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THE EFFECT OF PRIOR EXERCISE ON POSTPRANDIAL LIPAEMIA

by

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ABSTRACT

Coronary heart disease (CHD) remains the primary cause of death in the United Kingdom today and postprandial lipaemia (exaggerated elevation of the plasma triacylglycerol (TAG) concentration after intake of a fat-containing meal) is gaining recognition as an independent CHD risk factor. This thesis provides an overview of the effect that single bouts of exercise can exert on postprandial lipaemia. The conclusions from the experimental chapters within this thesis are that: prior moderate exercise reduces the lipaemia associated with moderate and high fat meals to a very similar extent in percentage terms; a single session of resistance exercise does not lower postprandial TAG concentrations in overweight, sedentary men, regardless of exercise intensity; *ad libitum* energy intake is not significantly increased on the morning after a brisk walk, with the exercise-induced lowering of lipaemia akin to percentage reductions from studies where fixed size meals were given; and aerobic exercise which lowers postprandial lipaemia, also increases postprandial hepatic portal vein and femoral artery blood flow. The general message from this thesis is that moderate-intensity aerobic exercise should be advocated as a strategy to lower cardiovascular disease risk, based on experimental evidence that postprandial lipaemia is consistently reduced after single bouts of brisk walking.

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CHAPTER ONE

GENERAL INTRODUCTION

1.1. Mortality and coronary heart disease

In the modern developed world, where mortality rates due to communicable disease are relatively low, most deaths occur due to non-communicable disease, with cardiovascular diseases being the most dominant. The World Health Organization (WHO) calculates the years of life lost (YLL) through taking into account the age at which deaths occur and giving greater weight to deaths occurring at younger ages. The percentage of YLL due to non-communicable diseases can generally be seen to increase as the income status of a country increases. For countries in the highest income band, the percentage of YLL due to communicable diseases is just 8%, with 77% due to non-communicable diseases and 15% due to injuries; the U.K. can therefore be seen to sit above most other high income countries as 84% of its YLL are due to non-communicable diseases (WHO, 2009). Within Europe, the most recently available data show that 48% of all deaths are due to cardiovascular disease (CVD), and the most prominent disease within CVD, coronary heart disease (CHD), accounts for 21.6% of all-cause mortality on its own, making it the most common cause of death for Europeans (Allender et al., 2008a). The situation in the U.K. is not radically different from that in Europe as a whole. For research purposes, the British Heart Foundation (BHF) considers the term "premature death" to mean death which occurs before reaching 75 years of age. Despite U.K. life expectancy reaching an all-time high in 2007 at 77 years for men and 82 years for women (WHO, 2009), tens of thousands of people still die prematurely every year from CHD in the U.K. The most recently published data show that 94,000 deaths occurred in the U.K. in 2006 due to CHD, of which 31,000 were considered premature. CVD

accounted for approximately 35% of all U.K. deaths in 2006, and CHD was the single greatest cause of death that year, representing 16.5% of total mortality (Allender et al., 2008b). Death rates from cardiovascular disease have been falling steadily since the early 1970s, and CHD deaths since the late 1970s, with reduction in major risk factors (primarily cigarette smoking) and improved treatment identified as the main reasons behind the lower death rates (Unal et al., 2004). However, as CHD remains the number one cause of death in the U.K. today, it clearly requires continued attention if death rates are not to plateau: an observation with some evidence among younger adults (Allender et al., 2008c; O'Flaherty et al., 2008).

1.2. The pathophysiology of early atherosclerosis

In simple terms, coronary heart disease refers to a partial or complete blockage of one or more of the coronary arteries. The blockage is initially brought on by deposition of lipids and leukocytes in the arterial walls: a process known as atherosclerosis. One of the earliest acknowledged indicators that atherosclerosis is occurring or may be about to occur is endothelial dysfunction. The endothelium (the lining of the intima) forms the innermost layer of cells within the blood vessel wall, therefore defining the lumen of the vessel and presenting a barrier that prevents large molecules from entering the subendothelial space. Endothelial cells are highly vasoactive and play an important role in modulating vascular tone, mainly through production of the vasodilators nitric oxide (NO) and prostacyclin, but also through the release of the potent vasoconstrictor endothelin-1. Whilst the endothelium is healthy and functioning in a normal physiological manner, the action of locally produced vasodilators dominates over that of vasoconstrictors, and net relaxation of the underlying smooth muscle in initiated, leading to vasodilation of the vessel when required. If the endothelium becomes damaged, and the barrier between the blood and the subendothelial vascular layers is eroded,

the secretion of vasodilatory substances is reduced and net vasoconstriction results. Factors reported to contribute towards a dysfunctional endothelium include cigarette smoking, dyslipidaemia (particularly hypercholesterolaemia), diabetes and hypertension (Cacciola et al., 2007), with all of these factors thought to impact endothelial functioning in part through an increase in endothelial production of reactive oxygen species. After the integrity of the vascular endothelium is compromised, lipoproteins are able to permeate it and begin to accumulate within the intima. In particular, elevated levels of low density lipoproteins (LDLs) are known to correlate closely with atherosclerotic development (Gardner et al., 1996; St-Pierre et al., 2005; Stampfer et al., 1996). Once within the intima, LDL particles bind to the proteoglycans of the extracellular matrix and the concentration of LDLs within the subendothelial space starts to increase. The trapping of LDL within the vessel wall increases the risk of it becoming modified by glycation, or, more often, oxidation. Following modification, LDL is more likely to recruit leukocytes (particularly monocytes) to the vessel wall and combined with the expression of leukocyte adhesion molecules on the luminal surface of injured endothelial cells, inflammation is directly promoted. While there is strong evidence that both lipoproteins and leukocytes will enter the intima at sites where the endothelial barrier is damaged, the specific sequence in which these events unfold remains controversial. Atherosclerosis is an inflammatory process and injury to the endothelium leads to an increase in inflammatory cytokines. These cytokines attract leukocytes to the damaged endothelial site via chemotaxis and it may be through the action of such leukocytes that the endothelium is made permeable to invading lipoproteins. Regardless of the order in which these steps occur, it is clear than once monocytes have adhered to the luminal surface of the intima, they then differentiate to macrophages beneath the endothelium. Such macrophages are capable of ingesting the modified LDL through scavenger receptors and thus become

