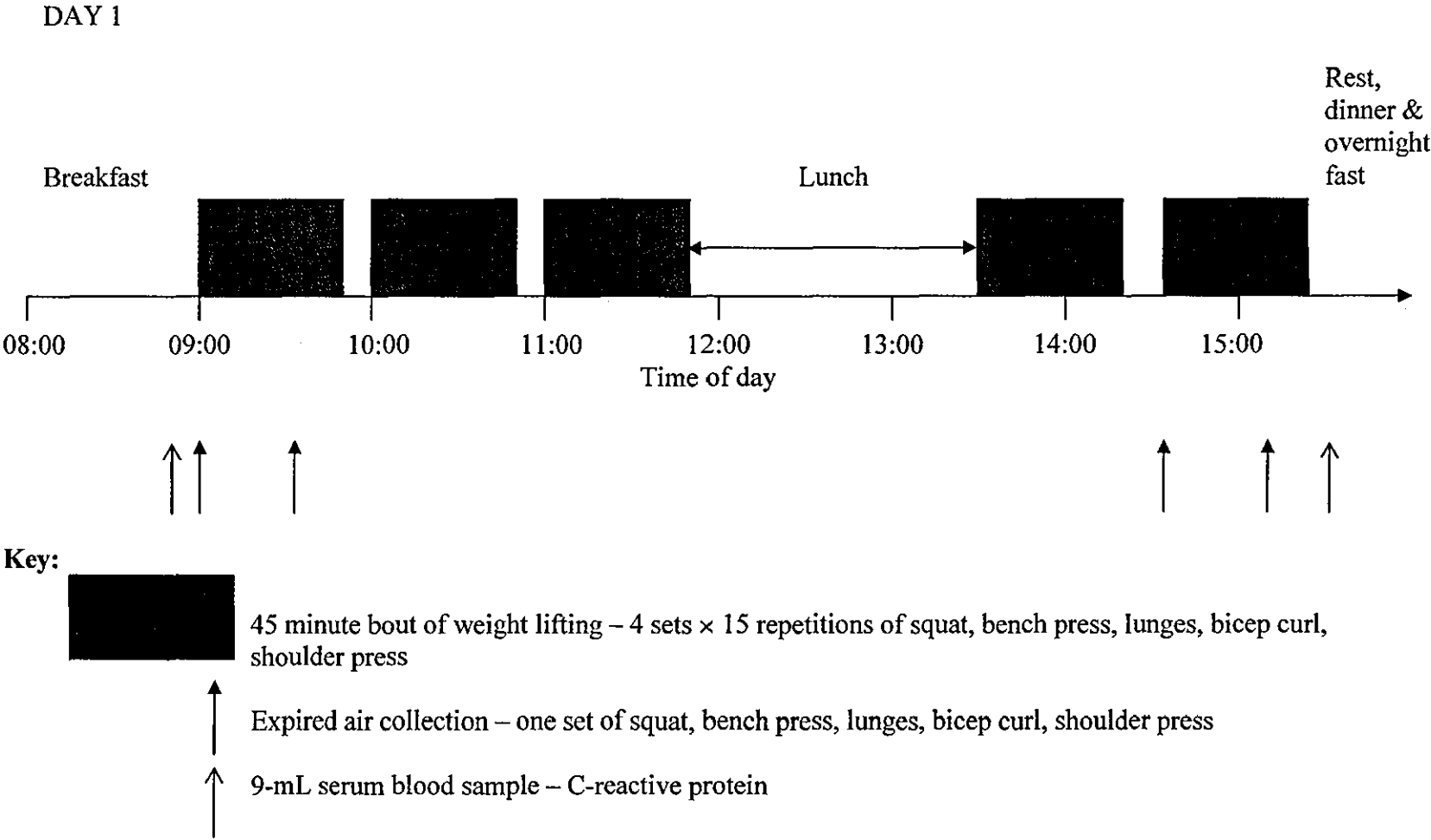
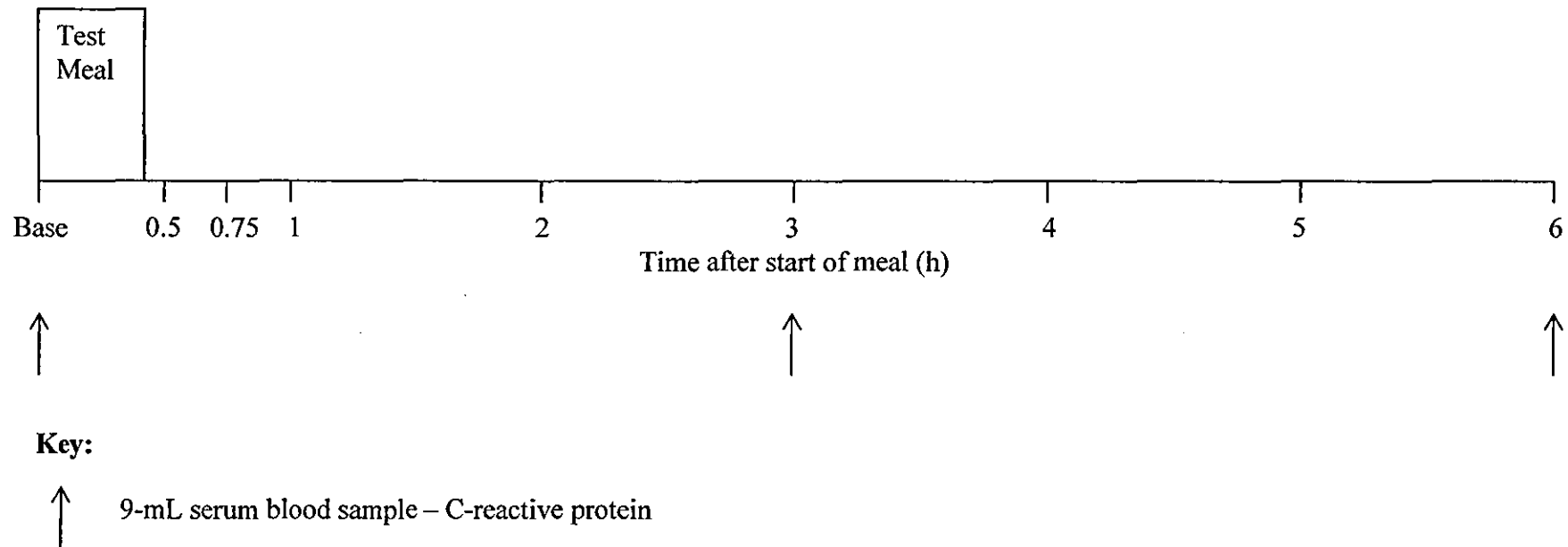


Figure 8.3. Schematic representation of the main trial protocol – day 2.

DAY 2



8.3 RESULTS

8.3.1 Resistance exercise session

The mean weight lifted over the course of the day was 34850 ± 1584 kg. The average weight lifted on each exercise was 23 ± 1 kg. There was no difference in oxygen uptake at each expired air sample measurement point ($P=0.363$). Oxygen uptake differed between exercises ($P<0.0005$) but did not show a different pattern of response at measurement points ($P=0.340$). The mean gross energy expenditure from the exercise was estimated to be 5.2 ± 0.4 MJ over the day. Mean net energy expenditure from exercise was estimated to be 3.5 ± 0.3 MJ.

8.3.2 Baseline serum concentrations

One participant who had serum CRP concentrations $> 10 \text{ mg}\cdot\text{L}^{-1}$ on the control trial was excluded from the study after analysis of the data. A CRP concentration $> 10 \text{ mg}\cdot\text{L}^{-1}$ may be indicative of infection or trauma. Such samples should be discarded and a new sample taken again at a later date (Ridker *et al.*, 2003; Pearson *et al.*, 2003). Further conversation with the participant revealed that he had been ill with a cold prior to the trial.

Baseline concentrations of CRP did not differ significantly between trials for CRP ($P=0.149$).

8.3.3. Changes in serum CRP

Serum CRP concentrations were not significantly different between trials or over time i.e. no main effect of trial or time. The pattern of CRP response was significantly different between trials: trial \times time interaction ($P=0.028$) (Figure 8.4). There was a

steady rise in CRP concentrations from baseline on day one of the exercise trial to baseline on day two. Thereafter, a sharp rise was observed in CRP concentrations at three hours into the postprandial period on day two. C-reactive protein then dropped slightly between three and six hours into the postprandial period. On the control trial CRP fell slightly between the morning of day one and two. The CRP concentration then remained steady throughout day two of the control trial. The mean peak CRP concentration on the exercise trial was $2.08 \text{ mg}\cdot\text{L}^{-1}$. This was 249% higher than the mean baseline value ($0.83 \text{ mg}\cdot\text{L}^{-1}$). Relationships between indices of CRP and indices of TAG for the nine subjects in this study were examined. No significant correlations were observed between CRP and TAG.

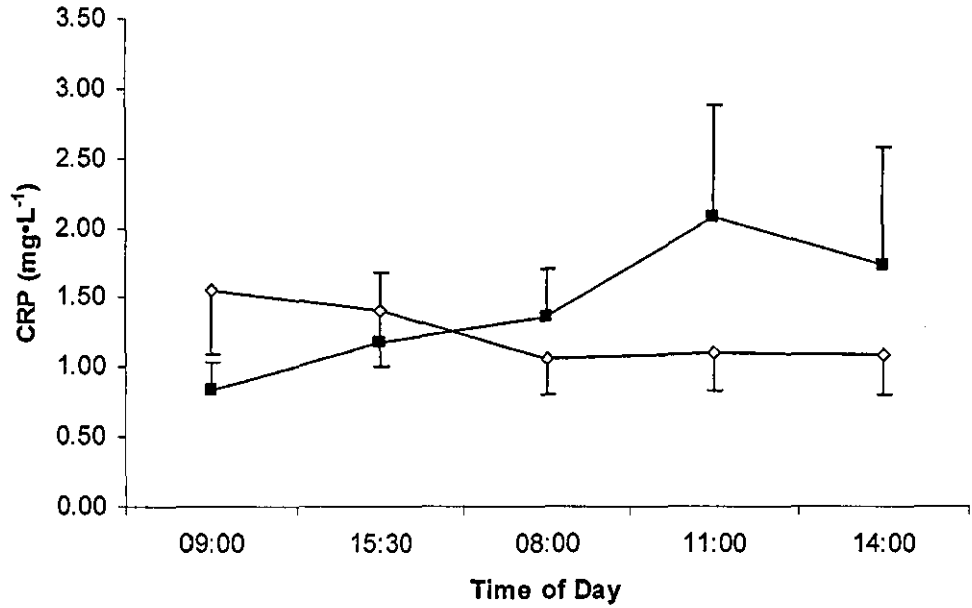


Figure 8.4. Serum C-reactive protein (CRP) concentrations in the control (◇) and resistance exercise (■) trials. Values are mean \pm SE, $n=9$. Trial \times time interaction ($P=0.028$).

Individual data on CRP concentrations are presented in Table 8.2. Baseline serum CRP concentrations were not consistent for all participants between trials i.e. a low/high baseline concentration on one trial was not necessarily matched by a similar value on the other trial. However, a Student's t-test showed no significant difference in CRP concentrations at baseline ($P=0.149$). Similarly a Wilcoxon matched pairs test for data which are not normally distributed showed no difference in baseline concentrations of CRP ($P=0.093$). On the control trial, serum CRP concentrations remained unchanged over time in the majority of individuals. On the exercise trial six out of nine participants exhibited a clear rise in serum CRP concentrations from baseline over the post-exercise observation period.

One participant on the exercise trial (participant number one) had high concentrations of serum CRP throughout the trial. It is possible that this participant skews the distribution of these data and therefore increases the likelihood of making a Type I error (false positive) - concluding that there is a difference between the control and exercise trial when one does not exist. Therefore Table 8.2 includes the median data for each participant alongside the mean data. If these data are logarithmically transformed CRP no difference is seen in the pattern of CRP response between trials: trial \times time interaction ($P=0.101$). Removal of participant one from these data shows a similar finding to the original data: trial \times time interaction ($P=0.004$). Serum CRP shows a sharp elevation on the exercise trial between day one and two of the trial and remained elevated throughout day two of the trial. In contrast on the control trial CRP falls slightly between the morning of day one and two. The CRP concentration then remains steady throughout day two of the control trial.

Table 8.2. Individual CRP data for nine participants on the control and resistance exercise trial.

	Day 1		Day 2				
Control	09:00 h	15:30 h	08:00 h	11:00	14:00 h	Mean	Median
1	1.00	1.40	1.20	1.20	0.80	1.12	1.20
2	3.80	3.50	2.10	2.30	2.10	2.76	2.30
3	3.40	2.40	2.00	2.00	2.50	2.46	2.40
4	0.20	0.10	0.10	0.10	0.10	0.12	0.10
5	2.10	2.30	1.30	1.40	1.30	1.68	1.40
6	0.20	0.20	0.30	0.30	0.20	0.24	0.20
7	0.20	0.20	0.20	0.20	0.40	0.24	0.20
8	1.80	1.60	1.70	1.50	1.70	1.66	1.70
9	1.20	0.90	0.70	0.90	0.70	0.88	0.90
Mean	1.54	1.40	1.07	1.10	1.09		
Median	1.20	1.40	1.20	1.20	0.80		
Exercise							
1	0.70	5.00	2.90	8.00	8.00	4.92	5.00
2	0.20	0.10	0.20	0.30	0.20	0.20	0.20
3	1.40	1.50	1.80	1.60	0.10	1.28	1.50
4	0.10	0.10	0.10	0.10	0.20	0.12	0.10
5	1.40	0.80	2.00	2.20	2.00	1.68	2.00
6	1.20	0.80	0.50	0.70	0.10	0.66	0.70
7	0.20	0.20	1.00	1.20	0.90	0.70	0.90
8	1.60	1.30	2.70	3.20	2.90	2.34	2.70
9	0.70	0.80	1.10	1.40	1.20	1.04	1.10
Mean	0.83	1.18	1.37	2.08	1.73		
Median	0.70	0.80	1.10	1.40	0.90		

All serum CRP concentrations are $\text{mg}\cdot\text{L}^{-1}$.

8.4 DISCUSSION

The main finding in the present study is that a high volume of resistance exercise caused a steep rise in serum CRP concentrations. Conversely, when no exercise was performed CRP concentrations remained unchanged throughout the period of observation. The rise in CRP post-exercise in the present study is in agreement with studies of strenuous aerobic exercise that have shown an acute increase in CRP post-exercise (Taylor *et al.*, 1987; Weight *et al.*, 1991; Fallon *et al.*, 2001; Siegel *et al.*, 2001). Moreover the present study is in agreement with the findings of Paulsen *et al.* (2005) who observed a significant increase in serum CRP concentrations in participants performing maximal eccentric contractions of the quadriceps. The findings in the present study differ, however, from those of Nosaka & Clarkson (1996) who observed no difference in plasma CRP concentrations after maximal eccentric actions of the elbow flexors.

Concentrations of CRP peaked at three hours into the postprandial period (approximately 11 a.m.) on day 2 of the present study. This was approximately 26 hours since the start of the resistance exercise (9 a.m. day 1) and 19.5 hours since the end of the resistance exercise (3.30 p.m. day 1). Paulsen and colleagues (2005) observed a peak 253% over baseline value increase in CRP concentrations 47 hours post-exercise in volunteers who performed 300 maximal eccentric contractions of the quadriceps muscle in one leg. However, a significant 168% over baseline value increase in serum CRP concentrations was observed by Paulsen and colleagues at 23 hours post-exercise. An increase in CRP concentrations in the present study was observed over the same time period and the magnitude of the increase is similar (249% higher, 149% over baseline). It should be noted that there was no control trial in the study by Paulsen *et al.* This means that it is difficult to determine whether the change in serum CRP concentrations was because of the exercise bout or some other

factor. However, it has previously been demonstrated that CRP does not vary throughout the day (Meier-Ewert *et al.*, 2001). Furthermore, in conjunction with the findings from the present study it seems likely that resistance exercise does produce a change in CRP concentrations.

The concentrations of CRP observed in the present study are similar to those in the study by Paulsen and colleagues (2005). Although Paulsen and co-workers do not state serum CRP concentrations in their study, values can be estimated from the CRP figure included in their paper. Mean serum CRP concentrations are approximately $0.80 \text{ mg}\cdot\text{L}^{-1}$ at baseline in the Paulsen *et al.* study. At 23 hours post exercise mean serum CRP concentrations are approximately $2.20 \text{ mg}\cdot\text{L}^{-1}$. These average values are very similar to those observed on the exercise trial in the present study (baseline $0.83 \text{ mg}\cdot\text{L}^{-1}$; 19.5 hours post-exercise $2.08 \text{ mg}\cdot\text{L}^{-1}$). The age, height and weight of the participants in the Paulsen *et al.* (27.5 ± 4.0 yr, $1.80 \pm 0.08\text{m}$, 82.6 ± 6.0 kg) study are similar to those of the participants in the present study (20.9 ± 0.2 yr, 1.81 ± 0.02 m, 80.5 ± 3.9 kg; $n=9$). Moreover, the baseline CRP concentrations on both studies are similar to that previously suggested to be in a low risk group for a cardiovascular event $<1 \text{ mg}\cdot\text{L}^{-1}$ (Ridker *et al.*, 2003; Pearson *et al.*, 2003), which is what would be expected in young healthy males.

Studies of aerobic exercise (Taylor *et al.*, 1987; Weight *et al.*, 1991; Fallon *et al.*, 2001; Siegel *et al.*, 2001) have demonstrated a transient increase in CRP concentrations post-exercise similar to that seen in the present study. The increase in CRP in these studies has been after acute strenuous aerobic exercise (marathons, ultra-marathons and triathlons). These types of exercise are known to induce significant muscle damage as evidenced by post-exercise increases in creatine kinase

(Taylor *et al.*, 1987; Tuominen *et al.*, 1996). Post-exercise muscle damage and increases in creatine kinase have been related to increases in interleukin-6, the main stimulant for hepatic CRP production (Bruunsgard *et al.*, 1997). Therefore, muscle damage may be one possible cause of the increase in CRP post-exercise. However, there is no evidence for this in the present study as creatine kinase or other markers of muscle damage were not measured.

Other studies have not confirmed an association between interleukin-6 and creatine kinase (Ostrowski *et al.*, 1998; Ostrowski *et al.*, 1999; Croisier *et al.*, 1999). Moreover, an interleukin-6 response has been observed during concentric exercise without any signs of muscle damage (Steensberg *et al.*, 2001). It has been postulated that contracting skeletal muscles stimulate the immediate increase in plasma interleukin-6 in response to exercise, independently of muscle damage (Pedersen *et al.*, 2001; Febbraio & Pedersen, 2002). Subsequently, muscle damage per se elicits repair mechanisms in the muscle leading to interleukin-6 production, which occurs later and is of a smaller magnitude than the interleukin-6 production related to muscle contractions. It is possible that the rise in CRP concentrations observed in the present study were a combination of injury-induced and contractile-induced rises in interleukin-6 which stimulated CRP. The increase in serum CRP concentrations seen immediately post-exercise (15:30), as well as the more pronounced, delayed peak on the second day of observation (11:00) may reflect this. There is no direct evidence for the relationship between the two, however, as interleukin-6 was not measured in the present study.

The findings in the present study and the study of Paulsen *et al.* (2005) differ from those seen by Nosaka & Clarkson (1996). It is likely that these different findings relate to the volume of muscle mass employed, the duration of exercise and the

number of muscle contractions performed in the studies. The interleukin-6 response has been related to all of these variables (Febbraio & Pedersen, 2002) and the magnitude of response of this cytokine is likely to influence subsequent CRP responses. Participants in the present study and the study by Paulsen and co-workers performed a large number of muscle contractions (300 per exercise in the present study and 300 in total in the Paulsen *et al.* study). Participants in the study by Nosaka & Clarkson completed 24 maximal eccentric contractions of the elbow flexors in the non-dominant arm. The duration of exercise was prolonged in the present study (over a 5 hour period) and lasted 35 minutes in the study by Paulsen and colleagues. The exercise duration in the study by Nosaka & Clarkson lasted just over seven minutes. Lastly the volume of muscle mass employed in the present study (multiple muscle groups) and the study by Paulsen and colleagues (quadriceps) is larger than the small muscle mass used in the Nosaka & Clarkson study. Thus, in summary the present study and that of Paulsen *et al.* (2005) involved a higher volume of exercise and this may explain why these studies observed an increase in CRP concentrations post-exercise and why Nosaka and Clarkson (1996) failed to observe any change in CRP concentrations in their study.

It is interesting to note that Nosaka & Clarkson (1996) observed no difference in CRP concentrations in their study despite over 100-fold increases in creatine kinase during the five day post-exercise testing period. Furthermore no change was seen in interleukin-6 in their study. These observations lend weight to the hypothesis that increases in serum CRP post-exercise are a result of contractile-induced increases in interleukin-6. This seems even more likely when considering the small number of muscle contractions completed by volunteers in the Nosaka & Clarkson study and the large number of muscle contractions completed in the present study and the Paulsen *et al.* study (2005). It would be interesting to see if the post-exercise increase in CRP

with high volumes of resistance exercise remained after several weeks of training. Training should have a prophylactic effect on muscle damage (Byrnes *et al.*, 1985) and therefore any failure to decrease CRP after training lends weight to the hypothesis that an increase in serum CRP concentrations is due to muscle contractions per se. Although Castaneda and colleagues (2004) saw a decrease in CRP with resistance training they do not state how long after the last bout of exercise the CRP measurement was made.

The absence of any change in serum CRP concentrations on the control trial has been observed previously. Meier-Ewert and colleagues (2001) demonstrated the stability of CRP concentrations throughout the course of a 24 hour day and the lack of diurnal variation in CRP, in the short term at least. This provides support for the fact that resistance exercise is the variable causing the change in CRP concentrations in the present study as food intake has no effect on CRP concentrations (Meier-Ewert *et al.*, 2001; Lichtenstein *et al.*, 2003). Furthermore, dietary intake was matched on both trials and so any change with food intake would be seen on the control trial. The absence of any significant correlation between CRP and postprandial TAG concentrations is probably also explained by the lack of change in CRP with food intake.

Individual data from participants revealed that baseline concentrations of CRP were not consistent between trials. This could be offered as an explanation to why no main effect of trial was observed in the present study if participants on the control trial started at a higher point than those on the exercise trial. This means that even with a rise in CRP concentrations on the exercise trial there would be no difference in the overall means between the two trials. However, the possibility that some of the baseline data were not normally distributed was not confirmed by a Wilcoxon

matched pairs test. The absence of any change in serum CRP concentrations in the majority of individuals on the control trial probably explains why no main effect of time was observed in the present study, despite changes during the exercise trial. The rise in CRP concentrations in six out of nine participants supports the main finding in the present study. However, one participant showed a decrease in serum CRP concentrations after exercise and two participants showed no change. This suggests that the post-exercise inflammatory response to resistance training is not the same for all individuals. Whether this is a product of previous training volume, some genetic or other factor is impossible to speculate from the evidence in the present study.

Participant number one on the exercise trial had high concentrations of serum CRP throughout the trial. It is possible that this participant skews the distribution of data. The high serum CRP concentrations ($8.0 \text{ mg}\cdot\text{L}^{-1}$) of this participant on day two of the exercise trial increase the overall mean concentrations on the exercise trial. Comparison of the median CRP concentrations between the control and exercise trial shows less difference between the two trials over the two days (Table 8.2). Thus, those data that show a steep rise in serum CRP concentrations after exercise might have been skewed. However, a closer look at the median CRP concentrations in Table 8.2 still indicates that there is a rise in CRP concentrations between day one and day two of the exercise trial. Furthermore, applying a logarithmic transformation to data in the present study in order to account for a skewed distribution may not also be necessary. An ANOVA is considered by some statisticians (Maxwell & Delaney, 1990; Vincent, 1995) to be generally robust to violations of the normality assumption and, therefore, if data in the present study is not logarithmically transformed the main finding presented here is correct. Finally, if participant number one is excluded from these data the main finding in the present study does not change. Nonetheless,

because of the nature of these data, further study of this area is necessary to conclude whether resistance exercise increases serum CRP concentrations.

In summary the present study demonstrates a steep post-exercise rise in CRP concentrations with resistance exercise in comparison to a control trial. However, because of skewed data this finding requires confirmation in future studies. The reason for a rise in serum CRP concentrations could be related to contractile-induced or injury induced increases in interleukin-6 which promote a subsequent increase in CRP. These data confirm the findings of studies involving acute strenuous aerobic exercise and one previous study of acute resistance exercise which have shown similar increases in CRP post-exercise. As a strategy for reducing cardiovascular disease this aspect of resistance exercise does not appear to be beneficial. However, increases in CRP similar to those seen in the present study may not be seen with smaller volumes of resistance exercise. Further studies are needed to determine this.