engorged with lipid, at which stage they are often referred to as “foam cells”. Foam cells are the primary constituent of the atherosclerotic lesion known as the “fatty streak” and fatty streaks represent the earliest visible sign of atherosclerosis.

1.3. Lipoprotein metabolism, triacylglycerol, and CHD risk factors

1.3.1. Residual cardiovascular risk

While the relationship between high concentrations of LDL-C and development of atherosclerotic disease is strong, and reducing LDL-C justifiably remains the primary treatment step, CHD is perhaps the most multifactorial of all diseases, with literally hundreds of factors reported to “independently predict” the disease in an article dating back almost 30 years (Hopkins and Williams, 1981). The true independence of the relationship between some of these 246 factors and CHD has rightly been questioned (Smith and Phillips, 1990), but it remains the case that lowering the plasma LDL-C concentration is not sufficient to lower risk for everyone and a so-called “residual cardiovascular risk” (Hausenloy and Yellon, 2008) is still evident when LDL-C is reduced to within the prescribed range. Moreover, while the use of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins) to combat high LDL-C concentrations is now standard practice, the risk of encountering serious skeletal muscle complaints from such pharmaceuticals, termed “statin myopathy”, is widely documented (Radcliffe and Campbell, 2008; Thompson et al., 2003). Therefore, other risk factors must be considered alongside LDL-C, and alternative treatments, free from the side effects of statins, require investigation. The notion of an “atherogenic lipoprotein phenotype” or “atherogenic lipid triad” has developed, within which the triumvirate of high plasma triacylglycerol (TAG) concentrations, small, dense LDL, and low concentrations of high density lipoprotein (HDL), combine to elevate cardiovascular risk (Austin et al., 1990;

Grundy, 1998; Krauss, 1998). To understand the strength of the residual cardiovascular risk posed by lipoproteins other than LDL, it is first necessary to introduce the lipoproteins and present an overview of lipoprotein metabolism.

1.3.2. Basics of lipoprotein composition and metabolism

Lipoproteins are macromolecular structures composed of triacylglycerol (TAG), cholesteryl esters (CE), phospholipids, free cholesterol and apolipoproteins. They form a heterogeneous family of particles which allow transport of TAG and CE within the plasma. Most often, lipoproteins are divided into four major classes, with each class containing particles within a different density range, as defined by Svedberg flotation rate (Sf). The largest and least dense particles are known as chylomicrons ($S_f > 400$). Chylomicrons are formed in the intestine, and, by weight, are 85 – 90% TAG. Nascent chylomicrons (containing apolipoprotein-B_{48}; apo-B_{48}) pick up exogenous TAG within the enterocyte, and then exit the intestine into the lymphatic system. Later, they enter the bloodstream at the point where the thoracic duct empties into the left subclavian vein. For a healthy individual, in the postabsorptive state the concentration of chylomicrons circulating in the plasma is extremely low; after a fat-containing meal, however, the concentration of chylomicrons (and therefore, chylomicron-TAG) can increase several fold. The enzyme lipoprotein lipase (LPL), which is abundant within the capillaries of adipose tissue, skeletal muscle and the heart, cleaves TAG from the core of chylomicrons, thereby reducing the particles in size and lipid content. These lipid-depleted particles are referred to as chylomicron remnants, and, after further delipidation by hepatic lipase (HL), their removal from the circulation is thought to occur primarily via the LDL-receptor related protein in the liver. In contrast to chylomicrons, very low density lipoproteins (VLDLs, S_f 20 – 400) are formed in the liver and contain apolipoprotein-B_{100}

(apo-B_{100}). They are smaller and denser than chylomicrons, but still consist primarily of TAG (65% by weight). Due to the high TAG content of both chylomicrons and VLDLs, the two species are sometimes collectively known as the TAG-rich lipoproteins (TRLs). Like chylomicrons, VLDLs lose TAG when passing through the capillaries of tissues expressing LPL, becoming smaller, denser and more CE-rich in the process. VLDLs are either removed from the bloodstream by the LPL receptor, or, for those particles which have been repeatedly delipidated by LPL and HL, they become so TAG-depleted and CE-enriched that they require reclassification as part of a different lipoprotein species: the low density lipoproteins (LDLs). LDL particles (S_f 0 - 12) are primarily composed of cholesteryl ester (~45% by weight) and contain very little TAG. Their main function is to supply cholesterol to tissues and they leave the circulation via the LDL receptor. The final major class of lipoproteins are the high density lipoproteins (HDLs). These particles begin as apolipoprotein-AI (apo-AI) molecules, associated with some phospholipid, and, even after gaining cholesterol, they remain primarily composed of protein and phospholipid. The pre-β HDLs collect excess cholesterol from cells and pick up unesterified cholesterol which is discarded when TAG-rich lipoproteins undergo hydrolysis by LPL, thus forming discoidal HDL. These particles become fully mature HDLs after esterification of their free cholesterol to cholesteryl esters by lecithin-cholesterol acyl transferase (LCAT). HDLs are active in removing cholesterol from tissues and transporting it to the liver: a process known as reverse cholesterol transport.