Chapter 9

General Discussion

9.1 Discussion

The aim of this thesis was to examine the effect of resistance exercise on postprandial TAG concentrations and serum CRP concentrations. The main hypothesis of the studies presented was that if energy expenditure from resistance exercise was sufficiently high then a reduction in postprandial TAG concentrations would be seen. The secondary hypothesis was that completing a single, high volume session of resistance exercise would produce an acute increase in serum CRP concentrations.

The studies presented in this thesis examined these questions in a systematic way. Study one (Chapter 4) found that a single session of resistance exercise had no influence on postprandial TAG concentrations the following day. A reduction in postprandial TAG concentrations was found in study two (Chapter 5) when the energy expenditure of exercise was increased by performing multiple bouts of weight lifting the day before a test meal. The third study (Chapter 6) examined whether current involvement in resistance training and the timing of the resistance exercise in relation to the test meal influenced the reduction in postprandial TAG concentrations. The results were perhaps surprising as an increase in postprandial TAG concentrations was observed when the test meal was given one hour post-exercise. It was hypothesized that muscle damage may have been the cause of the increase in TAG concentrations. Therefore, study four (Chapter 7) compared the effect of a single session of resistance exercise with downhill

running (aerobic exercise with a high number of eccentric contractions) on postprandial TAG concentrations. Increased postprandial TAG concentrations were seen one hour post-exercise with resistance exercise but not with downhill running suggesting that muscle damage was not the cause of the increased lipaemic response. The final study (Chapter 8) examined the influence of resistance exercise on serum CRP concentrations. An increase in serum CRP concentrations was observed 20 hours post-exercise.

There had been no previous investigations into resistance exercise and postprandial TAG concentrations when the work in the present thesis began. Since that time only two studies (Petitt *et al.*, 2003; Shannon *et al.*, 2005) have examined the issue. Petitt and colleagues (2003) published the first study in this area and reported a reduction in TAG concentrations with resistance exercise that was greater (18% lower) than that seen with aerobic exercise of approximately the same estimated energy expenditure (1.7 MJ and 1.6 MJ respectively). Shannon and co-workers (2005) observed no reduction in postprandial TAG concentrations in comparison with a control trial after participants performed a resistance exercise session consisting of one, three or five sets of eight different resistance exercises.

The findings of Petitt and colleagues are difficult to reconcile with those in the present thesis. As noted previously (Chapter 4, Pgs.58-59) the total energy expenditure of exercise was greater in study one of this thesis (2.3 MJ) than in the study by Petitt *et al.* (2003) (1.7 MJ). However, the duration of the exercise bout, the gap between the end of the exercise and consumption of the test meal and the macronutrient content of the two

test meals was similar. Despite these similarities in protocol there is disparity in the results. The total energy expenditure (5.1 MJ) of the resistance exercise in the second study of this thesis (i.e. Chapter 5, examining multiple bouts of resistance exercise) was three times greater than that seen in the Petitt *et al.* study. There was a 12 % reduction in the total lipaemic response in study two in comparison with a control trial, whereas Petitt *et al.* found a 14% reduction in comparison with a control trial. The attenuated postprandial TAG response seen by Petitt and co-workers has not been observed by any of the studies in this thesis or in the study by Shannon and co-workers (2005) when the energy expenditure from exercise was so low.

The combined findings from studies one and two (Chapters 4 and 5 respectively) in this thesis suggest that energy expenditure of prior exercise is the main determinant of the exercise induced reduction in postprandial TAG concentrations after resistance exercise. This agrees with the evidence from studies of aerobic exercise (Tsetsonis & Hardman, 1996b; Gill *et al.*, 2002a). Tsetsonis & Hardman observed a similar reduction in postprandial TAG concentrations with 90 minutes of walking at 63% of maximum oxygen uptake, or 3 hours walking at 32% of maximum oxygen uptake. Gill and colleagues found that walking for one hour reduced postprandial lipaemia by a mean of 9.3%, whereas a two hour walk reduced it by 22.8%. A significant relationship between effect size and energy expenditure was also observed by Petitt & Cureton (2003) who examined the effect of prior exercise on postprandial lipaemia using meta-analytic methods to quantify effect sizes. The findings of the studies in this thesis and from previous studies of aerobic exercise (Tsetsonis & Hardman, 1996b; Gill *et al.*, 2002;

Petitt & Cureton (2003) do not explain the reduction in postprandial TAG concentrations seen in the study by Petitt *et al.* (2003).

Petitt and colleagues (2003) observed a strong effect size ($d=-0.78$) (see note on calculations of effect size at the end of this chapter Pg.174) from the resistance exercise in their study. They go on to state that the resistance exercise bout in their study produced a reduction in postprandial lipaemia equivalent to a session of aerobic exercise with double the energy expenditure. Indeed the mean weighted effect for aerobic exercise on postprandial lipaemia calculated by Petitt & Cureton (2003) in their meta-analytic review of 555 participants in 29 studies was moderate ($d=-0.57$) (Petitt & Cureton, 2003). A 12% reduction in TAG concentrations in comparison with the control trial was seen in study two (Chapter 5) of this thesis. The effect size (see note Pg.174) calculated for study two was low ($d=-0.34$). This suggests that resistance exercise is not as effective as aerobic exercise in reducing postprandial TAG concentrations – a stark contrast to the finding of Pettit *et al.* (2003).

Of the studies cited by Petitt and Cureton (2003) in their meta-analysis the effect size ($d=-0.38$) for the study by Koutsari & Hardman (2001) was similar to that seen in study two of this thesis. The Koutsari & Hardman study was conducted in young healthy men (aged 33 SD 4). Participants followed a high-carbohydrate diet for three days before the test meal and exercised for 30 minutes on a treadmill at 61% of maximum oxygen uptake for each of those three days. The mean energy expenditure of the exercise was 1.6 MJ per session. Per exercise session this is considerably less than the 5.2 MJ expended by the

participants in the second study (Chapter 5) in the present thesis. Furthermore the duration of the exercise completed was considerably less even over the three days. Whilst it could be argued that accumulating exercise over three days may have had effects over and above performing exercise on a single day, such as in study two, the total duration of the exercise performed over the three days was still considerably less than the duration of the exercise in study two. However, several studies have demonstrated that accumulating exercise in a number of bouts, over one day at least, has the same effect as a single exercise session if the total energy expenditure of the sessions is equal (Murphy *et al.*, 2000; Gill *et al.*, 1998; Miyashita *et al.*, 2006a; Miyashita *et al.*, 2006b).

From the final argument in the preceding paragraph it should be noted that despite energy expenditure in each daily exercise session being considerably less in the Koutsari & Hardman (2001) study than the energy expenditure of resistance exercise in study two of this thesis, the total energy expenditure (4.8 MJ) in the Koutsari and Hardman study was similar to study two. This would suggest that the effect of resistance and aerobic exercise on postprandial TAG concentrations is similar. It is difficult to judge though, how much of the postprandial TAG reduction in the Koutsari and Hardman study was from the last bout of exercise performed and how much was a product of accumulated exercise over the three days. Certainly an increased lipaemic response to a test meal has been observed after only 60 hours since the last bout of exercise (Hardman *et al.*, 1998) suggesting that the effect of each exercise bout only influences TAG concentrations over a short period of time.

It is difficult to determine the minimum energy expenditure needed to reduce postprandial TAG concentrations with a single session of resistance exercise from the current evidence. The study by Petitt and colleagues (2003) was considered above and so will not be addressed further. The first study (Chapter 4) in this thesis saw no reduction in postprandial TAG concentrations with an estimated energy expenditure of 2.3 MJ. Shannon and colleagues (2005) also found no reduction in postprandial TAG concentrations when participants expended an estimated 2.58 MJ in resistance exercise. However, the effect of re-feeding the energy debt of exercise in the experiment by Shannon and colleagues confuses the evidence. The second study in this thesis found a 12% reduction with an estimated energy expenditure of 5.1 MJ. It is possible that a reduction in postprandial TAG concentrations would be seen with a lesser energy expenditure than this. Certainly the long duration of the exercise means that the net energy expenditure (3.5MJ) was considerably less than the gross. Therefore, a shorter more intense bout of resistance exercise rather than the multiple bouts used in study two may produce a similar response. However, it needs to be considered that both study one in the present thesis and the study by Shannon and colleagues used large volumes of resistance exercise when investigating its effects on postprandial TAG concentrations. It seems unlikely when recommending resistance exercise from a public health standpoint that many individuals would be capable of completing such large volumes and so increasing the volume further in an effort to reduce TAG concentrations may not be worthwhile.

One proposed mechanism cited by Petitt *et al.* (2003) for the greater reduction produced by resistance exercise on postprandial TAG concentrations is an increase in skeletal muscle lipoprotein lipase. Petitt and colleagues state that high intensity low repetition resistance exercise may have had a greater effect on skeletal muscle lipoprotein lipase than low intensity high repetition aerobic exercise. They have no evidence to support their hypothesis however, as skeletal muscle lipoprotein lipase activity was not measured in their study. Moreover, to the author's knowledge, there are no studies documenting the effect of resistance exercise on lipoprotein lipase per se. In study two of this thesis lipoprotein lipase activity was also not measured. There is some indirect evidence to support the hypothesis that lipoprotein lipase activity may have increased, however. Ferguson *et al.* (1998) have suggested that a minimum of 4.6 MJ needs to be expended through exercise to induce significant increases in lipoprotein lipase activity 24 hours post-exercise. The total energy expenditure in study two was above this value and could be one reason why a reduction in TAG concentrations was seen in study two and not in study one or the study by Shannon and colleagues (2005). It should be noted, however, this effect on lipoprotein lipase was only observed by Ferguson and co-workers after aerobic exercise of 4.6 MJ and greater. The effect of resistance exercise on this enzyme is still unclear. Secondly, insulin concentrations in study two were decreased on the exercise trial. The activity of lipoprotein lipase in muscle is decreased by insulin (Kiens *et al.*, 1989; Richelsen *et al.*, 1993). However, despite the decreased insulin concentrations with exercise in study two, indices of insulin and TAG were not significantly related. This agrees with the findings from aerobic exercise (Gill *et al.*, 2002b).

There was also some indirect evidence for a decreased hepatic VLDL output in study two. The significant increase in 3-hydroxybutyrate concentrations post-exercise suggested that NEFA delivered to the liver in the postprandial period were being directed toward oxidation rather than esterified into VLDL. Increased hepatic NEFA oxidation would be in response to the energy debt created by the exercise. Similar increases in 3-hydroxybutyrate alongside reductions in TAG have been observed with aerobic exercise (Malkova *et al.*, 2000). Furthermore, there is a body of evidence to suggest that decreased VLDL is at least partially responsible for the reduction in postprandial TAG with aerobic exercise (Gill *et al.*, 2001a; Gill *et al.*, 2001b; Gill *et al.*, 2003). In balance it is probable that the reduction in TAG in study two was due to a combination of decreased VLDL output and increased skeletal muscle lipoprotein lipase activity, although direct evidence for a role of either remains unavailable for resistance exercise.

It is difficult to provide any clear hypothesis for the findings of an increased postprandial TAG concentration in studies three and four (Chapters 7 and 8 respectively) of this thesis. The initial hypothesis that muscle damage may have interfered with TAG transport into the muscle was not confirmed by the comparison with a bout of downhill running. Myoglobin concentrations after downhill running were similar to those seen after resistance exercise suggesting that muscle damage was similar on both trials. Furthermore, no difference in the insulin or glucose responses between downhill running and resistance exercise was observed in either study three or four which suggests that

insulin resistance from muscle damage was not a cause of the impaired TAG uptake into the muscle.

The finding in both study three and four of increased TAG concentrations suggests that this effect is consistently observed with a single heavy session of resistance exercise. It appears to be only a transient effect, however, as no studies using two day models (i.e. resistance exercise day one, test meal consumed day two) have seen an increase in postprandial lipaemia with resistance exercise. Moreover, no studies of aerobic exercise using one-day models have observed an increase in postprandial TAG concentrations (Zhang *et al.*, 1998; Murphy *et al.*, 2000; Katsanos *et al.*, 2004; Katsanos & Moffatt, 2004; Petridou *et al.*, 2004; Pfeiffer *et al.*, 2005) and so the mechanism behind this increase remains to be elucidated. However, the two primary mechanisms proposed to elicit a reduction in postprandial TAG concentrations are an increase in skeletal muscle lipoprotein lipase activity and a reduced hepatic VLDL-TAG output. It seems possible therefore, that resistance exercise could interfere with one or both of these mechanisms to increase postprandial TAG concentrations in the short term.