1.3.3. High density lipoproteins and risk of CVD/CHD

The physiological function of HDLs in removing cholesterol from the foam cells of atherosclerotic lesions suggests that such particles have a direct anti-atherogenic effect. Numerous epidemiological studies have reported an inverse relationship between the

concentration of HDL cholesterol and risk of cardiovascular disease (Assmann et al., 1996; Castelli et al., 1986; de Backer et al., 1998; Despres et al., 2000; Goldbourt et al., 1997; Gordon et al., 1989; Jousilahti et al., 1999; Koro et al., 2006; Lewington et al., 2007; Perova et al., 1995; Ridker et al., 2005; Sharrett et al., 2001; Stamler et al., 1986; Stampfer et al., 1991; Stensvold et al., 1992; Walldius et al., 2001), and a recent study, analysing data from over 100,000 individuals, found that HDL protects both men and women against both CVD and CHD across the age range (Cooney et al. 2009). Such findings have led to low HDL-C being viewed as strong, independent risk factor for CHD (NCEP ATP III guidelines, 2002) and interventions to increase HDL-C are encouraged as a means to atherosclerosis prevention (Wierzbicki, 2005).

1.3.4. Fasting TAG concentrations and risk of CHD

The observation that plasma TAG concentrations are associated with myocardial infarction (MI) was first made half a century ago (Albrink and Man, 1959). Many studies thereafter have shown a positive univariate correlation between TAG and CHD (see Austin (1991) for an excellent review) and in those studies which controlled for either total or LDL cholesterol during analysis, the association was found to persist. However, as these studies were not prospective in nature, the direction of the association between TAG and MI could not be stated confidently, particularly as lipid levels have been shown to increase during the year after MI (Tibblin and Cramer, 1963). Subsequent investigations with prospective designs generally added support to the idea that increased concentrations of plasma/serum TAG were correlated with CHD, but high density lipoprotein cholesterol (HDL-C) proved to be a confounding factor in the relationship i.e. when HDL-C concentration was entered into multivariate analyses, the association between TAG and CHD often disappeared (Austin,

1991). The fact that HDL-C stills shows an inverse correlation with CHD after controlling for TAG, but the opposite is generally not found (Smith and Phillips, 1990), is confusing, particularly as HDL-C concentrations are largely determined by metabolism of TAG-rich lipoproteins (Miesenböck and Patsch, 1990; Nikkilä et al., 1987; Patsch et al., 1987; Tall, 1990). The intra-individual variability shown when analysing plasma samples taken on two separate occasions has been shown to be higher for TAG than for cholesterol (Jacobs and Barrett-Connor, 1982) and may result in hypertriglyceridaemic individuals being misclassified as having normal TAG concentrations (Austin, 1991). If a non-differential bias for misclassification to occur does exist, then this may explain why the extent of the association between TAG and CHD is lowered in multivariate analyses by HDL-C, but TAG does not render the correlation between HDL-C and CHD non-significant (Kelsey et al., 1986). Review articles continue to disagree to this day whether TAG is an independent predictor of CHD after controlling for possible confounders (particularly HDL-C), with plasma TAG showing no independent association with coronary mortality in a review from the early 1990s (Criqui et al., 1993), whereas a recent review declared that "Epidemiologic studies provide evidence of an association between triglycerides and the development of primary CHD independently of HDL-C" (Morrison and Hokanson, 2009). However, due to the fact that controversy still exists regarding the strength of association between TAG concentrations and cardiac events, the prevailing mood has been for TAG not to be considered an independent risk factor for development of CHD. The use of fasting measures to predict subsequent risk of disease is limited by the fact that disease risk is more strongly associated with the usual value of a variable that is observed, i.e. non-fasting measures (Sarwar and Sattar, 2009), leading to baselines values underestimating risk through regression dilution bias. As the postprandial state reflects the norm for most adults across the day,

measurement of postprandial, not fasting, TAG concentrations may be of more relevance in assessing disease risk.

1.3.5. Plasma TAG concentrations in the postprandial state and risk of CHD

Over 90% of dietary fat exists in the form of TAG (Frayn, 2003) and intake of a single fat-containing meal transiently elevates the plasma TAG concentration. Exogenous TAG is hydrolysed to fatty acids and 2-monoacylglycerol, primarily by the action of pancreatic lipase in the small intestine, with these constituents then reesterified to TAG within the enterocyte. TAG is a hydrophobic compound and consequently the majority of newly synthesised TAG is incorporated within chylomicrons, forming the core of these intestinally-derived lipoprotein particles. Chylomicrons exit the intestine into the lymphatic system, after which they enter the bloodstream at the point where the thoracic duct empties into the left subclavian vein. As most people will eat several meals across the course of an average day, and plasma TAG does not return to pre-meal concentrations until many hours after eating, the majority of individuals will be in a near daylong postprandial state from breakfast onwards and the plasma TAG concentration will not return to baseline until the fast brought on by nighttime sleep. In addition to chylomicrons, which are formed in the intestine and contain only exogenous TAG, another class of lipoprotein particles is produced which also carries large quantities of TAG. VLDLs are secreted by the liver, and, postabsorptively at least, the fatty acids which compose TAG within a VLDL particle come from endogenous sources. Dietary fatty acids have however been reported to make a sizeable contribution to VLDL-TAG after a fat meal (Heath et al., 2007). In the hours after fat ingestion, the concentration of TAG within both chylomicrons and very low density lipoproteins (VLDLs) is increased substantially (Cohn et al., 1988; Cohn et al., 1989; Genest et al., 1986). Intravascular hydrolysis of TAG is effected