The increase in postprandial TAG concentrations observed in studies three and four may not have occurred if a test meal with a lower fat load had been given. At least two studies (Petridou *et al.*, 2004; Pfeiffer *et al.*, 2005) using one-day models with aerobic exercise have found no reduction in postprandial TAG concentrations when meals with a moderate fat content (35% and 33% respectively) were given post-exercise. It is possible that a significant rise in TAG would not be seen if the test meal were of a more moderate

fat content although more evidence for this hypothesis is needed. However, postprandial lipaemia has been shown to be positively related to the amount fat ingested (Dubois *et al.*, 1998). More studies with applicability to everyday conditions are required.

The increase in serum CRP concentrations after resistance exercise observed in the fifth study of this thesis (Chapter 8) does not appear beneficial if resistance exercise is to be considered as a strategy for the reduction of cardiovascular disease. A similar increase with resistance exercise has been observed previously by Paulsen *et al.* (2005) and in studies of acute strenuous aerobic exercise (Taylor *et al.*, 1987; Weight *et al.*, 1991; Fallon *et al.*, 2001; Siegel *et al.*, 2001). However, the increased serum CRP concentrations with resistance exercise observed in these studies (Study Five; Paulsen *et al.*, 2005) needs to be kept in context. People undertaking a program of resistance exercise are unlikely to complete the volume of exercise used in Study Five of this thesis and in the Paulsen *et al.* study in a single exercise session. Indeed Nosaka & Clarkson (1996) observed no increase in CRP in their study when a much smaller volume than that completed in study five or the Paulsen *et al.* study was used. Secondly, the effect of resistance training may be to decrease serum CRP concentrations (Castaneda *et al.* 2004). However, more randomised, prospective, controlled studies are needed. The picture is certainly unclear with aerobic exercise because of these failures. Finally, other benefits of resistance training on cardiovascular disease may outweigh any potential decrement caused by transiently increased serum CRP concentrations. Epidemiological studies (Paffenbarger *et al.*, 1975; Tanasescu *et al.*, 2002; Jurca *et al.*, 2004) suggest that resistance exercise has potential as a strategy in the prevention of cardiovascular disease.

Indeed the study by Tanasescu and colleagues (2002) suggests that only a small volume of resistance exercise (30 minutes a week) is needed each week to have a potential benefit on coronary heart disease risk. The volume of resistance exercise associated with this duration may also not increase serum CRP concentrations. Further investigation is needed to assess the effect of resistance exercise on CRP.

In summary, there are three main findings from the studies in this thesis. Firstly, that resistance exercise can cause a decrease in postprandial TAG concentrations if the energy expenditure from exercise is high enough. Secondly, that resistance exercise causes a transient rise in postprandial TAG concentrations when the test meal is given early in the post-exercise period. Finally, that resistance exercise causes a post-exercise increase in serum CRP concentrations. Whilst the finding from study two that resistance exercise can reduce postprandial lipaemia is beneficial, in reality it is unlikely that most individuals would be able complete the volume of exercise used in this study either because of fitness or time constraints. Certainly aerobic exercise of a much shorter duration produces similar or greater reductions on postprandial TAG concentrations. The transient rise observed in TAG concentrations in studies three and four of this thesis may not occur when meals with a lower fat load than the ones in the present thesis are given. Nonetheless, this hypothesis needs testing and further investigation into the cause of the rise in TAG is needed. Whilst the increase in CRP with resistance exercise is not beneficial, again most individuals are unlikely to complete the volume of exercise used in the study. Again further investigation is needed to see if smaller volumes of resistance exercise produce a rise in CRP and if resistance training attenuates the rise. Whilst some

of these findings are not beneficial when recommending resistance exercise as a strategy to reduce cardiovascular disease it is possible resistance exercise produces improvements in risk factors for cardiovascular disease that were not addressed by this thesis e.g. blood pressure and obesity. Furthermore, its role in maintaining other aspects of a healthy body or healthy lifestyle may outweigh any negative benefits and need to be considered before any changes to current exercise recommendations are made.

9.2 Further Research

The studies in the present thesis suggest that a large volume of exercise is needed to reduce postprandial TAG concentrations in young males. Although, it is unlikely few people in the population will be able to complete this volume of weight lifting, resistance exercise may still have a role as a strategy to reduce postprandial TAG concentrations and possibly cardiovascular disease. As mentioned in the introduction to this work (Chapter 1, Pg.5) at least two studies (Ades *et al.*, 1996, Brochu *et al.*, 2002) have demonstrated that resistance exercise can lead to improved endurance capacity and performance in older adults. Whether resistance exercise per se, or whether the improvement in endurance exercise capacity that comes from resistance exercise leads to improved lipid metabolism in older adults needs to be investigated. It may simply be that resistance exercise can lead to improvements in independent living for elder adults which leads to increased physical activity. Nonetheless, the benefits of resistance exercise for the elder population needs researching.

The second area where resistance exercise may be able to play a role is as part of a lifestyle intervention for obese adults or children. Resistance exercise may be able to contribute to the total energy expenditure of an exercise program for obese people. Alternatively, obese people may not be very mobile at the start of a lifestyle intervention program. Resistance exercise may therefore be an exercise modality which obese people can take part in more easily than jogging or cycling. It may also simply be more enjoyable for them and they may well be good at it as obese people tend to have a large amount of muscle mass. This may improve their confidence to take part in other modes of exercise. Lastly resistance exercise may be a method to maintain muscle mass during weight loss. Whether resistance exercise contributes to improvements in lipid metabolism directly or because it aids weight loss or improves other areas of exercise capacity needs investigating.

Note: Calculation of effect size

The equation used to calculate the effect size (ES) for Study Two (Chapter 5) was:

$$ES = (M_1 - M_2)/s$$

Where M_1 = the mean incremental area under the TAG concentration *versus* time curve on the resistance exercise trial

M_2 = the mean incremental area under the TAG concentration *versus* time curve on the control trial

s = the standard deviation of the incremental area under the TAG concentration *versus* time for curve the control group.

(Thomas & Nelson, 1996)

Petitt *et al.* (2003) do not state exactly how they calculated effect size in their paper on resistance exercise and postprandial lipaemia. They simply state that effect size was calculated on the basis of adjusted means. However, in the meta-analytic paper by Petitt & Cureton (2003) they state that the effect size, “was calculated by subtracting the control or baseline mean response from the intervention mean response and dividing the difference by the baseline response standard deviation, or the pooled response standard deviation for studies with a cross-sectional design.” This is the same as the method described above for study two and therefore a good comparison can be made between study two and the data taken from this paper. Moreover, it seems likely that this would have been the method Petitt *et al.* (2003) used in their paper on resistance exercise and postprandial lipaemia and so it is likely that the comparisons made in this chapter are suitable.

References

- Abbott, W. G., S. Lillioja, A. A. Young, J. K. Zawadzki, H. Yki-Jarvinen, L. Christin, and B. V. Howard. Relationships between plasma lipoprotein concentrations and insulin action in an obese hyperinsulinemic population. *Diabetes*. 36:897-904, 1987.
- Ades, P. A., D. L. Ballor, T. Ashikaga, J. L. Utton, and K. S. Nair. Weight training improves walking endurance in healthy elderly persons. *Ann Intern Med*. 124:568-572, 1996.
- Albert, M. A., R. J. Glynn, and P. M. Ridker. Effect of physical activity on serum C-reactive protein. *Am J Cardiol*. 93:221-225, 2004.
- Aldred, H. E., A. E. Hardman, and S. Taylor. Influence of 12 weeks of training by brisk walking on postprandial lipemia and insulinemia in sedentary middle-aged women. *Metabolism*. 44:390-397, 1995.
- Aldred, H. E., I. C. Perry, and A. E. Hardman. The effect of a single bout of brisk walking on postprandial lipemia in normolipidemic young adults. *Metabolism*. 43:836-841, 1994.
- Alessio, H. M., A. E. Hagerman, B. K. Fulkerson, J. Ambrose, R. E. Rice, and R. L. Wiley. Generation of reactive oxygen species after exhaustive aerobic and isometric exercise. *Med Sci Sports Exerc*. 32:1576-1581, 2000.

Altekruse, E. B. and J. H. Wilmore. Changes in blood chemistries following a controlled exercise program. *J Occup Med.* 15:110-113, 1973.

American College of Sports Medicine. *ACSM's Guidelines for Exercise Testing and Prescription*. 7th ed: Lippincott Williams and Wilkins, 2005.

Aronson, D., M. Sheikh-Ahmad, O. Avizohar, A. Kerner, R. Sella, P. Bartha, W. Markiewicz, Y. Levy, and G. J. Brook. C-Reactive protein is inversely related to physical fitness in middle-aged subjects. *Atherosclerosis*. 176:173-179, 2004.

Austin, M. A., J. L. Breslow, C. H. Hennekens, J. E. Buring, W. C. Willett, and R. M. Krauss. Low-density lipoprotein subclass patterns and risk of myocardial infarction. *Jama*. 260:1917-1921, 1988.

Banz, W. J., M. A. Maher, W. G. Thompson, D. R. Bassett, W. Moore, M. Ashraf, D. J. Keefer, and M. B. Zemel. Effects of resistance versus aerobic training on coronary artery disease risk factors. *Exp Biol Med (Maywood)*. 228:434-440, 2003.

Barter, P. J. and K. A. Rye. High density lipoproteins and coronary heart disease. *Atherosclerosis*. 121:1-12, 1996.

Behall, K. M., J. C. Howe, G. Martel, W. H. Scott, and C. R. Dooly. Comparison of resistive to aerobic exercise training on cardiovascular risk factors of sedentary, overweight premenopausal and postmenopausal women. *Nutrition Research*. 23:607-619, 2003.

Bergeron, N. and R. J. Havel. Influence of diets rich in saturated and omega-6 polyunsaturated fatty acids on the postprandial responses of apolipoproteins B-48, B-100, E, and lipids in triglyceride-rich lipoproteins. *Arterioscler Thromb Vasc Biol.* 15:2111-2121, 1995.

Berliner, J., N. Leitinger, A. Watson, J. Huber, A. Fogelman, and M. Navab. Oxidized lipids in atherogenesis: formation, destruction and action. *Thromb Haemost.* 78:195-199, 1997.

Bjorkegren, J., C. J. Packard, A. Hamsten, D. Bedford, M. Caslake, L. Foster, J. Shepherd, P. Stewart, and F. Karpe. Accumulation of large very low density lipoprotein in plasma during intravenous infusion of a chylomicron-like triglyceride emulsion reflects competition for a common lipolytic pathway. *J Lipid Res.* 37:76-86, 1996.

Blackburn, R., P. Giral, E. Bruckert, J. M. Andre, S. Gonbert, M. Bernard, M. J. Chapman, and G. Turpin. Elevated C-reactive protein constitutes an independent predictor of advanced carotid plaques in dyslipidemic subjects. *Arterioscler Thromb Vasc Biol.* 21:1962-1968, 2001.

Blair, S. N., J. B. Kampert, H. W. Kohl, 3rd, C. E. Barlow, C. A. Macera, R. S. Paffenbarger, Jr., and L. W. Gibbons. Influences of cardiorespiratory fitness and other precursors on cardiovascular disease and all-cause mortality in men and women. *Jama.* 276:205-210, 1996.

Borg, G. A. Perceived exertion: a note on "history" and methods. *Med Sci Sports*. 5:90-93, 1973.

Borsheim, E., S. Knardahl, and A. T. Hostmark. Short-term effects of exercise on plasma very low density lipoproteins (VLDL) and fatty acids. *Med Sci Sports Exerc*. 31:522-530, 1999.

British Heart Foundation. *Coronary heart disease statistics*. London: BHF, 2005.

Brochu, M., P. Savage, M. Lee, J. Dee, M. E. Cress, E. T. Poehlman, M. Tischler, and P. A. Ades. Effects of resistance training on physical function in older disabled women with coronary heart disease. *J Appl Physiol*. 92:672-678, 2002.

Brown, S. A., L. E. Chambless, A. R. Sharrett, A. M. Gotto, Jr., and W. Patsch. Postprandial lipemia: reliability in an epidemiologic field study. *Am J Epidemiol*. 136:538-545, 1992.

Brunzell, J. D., W. R. Hazzard, D. Porte, Jr., and E. L. Bierman. Evidence for a common, saturable, triglyceride removal mechanism for chylomicrons and very low density lipoproteins in man. *J Clin Invest*. 52:1578-1585, 1973.

Bruunsgaard, H., H. Galbo, J. Halkjaer-Kristensen, T. L. Johansen, D. A. MacLean, and B. K. Pedersen. Exercise-induced increase in serum interleukin-6 in humans is related to muscle damage. *J Physiol*. 499 (Pt 3):833-841, 1997.

Byrnes, W. C., P. M. Clarkson, J. S. White, S. S. Hsieh, P. N. Frykman, and R. J. Maughan. Delayed onset muscle soreness following repeated bouts of downhill running. *J Appl Physiol.* 59:710-715, 1985.

Castaneda, C., P. L. Gordon, R. C. Parker, K. L. Uhlin, R. Roubenoff, and A. S. Levey. Resistance training to reduce the malnutrition-inflammation complex syndrome of chronic kidney disease. *Am J Kidney Dis.* 43:607-616, 2004.

Cohen, J. C., T. D. Noakes, and A. J. Benade. Postprandial lipemia and chylomicron clearance in athletes and in sedentary men. *Am J Clin Nutr.* 49:443-447, 1989.

Cohen, J. C., T. D. Noakes, and A. J. Benade. Serum triglyceride responses to fatty meals: effects of meal fat content. *Am J Clin Nutr.* 47:825-827, 1988.

Cohn, J. S. Postprandial lipemia: emerging evidence for atherogenicity of remnant lipoproteins. *Can J Cardiol.* 14 Suppl B:18B-27B, 1998.

Cohn, J. S., J. R. McNamara, S. D. Cohn, J. M. Ordovas, and E. J. Schaefer. Postprandial plasma lipoprotein changes in human subjects of different ages. *J Lipid Res.* 29:469-479, 1988.

Croisier, J. L., G. Camus, I. Venneman, G. Deby-Dupont, A. Juchmes-Ferir, M. Lamy, J. M. Crielaard, C. Deby, and J. Duchateau. Effects of training on exercise-

induced muscle damage and interleukin 6 production. *Muscle Nerve*. 22:208-212, 1999.

Das, I. Raised C-reactive protein levels in serum from smokers. *Clin Chim Acta*. 153:9-13, 1985.

Del Aguila, L. F., R. K. Krishnan, J. S. Ulbrecht, P. A. Farrell, P. H. Correll, C. H. Lang, J. R. Zierath, and J. P. Kirwan. Muscle damage impairs insulin stimulation of IRS-1, PI 3-kinase, and Akt-kinase in human skeletal muscle. *Am J Physiol Endocrinol Metab*. 279:E206-212, 2000.

Department of Health. *At least five a week - Evidence on the impact of physical activity and its relationship to health*. Crown Copyright, 2004.

Dill, D. B. and D. L. Costill. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol*. 37:247-248, 1974.

Di Massimo, C., P. Scarpelli, M. Penco, and M. G. Tozzi-Ciancarelli. Possible involvement of plasma antioxidant defences in training-associated decrease of platelet responsiveness in humans. *Eur J Appl Physiol*. 91:406-412, 2004.

Durnin, J.V. and J. Womersley. Body fat assessed from total body density and its estimation from skinfold thickness: measurements on 481 men and women aged from 16 to 72 years. *Br J Nutr*. 32:77-97, 1974.

Dufaux, B., U. Order, H. Geyer, and W. Hollmann. C-reactive protein serum concentrations in well-trained athletes. *Int J Sports Med.* 5:102-106, 1984.

Elliott, K. J., C. Sale, and N. T. Cable. Effects of resistance training and detraining on muscle strength and blood lipid profiles in postmenopausal women. *Br J Sports Med.* 36:340-344, 2002.

Essen-Gustavsson, B. and P. A. Tesch. Glycogen and triglyceride utilization in relation to muscle metabolic characteristics in men performing heavy-resistance exercise. *Eur J Appl Physiol Occup Physiol.* 61:5-10, 1990.

Fallon, K. E. The acute phase response and exercise: the ultramarathon as prototype exercise. *Clin J Sport Med.* 11:38-43, 2001.

Febbraio, M. A. and B. K. Pedersen. Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. *Faseb J.* 16:1335-1347, 2002.

Ferguson, M. A., N. L. Alderson, S. G. Trost, D. A. Essig, J. R. Burke, and J. L. Durstine. Effects of four different single exercise sessions on lipids, lipoproteins, and lipoprotein lipase. *J Appl Physiol.* 85:1169-1174, 1998.

Frayn, K. N. Calculation of substrate oxidation rates in vivo from gaseous exchange. *J Appl Physiol.* 55:628-634, 1983.

Gill, J. M., M. J. Caslake, C. McAllister, F. Tsofliou, W. R. Ferrell, C. J. Packard, and D. Malkova. Effects of short-term detraining on postprandial metabolism, endothelial function, and inflammation in endurance-trained men: dissociation between changes in triglyceride metabolism and endothelial function. *J Clin Endocrinol Metab.* 88:4328-4335, 2003b.

Gill, J. M., K. N. Frayn, S. A. Wootton, G. J. Miller, and A. E. Hardman. Effects of prior moderate exercise on exogenous and endogenous lipid metabolism and plasma factor VII activity. *Clin Sci (Lond)*. 100:517-527, 2001b.

Gill, J. M. and A. E. Hardman. Postprandial lipemia: effects of exercise and restriction of energy intake compared. *Am J Clin Nutr.* 71:465-471, 2000.

Gill, J. M., S. L. Herd, and A. E. Hardman. Moderate exercise and post-prandial metabolism: issues of dose-response. *J Sports Sci.* 20:961-967, 2002.

Gill, J. M., S. L. Herd, N. V. Tsetsonis, and A. E. Hardman. Are the reductions in triacylglycerol and insulin levels after exercise related? *Clin Sci (Lond)*. 102:223-231, 2002b.

Gill, J. M., S. L. Herd, V. Vora, and A. E. Hardman. Effects of a brisk walk on lipoprotein lipase activity and plasma triglyceride concentrations in the fasted and postprandial states. *Eur J Appl Physiol.* 89:184-190, 2003a.

Gill, J. M., D. Malkova, and A. E. Hardman. Reproducibility of an oral fat tolerance test is influenced by phase of menstrual cycle. *Horm Metab Res.* 37:336-341, 2005.

Gill, J.M., G.P. Mees, K.N. Frayn, and A.E. Hardman. Moderate exercise, postprandial lipaemia and triacylglycerol clearance. *Eur J Clin Invest.* 31:201-207, 2001a.

Gill, J.M., M.H. Murphy, and A.E. Hardman. Postprandial lipemia: effects of intermittent versus continuous exercise. *Med Sci Sports Exerc.* 30:1515-1520, 1998.

Goldberg, L., D. L. Elliot, R. W. Schutz, and F. E. Kloster. Changes in lipid and lipoprotein levels after weight training. *Jama.* 252:504-506, 1984.

Greiwe, J. S., J. O. Holloszy, and C. F. Semenkovich. Exercise induces lipoprotein lipase and GLUT-4 protein in muscle independent of adrenergic-receptor signaling. *J Appl Physiol.* 89:176-181, 2000.

Griffin, B. A. Low-density lipoprotein subclasses: mechanisms of formation and modulation. *Proc Nutr Soc.* 56:693-702, 1997.

Griffin, B. A., D. J. Freeman, G. W. Tait, J. Thomson, M. J. Caslake, C. J. Packard, and J. Shepherd. Role of plasma triglyceride in the regulation of plasma low density lipoprotein (LDL) subfractions: relative contribution of small, dense LDL to coronary heart disease risk. *Atherosclerosis.* 106:241-253, 1994.

Griffin, B. A. and A. Zampelas. Influence of dietary fatty acids on the atherogenic lipoprotein phenotype. *Nutrition Research Reviews*. 8:1-26, 1995.

Groot, P. H., W. A. van Stiphout, X. H. Krauss, H. Jansen, A. van Tol, E. van Ramshorst, S. Chin-On, A. Hofman, S. R. Cresswell, and L. Havekes. Postprandial lipoprotein metabolism in normolipidemic men with and without coronary artery disease. *Arterioscler Thromb*. 11:653-662, 1991.

Hammett, C. J., H. C. Oxenham, J. C. Baldi, R. N. Doughty, R. Ameratunga, J. K. French, H. D. White, and R. A. Stewart. Effect of six months' exercise training on C-reactive protein levels in healthy elderly subjects. *J Am Coll Cardiol*. 44:2411-2413, 2004.

Hammett, C. J., H. Prapavessis, J. C. Baldi, N. Varo, U. Schoenbeck, R. Ameratunga, J. K. French, H. D. White, and R. A. Stewart. Effects of exercise training on 5 inflammatory markers associated with cardiovascular risk. *Am Heart J*. 151:367 e367-367 e316, 2006.

Hardman, A. E. The influence of exercise on postprandial triacylglycerol metabolism. *Atherosclerosis*. 141 Suppl 1:S93-100, 1998.

Hardman, A. E. and H. E. Aldred. Walking during the postprandial period decreases alimentary lipaemia. *J Cardiovasc Risk*. 2:71-78, 1995.

Hardman, A. E., J. E. Lawrence, and S. L. Herd. Postprandial lipemia in endurance-trained people during a short interruption to training. *J Appl Physiol.* 84:1895-1901, 1998.

Herd, S. L., A. E. Hardman, L. H. Boobis, and C. J. Cairns. The effect of 13 weeks of running training followed by 9 d of detraining on postprandial lipaemia. *Br J Nutr.* 80:57-66, 1998.

Jeppesen, J., C. B. Hollenbeck, M. Y. Zhou, A. M. Coulston, C. Jones, Y. D. Chen, and G. M. Reaven. Relation between insulin resistance, hyperinsulinemia, postheparin plasma lipoprotein lipase activity, and postprandial lipemia. *Arterioscler Thromb Vasc Biol.* 15:320-324, 1995.

Jurca, R., M. J. Lamonte, T. S. Church, C. P. Earnest, S. J. Fitzgerald, C. E. Barlow, A. N. Jordan, J. B. Kampert, and S. N. Blair. Associations of muscle strength and fitness with metabolic syndrome in men. *Med Sci Sports Exerc.* 36:1301-1307, 2004.

Karpe, F., S. Boquist, R. Tang, G. M. Bond, U. de Faire, and A. Hamsten. Remnant lipoproteins are related to intima-media thickness of the carotid artery independently of LDL cholesterol and plasma triglycerides. *J Lipid Res.* 42:17-21, 2001.

Karpe, F., U. de Faire, M. Mercuri, M. G. Bond, M. L. Hellenius, and A. Hamsten. Magnitude of alimentary lipemia is related to intima-media thickness of the common carotid artery in middle-aged men. *Atherosclerosis.* 141:307-314, 1998.

Karpe, F. and A. Hamsten. Postprandial lipoprotein metabolism and atherosclerosis. *Curr Opin Lipidol.* 6:123-129, 1995.

Kasapis, C. and P. D. Thompson. The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review. *J Am Coll Cardiol.* 45:1563-1569, 2005.

Kashyap, M. L., R. L. Barnhart, L. S. Srivastava, G. Perisutti, C. Allen, E. Hogg, C. J. Glueck, and R. L. Jackson. Alimentary lipemia: plasma high-density lipoproteins and apolipoproteins CII and CIII in healthy subjects. *Am J Clin Nutr.* 37:233-243, 1983.

Katja, B., L. Tiina, S. Veikko, and J. Pekka. Associations of leisure time physical activity, self-rated physical fitness, and estimated aerobic fitness with serum C-reactive protein among 3803 adults. *Atherosclerosis*, 2005.

Katsanos, C. S., P. W. Grandjean, and R. J. Moffatt. Effects of low and moderate exercise intensity on postprandial lipemia and postheparin plasma lipoprotein lipase activity in physically active men. *J Appl Physiol.* 96:181-188, 2004.

Katsanos, C. S. and R. J. Moffatt. Acute effects of premeal versus postmeal exercise on postprandial hypertriglyceridemia. *Clin J Sport Med.* 14:33-39, 2004.

Kiens, B. and H. Lithell. Lipoprotein metabolism influenced by training-induced changes in human skeletal muscle. *J Clin Invest.* 83:558-564, 1989.

Kiens, B., H. Lithell, K. J. Mikines, and E. A. Richter. Effects of insulin and exercise on muscle lipoprotein lipase activity in man and its relation to insulin action. *J Clin Invest.* 84:1124-1129, 1989.

Kiens, B. and E. A. Richter. Utilization of skeletal muscle triacylglycerol during postexercise recovery in humans. *Am J Physiol.* 275:E332-337, 1998.

King, D.S., G.P. Dalsky, W.E. Clutter, D.A. Young, M.A. Staten, P.E. Cryer, and J.O. Holloszy. Effects of exercise and lack of exercise on insulin sensitivity and responsiveness. *J Appl Physiol.* 64:1942-1946, 1988.

Kirwan, J. P. and M. Jing. Modulation of insulin signaling in human skeletal muscle in response to exercise. *Exerc Sport Sci Rev.* 30:85-90, 2002.