by the enzyme lipoprotein lipase (LPL), with chylomicron- and VLDL-TAG both cleared from the circulation by this common saturable mechanism (Brunzell et al., 1973). Chylomicrons appear to be favoured over VLDLs as a substrate by LPL (Potts et al., 1991a,b), with *in vitro* work suggesting the affinity for LPL to bind chylomicrons is approximately 45 times greater than that for VLDLs (Xiang et al., 1999). The preference of LPL for chylomicrons over VLDLs leads to an accumulation of large VLDL particles (Cohn et al., 1993; Karpe et al., 1993). Indeed, while absolute TAG content per particle is higher for chylomicrons than VLDLs, 80% of the rise in TAG-rich lipoprotein particle number after a standard mixed meal is due to apoB-100 containing particles (Schneeman et al., 1993) and more than 90% of postprandial TRL particles are of endogenous origin (Karpe, 1999). In the blood, chylomicron-TAG has an approximate half-life of just 5 minutes (Grundy and Mok, 1976), with chylomicron particles being rapidly delipidated by LPL, thus leaving chylomicron remnants. Over 30 years ago, Donald Zilversmit hypothesised a mechanistic rationale outlining the atherogenic potential of TAG-rich lipoproteins, and in particular, chylomicron remnants (Zilversmit, 1979). With apolipoproteins from chylomicrons having been detected in lesions found in human arteries, Zilversmit proposed that atherogenesis was a postprandial phenomenon, with the formation of chylomicron remants from chylomicrons suggested to be a process which promoted atherosclerosis. A case-control study in humans later reported that postprandial plasma total TAG concentrations, particularly the concentrations 6 h and 8 h after a meal, independently predicted whether study participants were patients with coronary artery disease (CAD) or controls (Patsch et al., 1992). Postprandial TAG was far more discriminatory than fasting TAG in this sense, with over two-thirds of participants correctly identified purely by their TAG concentrations in the late postprandial period. Postprandial TAG was found to be predictive of CAD even after controlling for HDL-C, therefore making

it a superior measure to the TAG concentration in the fasted state. Investigation of patients with familial chylomicronaemia has suggested that chylomicrons and/or large VLDL are directly atherogenic (Benlian et al., 1996). These individuals are deficient in LPL and display low concentrations of remnant particles and low density lipoprotein (LDL), yet severe coronary atherosclerosis is still evident. The high concentration of TAG-rich lipoproteins seen in this group may therefore accelerate the atherosclerotic process. Small chylomicron remnants have also been implicated in the progression of coronary heart disease (CHD) (Karpe et al., 1994). Further evidence for the atherogenic potential of postprandial lipoproteins was provided by an article reporting a positive association between TAG concentrations 6 h and 7 h after a fat-rich meal and the intima-media thickness (IMT) of the common carotid artery (Karpe et al., 1998). The IMT of the common carotid artery provides a surrogate marker of early atherosclerosis and as the men investigated in the study of Karpe et al. were not CHD patients, the positive correlation between postprandial TAG concentrations and IMT signifies the potential importance of TAG-rich lipoproteins in atheromatous plaque formation. Recent epidemiological studies also support the notion that an elevated posprandial TAG concentration is associated with greater likelihood of atherosclerotic disease. Non-fasting triglycerides were shown to correlate (after multivariate analysis) with myocardial infarction, ischaemic heart disease and death in a prospective cohort study involving almost 14,000 men and women (Nordestgaard et al., 2007). The results of a separate prospective study involving 26,500 women were published in the same issue of JAMA; this study concluded that non-fasting triglyceride levels were associated with incident cardiovascular events, independent of traditional cardiac risk factors, whereas fasting triglyceride levels showed little independent relationship (Bansal et al., 2007). These studies appear to have generated interest in the relationship between TAG-rich lipoproteins in the

postprandial period (particularly, lipoprotein remnants) and cardiovascular events, with three review articles published on this topic within the last 18 months (Kannel and Vasan, 2009; Nordestgaard et al., 2009; Stalenhoef and de Graaf, 2008). Postprandial lipaemia is therefore an emerging risk factor for CHD and evidence implicating remnants of TAG-rich lipoproteins in the atherogenic process is steadily accumulating.

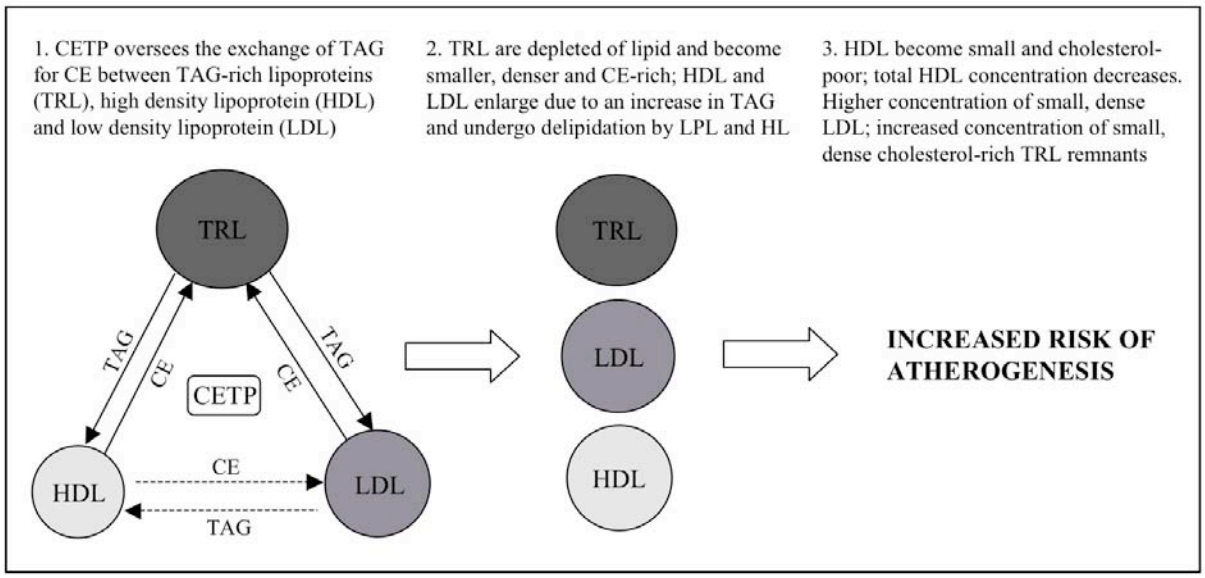
1.4 Integrative metabolism during the postprandial period and CHD risk

1.4.1. CETP and exchange between lipoproteins

It has been known for at least 45 years that exchange of hydrophobic lipids occurs between lipoprotein particles of different classes (Nichols and Smith, 1965). The isolation and characterisation of cholesteryl ester transfer protein (CETP) over 30 years ago (Chajek and Fielding, 1978) outlined a mechanism by which CE was transferred from HDL to VLDL and LDL, with TAG being transported in the opposite direction. More recent evidence suggests that CETP transfers cholesteryl esters from HDL particles to TAG-rich lipoproteins, with TAG travelling in the opposite direction (Assmann and Gotto Jr., 2004). Therefore, in addition to the proposed direct atherogenicity of chylomicrons after LPL has cleaved them of their TAG, and the elevation of VLDL-TAG in the plasma due to competition from chylomicrons, TAG-rich lipoproteins may also increase cardiovascular risk by lowering HDL concentrations through the action of CETP.

Figure 1.1 presents a summary of lipid exchange between lipoproteins via CETP, as outlined in recent reviews (Borggreve et al., 2003; Dullaart et al., 2007; Sarwar and Sattar, 2009).

Figure 1.1. Potential mechanism by which an elevated concentration of TAG-rich lipoproteins results in increased atherogenic risk



CETP facilitates exchange of TAG from TRL to HDL and LDL, and from LDL to HDL, with CE travelling in the opposite direction. Dotted arrows indicate that net mass transfer between HDL and LDL is not as great as for exchange between TRL and HDL/LDL. These exchanges result in TRLs becoming smaller, which could allow the particles to enter the subendothelial space through damaged regions of the endothelium. Accumulation of TAG within HDL and LDL increases their size, but the particles become smaller and denser due to loss of TAG after interaction with LPL and HL. Small, CE-poor HDLs are more likely to be removed from the circulation, whereas small, dense LDLs present an increased risk of atherogenesis as their smaller size allows greater access to the intima, thereby beginning the process of atherosclerosis. CE, cholesteryl ester; CETP, cholesteryl ester transfer protein; LPL, lipoprotein lipase; HL, hepatic lipase; TAG, triacylglycerol

It has been reported that VLDL-TAG concentration correlates strongly with net CE transfer in normolipidaemic individuals (Mann et al., 1991); a finding which the authors offer as evidence that VLDL concentrations determine the extent of CE transfer. By extension, this would suggest that in the fasted state, when concentrations of TRLs (and thus, plasma TAG concentrations) are generally low, exchange of hydrophobic lipids between species would also be expected to be low. However, during the period after ingestion of a fat-containing meal, when the concentration of TRLs (and plasma TAG) is elevated, exchange between lipoproteins is increased, therefore chylomicrons and VLDLs become small, dense and CE-

rich, while HDLs and LDLs increase in TAG content and size. Hydrolysis of the newly acquired TAG (particularly via the action of hepatic lipase) reduces the size of HDL and LDL particles, with the total HDL concentration reducing (due to subsequent removal of small, dense, CE-poor HDL) and the concentration of small, dense, atherogenic LDL particles becoming greater. It can therefore be seen that elevation of the plasma TAG concentration due to an increase in TRL-TAG after a fat-containing meal, has the potential to accelerate development of atherosclerosis through modification of TRLs themselves and other lipoprotein species (de Grooth et al., 2004).