Klocke, F.J., D.P. Copley, J.A. Krawczyk and M. Reichlin. Rapid renal clearance of immunoreactive canine plasma myoglobin. *Circulation.* 65:1522-1528, 1982.

Koutsari, C. and A. E. Hardman. Exercise prevents the augmentation of postprandial lipaemia attributable to a low-fat high-carbohydrate diet. *Br J Nutr.* 86:197-205, 2001.

Koutsari, C., F. Karpe, S. M. Humphreys, K. N. Frayn, and A. E. Hardman. Exercise prevents the accumulation of triglyceride-rich lipoproteins and their remnants seen when changing to a high-carbohydrate diet. *Arterioscler Thromb Vasc Biol.* 21:1520-1525, 2001.

Koutsari, C., D. Malkova and A.E. Hardman. Postprandial lipemia after short-term variation in dietary fat and carbohydrate. *Metabolism*. 49:1150-1155, 2000.

Kraemer, W. J., K. Adams, E. Cafarelli, G. A. Dudley, C. Dooly, M. S. Feigenbaum, S. J. Fleck, B. Franklin, A. C. Fry, J. R. Hoffman, R. U. Newton, J. Potteiger, M. H. Stone, N. A. Ratamess, and T. Triplett-McBride. American College of Sports Medicine position stand. Progression models in resistance training for healthy adults. *Med Sci Sports Exerc*. 34:364-380, 2002.

Kraemer, W. J., J. E. Dziados, L. J. Marchitelli, S. E. Gordon, E. A. Harman, R. Mello, S. J. Fleck, P. N. Frykman, and N. T. Triplett. Effects of different heavy-resistance exercise protocols on plasma beta-endorphin concentrations. *J Appl Physiol*. 74:450-459, 1993.

Kraemer, W. J., S. J. Fleck, C. M. Maresh, N. A. Ratamess, S. E. Gordon, K. L. Goetz, E. A. Harman, P. N. Frykman, J. S. Volek, S. A. Mazzetti, A. C. Fry, L. J. Marchitelli, and J. F. Patton. Acute hormonal responses to a single bout of heavy resistance exercise in trained power lifters and untrained men. *Can J Appl Physiol*. 24:524-537, 1999.

Kraemer, W. J. and N. A. Ratamess. Fundamentals of resistance training: progression and exercise prescription. *Med Sci Sports Exerc*. 36:674-688, 2004.

Kraemer, W.J., J.S. Volek, J.A. Bush, M. Putukian, and W.J. Sebastianelli. Hormonal responses to consecutive days of heavy resistance exercise with or without nutritional supplementation. *J Appl Physiol.* 75:1847-1853, 1998.

Kuller, L. H., R. P. Tracy, J. Shaten, and E. N. Meilahn. Relation of C-reactive protein and coronary heart disease in the MRFIT nested case-control study. Multiple Risk Factor Intervention Trial. *Am J Epidemiol.* 144:537-547, 1996.

LaMonte, M. J., B. E. Ainsworth, and J. L. Durstine. Influence of cardiorespiratory fitness on the association between C-reactive protein and metabolic syndrome prevalence in racially diverse women. *J Womens Health (Larchmt).* 14:233-239, 2005.

Lehtonen, A. and J. Viikari. Serum triglycerides and cholesterol and serum high-density lipoprotein cholesterol in highly physically active men. *Acta Med Scand.* 204:111-114, 1978.

Lemieux, I., A. Pascot, D. Prud'homme, N. Almeras, P. Bogaty, A. Nadeau, J. Bergeron, and J. P. Despres. Elevated C-reactive protein: another component of the atherothrombotic profile of abdominal obesity. *Arterioscler Thromb Vasc Biol.* 21:961-967, 2001.

LeMura, L. M., S. P. von Duvillard, J. Andreacci, J. M. Klebez, S. A. Chelland, and J. Russo. Lipid and lipoprotein profiles, cardiovascular fitness, body composition, and

diet during and after resistance, aerobic and combination training in young women. *Eur J Appl Physiol.* 82:451-458, 2000.

Levine, G. N., J. F. Keaney, Jr., and J. A. Vita. Cholesterol reduction in cardiovascular disease. Clinical benefits and possible mechanisms. *N Engl J Med.* 332:512-521, 1995.

Libby, P., P. M. Ridker, and A. Maseri. Inflammation and atherosclerosis. *Circulation.* 105:1135-1143, 2002.

Lichtenstein, A. H., A. T. Erkkila, B. Lamarche, U. S. Schwab, S. M. Jalbert, and L. M. Ausman. Influence of hydrogenated fat and butter on CVD risk factors: remnant-like particles, glucose and insulin, blood pressure and C-reactive protein. *Atherosclerosis.* 171:97-107, 2003.

Liesen, H., B. Dufaux, and W. Hollmann. Modifications of serum glycoproteins the days following a prolonged physical exercise and the influence of physical training. *Eur J Appl Physiol Occup Physiol.* 37:243-254, 1977.

Lithell, H., M. Cedermark, J. Froberg, P. Tesch, and J. Karlsson. Increase of lipoprotein-lipase activity in skeletal muscle during heavy exercise. Relation to epinephrine excretion. *Metabolism.* 30:1130-1134, 1981.

Lithell, H., J. Orlander, R. Schele, B. Sjodin, and J. Karlsson. Changes in lipoprotein-lipase activity and lipid stores in human skeletal muscle with prolonged heavy exercise. *Acta Physiol Scand.* 107:257-261, 1979.

Malkova, D., R. D. Evans, K. N. Frayn, S. M. Humphreys, P. R. Jones, and A. E. Hardman. Prior exercise and postprandial substrate extraction across the human leg. *Am J Physiol Endocrinol Metab.* 279:E1020-1028, 2000.

Malkova, D., A. E. Hardman, R. J. Bowness, and I. A. Macdonald. The reduction in postprandial lipemia after exercise is independent of the relative contributions of fat and carbohydrate to energy metabolism during exercise. *Metabolism.* 48:245-251, 1999.

Manolio, T. A., P. J. Savage, G. L. Burke, K. A. Liu, L. E. Wagenknecht, S. Sidney, D. R. Jacobs, Jr., J. M. Roseman, R. P. Donahue, and A. Oberman. Association of fasting insulin with blood pressure and lipids in young adults. The CARDIA study. *Arteriosclerosis.* 10:430-436, 1990.

Marcell, T. J., K. A. McAuley, T. Traustadottir, and P. D. Reaven. Exercise training is not associated with improved levels of C-reactive protein or adiponectin. *Metabolism.* 54:533-541, 2005.

Marti, B., M. Knobloch, W. F. Riesen, and H. Howald. Fifteen-year changes in exercise, aerobic power, abdominal fat, and serum lipids in runners and controls. *Med Sci Sports Exerc.* 23:115-122, 1991.

Martin, R. P., W. L. Haskell, and P. D. Wood. Blood chemistry and lipid profiles of elite distance runners. *Ann N Y Acad Sci.* 301:346-360, 1977.

Mattusch, F., B. Dufaux, O. Heine, I. Mertens, and R. Rost. Reduction of the plasma concentration of C-reactive protein following nine months of endurance training. *Int J Sports Med.* 21:21-24, 2000.

Maxwell, S.E. and H.D. Delaney. *Designing Experiments and Analysing Data: A Model Comparison Perspective.* Wadsworth, California, 1990.

McArdle, W. D., F. I. Katch, and V. L. Katch. *Exercise Physiology - Energy, Nutrition, and Human Performance.* 5th ed: Lippincott Williams and Wilkins, 2001.

Meier-Ewert, H. K., P. M. Ridker, N. Rifai, N. Price, D. F. Dinges, and J. M. Mullington. Absence of diurnal variation of C-reactive protein concentrations in healthy human subjects. *Clin Chem.* 47:426-430, 2001.

Merrill, J. R., R. G. Holly, R. L. Anderson, N. Rifai, M. E. King, and R. DeMeersman. Hyperlipemic response of young trained and untrained men after a high fat meal. *Arteriosclerosis.* 9:217-223, 1989.

Meyer, E., H. T. Westerveld, F. C. de Ruyter-Meijstek, M. M. van Greevenbroek, R. Rienks, H. J. van Rijn, D. W. Erkelens, and T. W. de Bruin. Abnormal postprandial apolipoprotein B-48 and triglyceride responses in normolipidemic women with

greater than 70% stenotic coronary artery disease: a case-control study. *Atherosclerosis*. 124:221-235, 1996.

Miesenbock, G. and J. R. Patsch. Postprandial hyperlipidemia: the search for the atherogenic lipoprotein. *Current Opinion in Lipidology*. 3:196-201, 1992.

Miyashita, M., S.F. Burns, and D.J. Stensel. Accumulating short bouts of running exercise throughout the day reduces postprandial plasma triacylglycerol concentrations and resting blood pressure in healthy young men. *Journal of Physical Activity and Health*. 3:112-123, 2006a.

Miyashita, M., S.F. Burns, and D.J. Stensel. Exercise and postprandial lipemia: effect of continuous compared with intermittent activity patterns. *Am J Clin Nutr*. 83:24-29, 2006b.

Morris, J. N., J. A. Heady, P. A. Raffle, C. G. Roberts, and J. W. Parks. Coronary heart-disease and physical activity of work. *Lancet*. 265:1111-1120; concl, 1953a.

Morris, J. N., J. A. Heady, P. A. Raffle, C. G. Roberts, and J. W. Parks. Coronary heart-disease and physical activity of work. *Lancet*. 265:1053-1057; contd, 1953b.

Murphy, M. H., A. M. Nevill, and A. E. Hardman. Different patterns of brisk walking are equally effective in decreasing postprandial lipaemia. *Int J Obes Relat Metab Disord*. 24:1303-1309, 2000.

Murtagh, E. M., C. Boreham, A. Nevill, G. Davison, T. Trinick, E. Duly, M. El-Agnaf, and M. Murphy. Acute responses of inflammatory markers of cardiovascular disease risk to a single walking session. *Journal of Physical Activity and Health*. 3:324-332, 2005.

Nosaka, K. and P. M. Clarkson. Changes in indicators of inflammation after eccentric exercise of the elbow flexors. *Med Sci Sports Exerc*. 28:953-961, 1996.

Nosaka, K. and P.M. Clarkson. Variability in serum creatine kinase response after eccentric exercise of the elbow flexors. *Int. J. Sports Med*. 17:120-127, 1996a.

Nosaka, K., P.M. Clarkson, and F.S. Apple. Time course of serum protein changes after strenuous exercise of the forearm flexors. *J. Lab Clin Med* 119:183-188, 1992.

Ohta, T., Y. Ikeda, R. Nakamura, and I. Matsuda. Lipoprotein-containing apolipoprotein A-I: sex-related quantitative and qualitative changes in this lipoprotein subspecies after ingestion of fat. *Am J Clin Nutr*. 56:404-409, 1992.

Ostrowski, K., C. Hermann, A. Bangash, P. Schjerling, J. N. Nielsen, and B. K. Pedersen. A trauma-like elevation of plasma cytokines in humans in response to treadmill running. *J Physiol*. 513 (Pt 3):889-894, 1998.

Ostrowski, K., T. Rohde, S. Asp, P. Schjerling, and B. K. Pedersen. Pro- and anti-inflammatory cytokine balance in strenuous exercise in humans. *J Physiol*. 515 (Pt 1):287-291, 1999.

Paffenbarger, R. S. and W. E. Hale. Work activity and coronary heart mortality. *N Engl J Med.* 292:545-550, 1975.

Paffenbarger, R. S., Jr., M. E. Laughlin, A. S. Gima, and R. A. Black. Work activity of longshoremen as related to death from coronary heart disease and stroke. *N Engl J Med.* 282:1109-1114, 1970.

Paffenbarger, R. S., Jr., A. L. Wing, and R. T. Hyde. Physical activity as an index of heart attack risk in college alumni. *Am J Epidemiol.* 108:161-175, 1978.

Parks, E. J. Effect of dietary carbohydrate on triglyceride metabolism in humans. *J Nutr.* 131:2772S-2774S, 2001.

Pasceri, V., J. T. Willerson, and E. T. Yeh. Direct proinflammatory effect of C-reactive protein on human endothelial cells. *Circulation.* 102:2165-2168, 2000.

Patsch, J. R., J. B. Karlin, L. W. Scott, L. C. Smith, and A. M. Gotto, Jr. Inverse relationship between blood levels of high density lipoprotein subfraction 2 and magnitude of postprandial lipemia. *Proc Natl Acad Sci U S A.* 80:1449-1453, 1983.