1.4.2. Insulin and postprandial lipid metabolism

In addition to the modifications which CETP can induce when TRL-TAG concentrations are elevated, insulin exerts control over postprandial TAG concentrations through its various effects in different body tissues. Fat-containing meals consumed in everyday life are rarely pure lipid, instead containing a mixture of fat, carbohydrate and protein. The carbohydrate content of the meal triggers an insulin response, with the pancreas increasing the dose of insulin it secretes into the portal vein, such that the acute increase in plasma glucose concentration is normalised. As well as modulating the extent to which glucose will be taken up by cells in skeletal muscle and adipose tissue, insulin governs the release of fatty acids from adipose tissue through its inhibitory effect on hormone sensitive lipase (HSL; Steinberg and Khoo, 1977) via cAMP-dependent (Enoksson et al., 1998) and -independent pathways (Strålfors and Honnor, 1989). The increase in circulating insulin after a mixed meal reduces lipolysis of TAG stored in adipose tissue, thereby lowering the plasma non-esterified fatty acid (NEFA) concentration in the early postprandial period. Insulin also regulates the activity of the enzyme responsible for cleaving fatty acids from TAG within the plasma, lipoprotein

lipase (LPL). The activity of LPL within adipose tissue is increased by insulin, therefore enhancing clearance of fatty acids from plasma TAG into adipose tissue, whereas skeletal muscle LPL activity is largely unaffected (Wang and Eckel, 2009). Following ingestion of a mixed meal, the addition of exogenous TAG to TAG already circulating endogenously results in a plentiful supply of plasma TAG which must be directed to storage depots or oxidised. As the liver is continuously releasing TAG into the plasma within VLDLs, postprandial increases in insulin act both directly, and indirectly (by lowering the concentration of NEFA available for reesterification to TAG, following inhibition of HSL) to downregulate hepatic VLDL secretion (Lewis et al., 1995). As insulin plays a crucial role in the regulation of postprandial TAG metabolism, a reduced sensitivity to the effect that insulin exerts when it binds to the insulin receptor can have negative consequences for the plasma TAG concentration (Reaven, 1988). An increased plasma TAG concentration is one of several factors which elevates the risk of CHD for individuals with type 2 diabetes (Reaven & Chen, 1996).

1.4.3. Coagulation and inflammation in the postprandial state

In addition to the direct and indirect effects of TRL on CHD risk, other cardiovascular risk factors are also increased in the postprandial state. Factor VII is an essential component regarding the initiation of coagulation, but elevated concentrations of Factor VII have been reported to increase risk of CHD (Junker et al., 1997). Intake of a high-fat mixed meal increases activation of Factor VII during the subsequent postprandial period (Silveira et al., 1994), with the extent of the activation seemingly related to the degree of LPL-mediated release of fatty acids from TAG-rich lipoproteins. Similarly, concentrations of the pro-inflammatory cytokine interleukin-6 (IL-6) have been shown to rise in the hours after an oral fat tolerance test (Lundman et al., 2007). As IL-6 has been shown to correlate positively with

CHD (Patterson et al., 2009), it appears the cardiovascular risk due to increased TRL after high-fat mixed meals is compounded further by elevation of circulating markers of coagulation and inflammation postprandially.

1.4.5. Summary

Based on current knowledge of postprandial metabolism, it appears that the combination of an elevated chylomicron remnant concentration and a preponderance of large VLDL particles reflects a situation in which dietary fat can contribute both directly and indirectly towards development of atherosclerosis (Cohn, 2008). The remodelling of lipoproteins in the postprandial period can drive an unfavourable change in strong cardiovascular risk factors (low HDL-C and increased concentrations of small, dense LDL), with fat-containing meals also shown to increase coagulatory and inflammatory processes. Interventions which attenuate the rise in TRL after a meal are therefore necessary if the risk of developing coronary heart disease (CHD), posed by postprandial lipaemia, is to be addressed.

1.5. Macronutrient intake and plasma TAG concentrations

Dietary intake of fat, and saturated fat in particular, has attracted attention in recent years, with growing evidence suggesting that lipids are implicated in the aetiology of cardiovascular events (Kris-Etherton et al., 2001). As plasma TAG concentrations are substantially elevated after fat loading (Cohen et al., 1988a; Dubois et al., 1994; Dubois et al., 1998), and postprandial TAG concentrations are correlated with both early atherosclerosis (Karpe et al., 1998) and CHD incidence (Patsch et al., 1992), reducing dietary fat intake could be viewed as a logical step to lower the risk of CHD. Indeed, the recent report of a joint WHO/FAO expert consultation reiterated the standpoint that fat should not constitute more than 15 - 30% of total

energy intake and saturated fat not more than 10% of total energy if cardiovascular disease risk is to be minimised (Joint WHO/FAO Expert Consultation on Diet, Nutrition and the Prevention of Chronic Diseases, 2003). Fat is however an essential part of the human diet and attention must be paid to which macronutrient will be substituted in if fat intake is reduced, as individuals switching to high-carbohydrate, low fat diets have been documented to experience negative changes to the lipid profile. Concentrations of HDL-C are reduced and for almost 50 years it has been known that high-carbohydrate diets cause hypertriacylglycerolaemia (Ahrens et al., 1961; Nestel, 1966). In fact, after just 3 days of a high-carbohydrate diet, fasting concentrations of plasma triacylglycerol (TAG) were found to be significantly elevated when compared with concentrations after intake of a high-fat diet (Koutsari et al., 2000). Switching a small percentage of dietary carbohydrate for dietary fat (whether it be polyunsaturated, monounsaturated or saturated) has been shown to increase HDL-C and reduce the serum TAG concentration (Mensink and Katan, 1992), and recent reports suggest that cardiovascular risk factors are more favourably affected by restriction of dietary carbohydrate than dietary fat (Volek et al., 2008; Volek et al., 2009). The article by Volek et al. (2009) provides evidence that saturated fatty acid levels within plasma TAG were actually reduced by a hypocaloric low-carbohydrate diet when compared with a hypocaloric low-fat diet, despite the low-carbohydrate diet containing three times more saturated fat. On balance, the literature suggests that low-fat, high-carbohydrate diets are not beneficial, and may be detrimental, in their effects on plasma TAG concentrations, therefore other strategies beside manipulation of dietary macronutrients are necessary if the elevations in potentially atherogenic lipoprotein remnants seen in the postprandial period are to be positively affected.