Patsch, J. R., G. Miesenbock, T. Hopferwieser, V. Muhlberger, E. Knapp, J. K. Dunn, A. M. Gotto, Jr., and W. Patsch. Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. *Arterioscler Thromb.* 12:1336-1345, 1992.

Patsch, J. R., S. Prasad, A. M. Gotto, Jr., and G. Bengtsson-Olivecrona. Postprandial lipemia. A key for the conversion of high density lipoprotein 2 into high density lipoprotein 3 by hepatic lipase. *J Clin Invest.* 74:2017-2023, 1984.

Paulsen, G., H. B. Benestad, I. Strom-Gundersen, L. Morkrid, K. T. Lappegard, and T. Raastad. Delayed leukocytosis and cytokine response to high-force eccentric exercise. *Med Sci Sports Exerc.* 37:1877-1883, 2005.

Pearson, T. A., G. A. Mensah, R. W. Alexander, J. L. Anderson, R. O. Cannon, 3rd, M. Criqui, Y. Y. Fadl, S. P. Fortmann, Y. Hong, G. L. Myers, N. Rifai, S. C. Smith, Jr., K. Taubert, R. P. Tracy, and F. Vinicor. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation.* 107:499-511, 2003.

Pedersen, B.E., A. Steensberg, and P. Schjerling. Muscle-derived interleukin-6: possible biological effects. *J. Physiol.* 536:329-337, 2001.

Petitt, D. S., S. A. Arngrimsson, and K. J. Cureton. Effect of resistance exercise on postprandial lipemia. *J Appl Physiol.* 94:694-700, 2003.

Petitt, D. S. and K. J. Cureton. Effects of prior exercise on postprandial lipemia: a quantitative review. *Metabolism.* 52:418-424, 2003.

Petridou, A., N. Gerkos, M. Kolifa, M. G. Nikolaidis, D. Simos, and V. Mougios. Effect of exercise performed immediately before a meal of moderate fat content on postprandial lipaemia. *Br J Nutr.* 91:683-687, 2004.

Pfeiffer, M., T. Ludwig, C. Wenk, and P. C. Colombani. The influence of walking performed immediately before meals with moderate fat content on postprandial lipemia. *Lipids Health Dis.* 4:24, 2005.

Podl, T. R., J. M. Zmuda, S. M. Yurgalevitch, M. C. Fahrenbach, L. L. Bausserman, R. B. Terry, and P. D. Thompson. Lipoprotein lipase activity and plasma triglyceride clearance are elevated in endurance-trained women. *Metabolism.* 43:808-813, 1994.

Poehlman, E.T., R.V. Dvorak, W.F. DeNino, M. Brochu, and P.A. Ades. Effects of resistance training and endurance training on insulin sensitivity in nonobese, young women: a controlled randomized trial. *J Clin Endocrinol Metab.* 85:2463-2468, 2000.

Poehlman, E.T., A.W. Gardner, P.A. Ades, S.M. Katzman-Rooks, S.M. Montgomery, O.K. Atlas, D.L. Ballor, and R.S. Tyzbir. Resting energy metabolism and cardiovascular disease risk in resistance-trained and aerobically trained males. *Metabolism.* 41:1351-1360, 1992.

Potts, J. L., S. M. Humphreys, S. W. Coppack, R. M. Fisher, G. F. Gibbons, and K. N. Frayn. Fasting plasma triacylglycerol concentrations predict adverse changes in lipoprotein metabolism after a normal meal. *Br J Nutr.* 72:101-109, 1994.

Prabhakaran, B., E. A. Dowling, J. D. Branch, D. P. Swain, and B. C. Leutholtz. Effect of 14 weeks of resistance training on lipid profile and body fat percentage in premenopausal women. *Br J Sports Med.* 33:190-195, 1999.

Pradhan, A. D., J. E. Manson, N. Rifai, J. E. Buring, and P. M. Ridker. C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *Jama.* 286:327-334, 2001.

Pratley, R., B. Nicklas, M. Rubin, J. Miller, A. Smith, M. Smith, B. Hurley and A. Goldberg. Strength training increases resting metabolic rate and norepinephrine levels in healthy 50- to 65-yr-old men. *J Appl Physiol.* 76:133-137, 1994.

Rashid, S., K. D. Uffelman, and G. F. Lewis. The mechanism of HDL lowering in hypertriglyceridemic, insulin-resistant states. *J Diabetes Complications.* 16:24-28, 2002.

Rawson, E. S., P. S. Freedson, S. K. Osganian, C. E. Matthews, G. Reed, and I. S. Ockene. Body mass index, but not physical activity, is associated with C-reactive protein. *Med Sci Sports Exerc.* 35:1160-1166, 2003.

Richelsen, B., S. B. Pedersen, T. Moller-Pedersen, O. Schmitz, N. Moller, and J. D. Borghlum. Lipoprotein lipase activity in muscle tissue influenced by fatness, fat distribution and insulin in obese females. *Eur J Clin Invest.* 23:226-233, 1993.

Ridker, P. M. Clinical application of C-reactive protein for cardiovascular disease detection and prevention. *Circulation*. 107:363-369, 2003.

Ridker, P. M., M. Cushman, M. J. Stampfer, R. P. Tracy, and C. H. Hennekens. Plasma concentration of C-reactive protein and risk of developing peripheral vascular disease. *Circulation*. 97:425-428, 1998.

Ridker, P. M., R. J. Glynn, and C. H. Hennekens. C-reactive protein adds to the predictive value of total and HDL cholesterol in determining risk of first myocardial infarction. *Circulation*. 97:2007-2011, 1998.

Ridker, P. M., C. H. Hennekens, J. E. Buring, and N. Rifai. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *N Engl J Med*. 342:836-843, 2000.

Roberts, W. L., L. Moulton, T. C. Law, G. Farrow, M. Cooper-Anderson, J. Savory, and N. Rifai. Evaluation of nine automated high-sensitivity C-reactive protein methods: implications for clinical and epidemiological applications. Part 2. *Clin Chem*. 47:418-425, 2001.

Roche, H. M. and M. J. Gibney. Effect of long-chain n-3 polyunsaturated fatty acids on fasting and postprandial triacylglycerol metabolism. *Am J Clin Nutr*. 71:232S-237S, 2000.

Roche, H. M. and M. J. Gibney. Long-chain n-3 polyunsaturated fatty acids and triacylglycerol metabolism in the postprandial state. *Lipids*. 34 Suppl:S259-265, 1999.

Roche, H. M. and M. J. Gibney. Postprandial triacylglycerolaemia: the effect of low-fat dietary treatment with and without fish oil supplementation. *Eur J Clin Nutr*. 50:617-624, 1996.

Roche, H. M., A. Zampelas, J. M. Knapper, D. Webb, C. Brooks, K. G. Jackson, J. W. Wright, B. J. Gould, A. Kafatos, M. J. Gibney, and C. M. Williams. Effect of long-term olive oil dietary intervention on postprandial triacylglycerol and factor VII metabolism. *Am J Clin Nutr*. 68:552-560, 1998.

Ross, R. Atherosclerosis--an inflammatory disease. *N Engl J Med*. 340:115-126, 1999.

Ryu, J. E., G. Howard, T. E. Craven, M. G. Bond, A. P. Hagaman, and J. R. Crouse, 3rd. Postprandial triglyceridemia and carotid atherosclerosis in middle-aged subjects. *Stroke*. 23:823-828, 1992.

Sady, S. P., E. M. Cullinane, A. Saritelli, D. Bernier, and P. D. Thompson. Elevated high-density lipoprotein cholesterol in endurance athletes is related to enhanced plasma triglyceride clearance. *Metabolism*. 37:568-572, 1988.

Sady, S. P., P. D. Thompson, E. M. Cullinane, M. A. Kantor, E. Domagala, and P. N. Herbert. Prolonged exercise augments plasma triglyceride clearance. *Jama*. 256:2552-2555, 1986.

Schlierf, G., A. Dinsenhacher, H. Kather, M. Kohlmeier, and W. Haberbosch. Mitigation of alimentary lipemia by postprandial exercise--phenomena and mechanisms. *Metabolism*. 36:726-730, 1987.

Seip, R. L., T. J. Angelopoulos, and C. F. Semenkovich. Exercise induces human lipoprotein lipase gene expression in skeletal muscle but not adipose tissue. *Am J Physiol*. 268:E229-236, 1995.

Seip, R. L., K. Mair, T. G. Cole, and C. F. Semenkovich. Induction of human skeletal muscle lipoprotein lipase gene expression by short-term exercise is transient. *Am J Physiol*. 272:E255-261, 1997.

Seip, R. L. and C. F. Semenkovich. Skeletal muscle lipoprotein lipase: molecular regulation and physiological effects in relation to exercise. *Exerc Sport Sci Rev*. 26:191-218, 1998.

Shannon, K. A., R. M. Shannon, J. N. Clore, C. Gennings, B. J. Warren, and J. A. Potteiger. Resistance exercise and postprandial lipemia: The dose effect of differing volumes of acute resistance exercise bouts. *Metabolism*. 54:756-763, 2005.

Sharrett, A. R., L. E. Chambless, G. Heiss, C. C. Paton, and W. Patsch. Association of postprandial triglyceride and retinyl palmitate responses with asymptomatic carotid artery atherosclerosis in middle-aged men and women. The Atherosclerosis Risk in Communities (ARIC) Study. *Arterioscler Thromb Vasc Biol.* 15:2122-2129, 1995.

Siegel, A. J., J. J. Stec, I. Lipinska, E. M. Van Cott, K. B. Lewandrowski, P. M. Ridker, and G. H. Tofler. Effect of marathon running on inflammatory and hemostatic markers. *Am J Cardiol.* 88:918-920, A919, 2001.

Simpson, H. S., C. M. Williamson, T. Olivecrona, S. Pringle, J. Maclean, A. R. Lorimer, F. Bonnefous, Y. Bogaievsky, C. J. Packard, and J. Shepherd. Postprandial lipemia, fenofibrate and coronary artery disease. *Atherosclerosis.* 85:193-202, 1990.

Siri, WE. Body composition from fluid spaces and density. Univ Calif Donner Lab Med Phys Rep, 1956.

Skoglund-Andersson, C., R. Tang, M. G. Bond, U. de Faire, A. Hamsten, and F. Karpe. LDL particle size distribution is associated with carotid intima-media thickness in healthy 50-year-old men. *Arterioscler Thromb Vasc Biol.* 19:2422-2430, 1999.

Smith, J. K., R. Dykes, J. E. Douglas, G. Krishnaswamy, and S. Berk. Long-term exercise and atherogenic activity of blood mononuclear cells in persons at risk of developing ischemic heart disease. *Jama.* 281:1722-1727, 1999.

Stampfer, M. J., R. M. Krauss, J. Ma, P. J. Blanche, L. G. Holl, F. M. Sacks, and C. H. Hennekens. A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. *Jama*. 276:882-888, 1996.

Steensberg, A., M. A. Febbraio, T. Osada, P. Schjerling, G. van Hall, B. Saltin, and B. K. Pedersen. Interleukin-6 production in contracting human skeletal muscle is influenced by pre-exercise muscle glycogen content. *J Physiol*. 537:633-639, 2001.

Stensvold, I., A. Tverdal, P. Urdal, and S. Graff-Iversen. Non-fasting serum triglyceride concentration and mortality from coronary heart disease and any cause in middle aged Norwegian women. *Bmj*. 307:1318-1322, 1993.

Strachan, A. F., T. D. Noakes, G. Kotzenberg, A. E. Nel, and F. C. de Beer. C reactive protein concentrations during long distance running. *Br Med J (Clin Res Ed)*. 289:1249-1251, 1984.

Tanasescu, M., M. F. Leitzmann, E. B. Rimm, W. C. Willett, M. J. Stampfer, and F. B. Hu. Exercise type and intensity in relation to coronary heart disease in men. *Jama*. 288:1994-2000, 2002.

Taylor, H. L., E. Buskirk, and A. Henschel. Maximal oxygen intake as an objective measure of cardio-respiratory performance. *J Appl Physiol*. 8:73-80, 1955.

Taylor, C., G. Rogers, C. Goodman, R. D. Baynes, T. H. Bothwell, W. R. Bezwoda, F. Kramer, and J. Hattingh. Hematologic, iron-related, and acute-phase protein responses to sustained strenuous exercise. *J Appl Physiol.* 62:464-469, 1987.

Thomas, J.R. and J.K. Nelson. *Research Methods in Physical Activity.* 3rd ed: Human Kinetics, Champaign, IL, 1996.