1.6. Exercise and postprandial lipaemia: What we know so far

1.6.1. Overview of the literature

The observation that endurance-trained athletes have lower fasting TAG concentrations (Durnstine and Haskell, 1994) and an attenuated lipaemic response to a fatty meal (Cohen et al., 1989; Merrill et al., 1989) when compared with their sedentary counterparts, suggested that aerobic exercise training could be a useful strategy by which to reduce plasma lipids. Indeed, several studies have reported a decrease in plasma TAG concentration after aerobic exercise training (Bieger et al., 1984; Drexel et al., 2008; Fenkci et al., 2006; Schlierf et al., 1988; Weintraub et al., 1989). As the postprandial state is the period when potentially atherogenic lipoprotein remnants abound (Cohn, 1998; Karpe et al., 1999; Zilversmit, 1979), and fasting TAG concentration (when in the normolipidaemic range) shows poor sensitivity as a predictor of the extent to which TAG concentrations will rise postprandially (Groot et al., 1991; Schrezenmeir et al., 1993), it is essential that studies attempting to investigate the effect of exercise on TAG metabolism, in relation to CHD risk, include measurements during the postprandial period. The first study designed to test the effect of a single exercise bout on postprandial lipaemia was conducted some 50 years ago and details of 72 studies which have investigated the impact of a single bout of aerobic exercise on postprandial TAG concentrations can be found in **Table 1.1**. The effect of single bouts of resistance exercise on postprandial lipaemia is not so widely reported in the literature (8 papers since 2003; **Table 1.2**), and the effect of a protocol combining aerobic and resistance exercise within the one session has only been documented in one article (**Table 1.3**).

Table 1.1. Studies investigating the effect of a single bout of aerobic exercise on postprandial TAG concentrations

Lead author(s) and date	Participants	Exercise	Exercise-meal delay (hours)	Main measure(s)	% Change with exercise (vs. control)
Cohen & Goldberg (1960)	22 (12 male) healthy medical students. Age: 20 - 25 y	• 6.44 km walk in 90 min, 45 min rest, then 3.22 km walk in 60 min (*N* = 6) • Continuous 9.66 km walk taking 120 - 180 min (*N* = 14) • Two 30 min cycles (*N* = 2)	• -1.25 h • -3 h • -3 h	Plasma turbidity (optical density) at 6 h or 7 h post-meal	-24.8% (*N* = 22) (*P* = 0.01 - 0.025)
Nikkilä & Konttinen (1962)	40 healthy male army recruits. Fasting TAG 1.13 mmol·l^{-1}	Cross-sectional design with 20 men marching for 16 km (120 min) and 20 men resting	-2 h (The march began 2 h after breakfast)	Serum TAG at 4 h and 6 h post-meal	28.0% lower for walking group at 4 h (*P* < 0.01) and 11.7% lower at 6 h (*P* not stated)
Zauner et al. (1968)	Experiment 1: 8 young, healthy Caucasian males with a mean age of 33 y Experiment 2: 7 young, healthy Caucasian males with a mean age of 21 y	Experiment 1: 20 min run to exhaustion (strenuous exercise) Experiment 2: 3.22 km walk in 45 min (mild exercise)	0 h (Fat meals eaten immediately after exercise)	Serum turbidity (optical density) at 3 h, 5 h and 7 h post-meal, with fasting value subtracted, i.e. delta optical density	1. Strenuous exercise reduced the delta turbidity at 7 h (*P* < 0.01) but not at 3 h or 5 h (*P* > 0.01). 2. Lower delta optical density at 3 h and 7 h with mild exercise (*P* < 0.01)
Chinnici and Zauner (1971)	12 physically active, Caucasian males. Age 22 - 48 y	6 men did no exercise. The other 6 men performed two different exercise bouts: separate 20 min treadmill walks at heart rates of 120 beats·min^{-1} and 160 beats·min^{-1}	-0 h (Fat meals eaten immediately before exercise)	Delta optical density of serum at 3 h, 5 h and 7 h post-meal	No statistically significant differences in delta optical density of serum were found between trials at any time-point (*P* values not reported)
Maruhama et al (1977)	6 healthy male students. Age 18 - 23 y; body mass 57 - 68 kg	6 x 2 min cycling at 720 kilopound metres per min (kpm/min), with the 2 min bouts performed at hourly intervals. Heart rate of 120 - 150 beats·min^{-1}	-0.5 h (First bout of cycling was 25 - 30 min after the fat meal)	Plasma total-, chylomicron- and lipoprotein-TAG in the fasted state and hourly for 6 h after a fat load	No significant difference for any TAG measure between exercise and control trials (*P* values not reported)
Feldman and Nixon (1982)	5 (2 male) healthy, untrained volunteers. Age 29.2 y (ranged from 19 - 49 y); VO_2max 24.2 ml·kg^{-1}·min^{-1} (ranged from 14.8 - 34.4 ml·kg^{-1}·min^{-1}); fasting TAG 0.87 mmol·l^{-1}	45 min cycle at 50 - 70% of maximum work load (mean exercise load of 384 kpm/min)	-0.75 h (Cycling began 45 min after the meal)	Serum TAG concentrations in the fasted state and 0.75 h, 1.5 h and 2 h after nasogastric feeding	Serum TAG concentrations were not different between exercise and control trials at any time point (*P* > 0.05)
Welle (1984)	6 (3 male) healthy, normal-weight volunteers. Age 19 - 29 y	Three 15 min bouts of cycling at 50 W, with 45 min recoveries between	-0.75 h (First bout began 45 min postmeal)	Plasma TAG in the fasted state and half-hourly for 3 h postmeal	TAG concentrations did not differ between trials at any time postprandially (*P* > 0.05)
Schlierf et al. (1987)	12 trained male students. Age 25 y; body mass 72 kg; 5 - 11 h of exercise performed per week Wmax of 270 - 360 W (cycle ergometer)	90 min cycle at 40% of maximal exercise capacity. Heart rate ~ 130 beats·min^{-1}	-1.5 h (Cycling started 1.5 h after breakfast)	Plasma TAG in the fasted state and 1.5, 3, 4.5 and 6 h after a high-fat mixed meal	Exercise lowered plasma TAG significantly at 3 h postprandially (*P* < 0.05), but not other time points