Tisi, P. V., M. Hulse, A. Chulakadabba, P. Gosling, and C. P. Shearman. Exercise training for intermittent claudication: does it adversely affect biochemical markers of the exercise-induced inflammatory response? *Eur J Vasc Endovasc Surg.* 14:344-350,

Tollin, C., M. Ericsson, O. Johnson, and C. Backman. Clearance of triglycerides from the circulation and its relationship to serum lipoproteins: influence of age and sex. *Scand J Clin Lab Invest.* 45:679-684, 1985.

Tracy, R. P. Inflammation in cardiovascular disease: cart, horse, or both? *Circulation.* 97:2000-2002, 1998.

Tracy, R. P., R. N. Lemaitre, B. M. Psaty, D. G. Ives, R. W. Evans, M. Cushman, E. N. Meilahn, and L. H. Kuller. Relationship of C-reactive protein to risk of cardiovascular disease in the elderly. Results from the Cardiovascular Health Study and the Rural Health Promotion Project. *Arterioscler Thromb Vasc Biol.* 17:1121-1127, 1997.

Tracy, R. P., B. M. Psaty, E. Macy, E. G. Bovill, M. Cushman, E. S. Cornell, and L. H. Kuller. Lifetime smoking exposure affects the association of C-reactive protein with cardiovascular disease risk factors and subclinical disease in healthy elderly subjects. *Arterioscler Thromb Vasc Biol.* 17:2167-2176, 1997.

Tsetsonis, N. V. and A. E. Hardman. Effects of low and moderate intensity treadmill walking on postprandial lipaemia in healthy young adults. *Eur J Appl Physiol Occup Physiol.* 73:419-426, 1996a.

Tsetsonis, N. V. and A. E. Hardman. Reduction in postprandial lipemia after walking: influence of exercise intensity. *Med Sci Sports Exerc.* 28:1235-1242, 1996b.

Tsetsonis, N. V., A. E. Hardman, and S. S. Mastana. Acute effects of exercise on postprandial lipemia: a comparative study in trained and untrained middle-aged women. *Am J Clin Nutr.* 65:525-533, 1997.

Tuominen, J. A., P. Ebeling, R. Bourey, L. Koranyi, A. Lamminen, J. Rapola, T. Sane, H. Vuorinen-Markkola, and V. A. Koivisto. Postmarathon paradox: insulin resistance in the face of glycogen depletion. *Am J Physiol.* 270:E336-343, 1996.

U.S. Department of Health and Human Services. *Physical Activity and Health: A Report of the Surgeon-General.* Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion., 1996.

Van Oostrom, A. J., T. P. Sijmonsma, C. Verseyden, E. H. Jansen, E. J. de Koning, T. J. Rabelink, and M. Castro Cabezas. Postprandial recruitment of neutrophils may contribute to endothelial dysfunction. *J Lipid Res.* 44:576-583, 2003.

Vincent, W.J. *Statistics in Kinesiology*. Human Kinetics: Champaign, IL, 1995.

Vodak, P. A., P. D. Wood, W. L. Haskell, and P. T. Williams. HDL-cholesterol and other plasma lipid and lipoprotein concentrations in middle-aged male and female tennis players. *Metabolism.* 29:745-752, 1980.

Volek, J. S., A. L. Gomez, and W. J. Kraemer. Fasting lipoprotein and postprandial triacylglycerol responses to a low-carbohydrate diet supplemented with n-3 fatty acids. *J Am Coll Nutr.* 19:383-391, 2000.

Weight, L. M., D. Alexander, and P. Jacobs. Strenuous exercise: analogous to the acute-phase response? *Clin Sci (Lond).* 81:677-683, 1991.

Weintraub, M. S., R. Zechner, A. Brown, S. Eisenberg, and J. L. Breslow. Dietary polyunsaturated fats of the W-6 and W-3 series reduce postprandial lipoprotein levels. Chronic and acute effects of fat saturation on postprandial lipoprotein metabolism. *J Clin Invest.* 82:1884-1893, 1988.

Weltan, S. M., A. N. Bosch, S. C. Dennis, and T. D. Noakes. Influence of muscle glycogen content on metabolic regulation. *Am J Physiol.* 274:E72-82, 1998.

Willerson, J. T. and P. M. Ridker. Inflammation as a cardiovascular risk factor. *Circulation*. 109:II2-10, 2004.

Wilmore, J.H. and D.L. Costill. *Physiology of Sport and Exercise*. 2nd ed: Human Kinetics, 1998.

Witztum, J. L. and J. A. Berliner. Oxidized phospholipids and isoprostanes in atherosclerosis. *Curr Opin Lipidol*. 9:441-448, 1998.

Wood, D., G. De Backer, O. Faergeman, I. Graham, G. Mancina, and K. Pyorala. Prevention of coronary heart disease in clinical practice: recommendations of the Second Joint Task Force of European and other Societies on Coronary Prevention. *Atherosclerosis*. 140:199-270, 1998.

World Health Organization. *The World Health Report*. Geneva: The World Health Organization, 2001.

Yaspelkis III, B. B. Resistance training improves insulin signaling and action in skeletal muscle. *Exerc Sport Sci Rev*. 34:42-46, 2006.

Yeh, E. T. and J. T. Willerson. Coming of age of C-reactive protein: using inflammation markers in cardiology. *Circulation*. 107:370-371, 2003.

Zampelas, A., H. Roche, J. M. Knapper, K. G. Jackson, M. Tornaritis, C. Hatzis, M. J. Gibney, A. Kafatos, B. J. Gould, J. Wright, and C. M. Williams. Differences in

postprandial lipaemic response between Northern and Southern Europeans. *Atherosclerosis*. 139:83-93, 1998.

Zauner, C. W. and N. Y. Benson. Effect of physical training upon postprandial lipemia in men with abnormal responses to the oral fat tolerance test. *J Sports Med Phys Fitness*. 17:381-386, 1977.

Zhang, J. Q., T. R. Thomas, and S. D. Ball. Effect of exercise timing on postprandial lipemia and HDL cholesterol subfractions. *J Appl Physiol*. 85:1516-1522, 1998.

Zilversmit, D. B. Atherogenesis: a postprandial phenomenon. *Circulation*. 60:473-485, 1979.

APPENDIX A

Ref No: R01/P23

LOUGHBOROUGH UNIVERSITY ETHICAL ADVISORY COMMITTEE

HUMAN BIOLOGICAL INVESTIGATION RESEARCH PROPOSAL

Title: The effect of a single bout of resistance exercise on postprandial lipaemia.

Applicants: Dr David Stensel, Mr Stephen Burns, Mr T Nightingale, Miss E Holder
and Miss R Carr

Departments: PE, SS & RM

Date of clearance: 27 September, 2001

Comments of the Committee:

The Committee asked that the following points be addressed:

1. The Committee asked that the proposal and the Participant Information Sheet be amended to state that a brief Health Questionnaire determining fitness to participate (ie no cold/flu symptoms) would be administered on each visit to the laboratory.
2. "Participant" should read "you" on the participant information sheet (BACKGROUND section).
3. Ruth Carr's contact details need adding to both the Participant Information Sheet and the poster used to recruit participants.

Once these points have been satisfactorily addressed the Committee agreed that the proposal should be granted clearance to proceed.

APPENDIX A

Ref No: R04/P36

LOUGHBOROUGH UNIVERSITY ETHICAL ADVISORY COMMITTEE

RESEARCH PROPOSAL INVOLVING HUMAN PARTICIPANTS

Title: The effect of resistance training on plasma ghrelin concentrations

Applicants: Dr D Stensel, S Burns, L Rosenberg, L Webster

Departments: School of Sport and Exercise Sciences

Date of clearance: 14 May 2004

Comments of the Committee:

The Committee agreed to issue clearance to proceed.

APPENDIX A

Ref No: R04/P137

LOUGHBOROUGH UNIVERSITY ETHICAL ADVISORY SUB-COMMITTEE

RESEARCH PROPOSAL INVOLVING HUMAN PARTICIPANTS

Title: The effect of resistance exercise on postprandial lipaemia

Applicants: Dr David Stensel, Stephen Burns, Lynsey Douglas, Chris Longhorn & Richard Pulsford

Department: SSES

Date of clearance: 21 December 2004

Comments of the Sub-Committee:

The Sub-Committee agreed to issue clearance to proceed.

APPENDIX A

Ref No: R05/P68

LOUGHBOROUGH UNIVERSITY ETHICAL ADVISORY SUB-COMMITTEE

RESEARCH PROPOSAL INVOLVING HUMAN PARTICIPANTS

Title: Comparison of the effects of resistance exercise and downhill treadmill running on postprandial lipaemia and C-reactive protein

Applicants: Dr D Stensel, S Burns, N Charles, E Kingzett

Department: SSES

Date of clearance: 1 July 2005

Comments of the Sub-Committee:

The Sub-Committee agreed to issue clearance to proceed.

APPENDIX B

COMPARISON OF THE EFFECTS OF RESISTANCE EXERCISE AND DOWNHILL TREADMILL RUNNING ON POSTPRANDIAL LIPAEMIA AND C-REACTIVE PROTEIN.

INFORMED CONSENT FORM

(to be completed after Participant Information Sheet has been read)

The purpose and details of this study have been explained to me. I understand that this study is designed to further scientific knowledge and that all procedures have been approved by the Loughborough University Ethical Advisory Committee.

I have read and understood the information sheet and this consent form.

I have had an opportunity to ask questions about my participation.

I understand that I am under no obligation to take part in the study.

I understand that I have the right to withdraw from this study at any stage for any reason, and that I will not be required to explain my reasons for withdrawing.

I understand that all the information I provide will be treated in strict confidence.

I agree to participate in this study.

Your name _____

Your signature _____

Signature of investigator _____

Date _____

APPENDIX C

HEALTH SCREEN FOR STUDY VOLUNTEERS

Name or Number

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is to ensure (i) their own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

Please complete this brief questionnaire to confirm fitness to participate:

1. At present, do you have any health problem for which you are:

- | | | |
|---|------------------------------|-----------------------------|
| (a) on medication, prescribed or otherwise..... | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| (b) attending your general practitioner..... | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| (c) on a hospital waiting list..... | Yes <input type="checkbox"/> | No <input type="checkbox"/> |

2. In the past two years, have you had any illness which require you to:

- | | | |
|--|------------------------------|-----------------------------|
| (a) consult your GP | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| (b) attend a hospital outpatient department..... | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| (c) be admitted to hospital | Yes <input type="checkbox"/> | No <input type="checkbox"/> |

3. Have you ever had or been diagnosed with any of the following:

- | | | |
|--|------------------------------|-----------------------------|
| (a) Convulsions/epilepsy..... | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| (b) Asthma..... | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| (c) Eczema..... | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| (d) Diabetes | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| (e) A blood disorder | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| (f) Head injury | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| (g) Digestive problems | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| (h) Heart problems | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| (i) Problems with bones or joints | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| (j) Disturbance of balance/coordination..... | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| (k) Numbness in hands or feet..... | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| (l) Disturbance of vision..... | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| (m) Ear / hearing problems..... | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| (n) Thyroid problems | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| (o) Kidney or liver problems..... | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| (p) Allergy to nuts | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| (q) High cholesterol | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
| (r) High triacylglycerol or any other form of dyslipidaemia..... | Yes <input type="checkbox"/> | No <input type="checkbox"/> |

4. Has any, otherwise healthy, member of your family under the

- | | | |
|--|------------------------------|-----------------------------|
| age of 35 died suddenly during or soon after exercise? | Yes <input type="checkbox"/> | No <input type="checkbox"/> |
|--|------------------------------|-----------------------------|

APPENDIX C

5. Are you:

- (a) Currently activeYes ☐ No ☐
- (b) Dieting Yes ☐ No ☐
- (c) A smoker or have you ever smoked Yes ☐ No ☐

If YES to any question, please describe briefly if you wish (eg to confirm problem was/is short-lived, insignificant or well controlled.)

.....

Thank you for your cooperation!

Loughborough University

APPENDIX D

LOUGHBOROUGH UNIVERSITY, SCHOOL OF SPORT & EXERCISE SCIENCES

Project title: Comparison of the effects of resistance exercise and downhill treadmill running on postprandial lipaemia and C-reactive protein.

It is important that you are free from illness/infection on the day of laboratory/exercise tests. This is to ensure your health and safety. Please do not give your consent to participate if you are currently suffering with a cold/flu or any other condition that might be exacerbated by engaging in laboratory/exercise tests.

Statement of consent to participate:

I understand that my permission to take part in these tests is voluntary and that I am free to withdraw from the experiment at any stage.

I declare that I am currently in good health and give my consent to participate in today's tests.

Signature of subject:.....Date:.....

Signature of witness:.....