Table 1.1. continued

Lead author(s) and date	Participants	Exercise	Exercise-meal delay (hours)	Main measure(s)	% Change with exercise (vs. control)
Klein et al. (1992)	6 healthy, sedentary men. Age 30.2 y; body mass 71.9 kg; BMI 24.5 $kg \cdot m^{-2}$; body fat 17.3 %; fasting TAG 0.89 $mmol \cdot l^{-1}$	Treadmill exercise. 5 min warm-up, 30 min of exercise at a heart rate corresponding to 75% of VO_2peak and 5 min cool-down.	-1 h (Exercise began 1 h after breakfast)	Plasma TAG concentration in the fasted state and at 4 h, 6 h and 8 h postprandially	Relative to control, exercise lowered plasma TAG at 4 h (-25.8%, $P < 0.05$), 6 h (-30.2%, $P < 0.02$) and 8 h (-26.3%, $P < 0.05$) after a fatty meal
Aldred et al. (1994)	12 (6 male) physically active, normolipidaemic nonsmokers. Age 25.8 y; body mass 69.0 kg; BMI 23.9 $kg \cdot m^{-2}$; body fat 19.6 %; VO_2max 48.6 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 0.87 $mmol \cdot l^{-1}$	120 min treadmill walk at 30.9% VO_2max	15 h	6 h incremental TAG AUC (*i*AUC)	-33.7% ($P < 0.01$)
Hardman and Aldred (1995)	12 (6 male) normolipidaemic young adults. Age 21 - 33 y.	90 min treadmill walk at 40% VO_2max	-1.5 h	6 h TAG AUC	-24% ($P < 0.05$)
Tsetsonis & Hardman (1996a)	12 (6 male) recreationally active, normolipidaemic nonsmokers. Age 26.9 y; BMI 23.7 $kg \cdot m^{-2}$; VO_2max 43.6 $ml \cdot kg^{-1} \cdot min^{-1}$	• 90 min walk at 31% VO_2max (LOW) • 90 min walk at 61% VO_2max (MOD)	15 h	6 h total TAG AUC (*t*AUC) and incremental TAG AUC (*i*AUC)	*t*AUC was significantly reduced after MOD (-25.5%, $P < 0.05$), but not after LOW (-15.7%, $P > 0.05$). *i*AUC showed a similar pattern (-31.2% for MOD, $P < 0.05$; -14.8% for LOW, $P > 0.05$)
Tsetsonis & Hardman (1996b)	9 (5 male) physically active, normolipidaemic volunteers. Age 27.7 y; body mass 71.8 kg; BMI 24.0 $kg \cdot m^{-2}$; VO_2max 50.2 $ml \cdot kg^{-1} \cdot min^{-1}$	• 180 min walk at 32% VO_2max • 90 min walk at 63% VO_2max	16 h	6 h total and incremental TAG AUC	Both lipaemic indices were reduced after both walks, relative to control ($P < 0.05$)
Tsetsonis et al. (1997)	• 13 untrained women. Age 43.8 y; body mass 62.2 kg; BMI 22.9 $kg \cdot m^{-2}$; VO_2max 31.7 $ml \cdot kg^{-1} \cdot min^{-1}$ • 9 trained women. Age 40.4 y; body mass 59.3 kg; BMI 22.2 $kg \cdot m^{-2}$; VO_2max 50.3 $ml \cdot kg^{-1} \cdot min^{-1}$	90 min treadmill walk at 62 - 63% VO_2max	16 h	6 h total and incremental TAG AUC	Total and incremental lipaemic responses were reduced for both groups, relative to control ($P < 0.05$)
Gill et al. (1998)	18 physically active, healthy, male nonsmokers. Age 30.1 y; body mass 72.2 kg; BMI 23.1 $kg \cdot m^{-2}$; VO_2max 57.8 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG < 2.0 $mmol \cdot l^{-1}$	• Three 30 min treadmill runs at 60.8% VO_2max • 90 min treadmill run at 61.8% VO_2max	15 - 16 h	6 h total TAG AUC	-17.7% for intermittent exercise and -18.1% for continuous exercise (both $P < 0.05$)
Hardman et al. (1998)	10 (9 male) normolipidaemic, endurance-trained nonsmokers. Age 37.7 y; body mass 68.8 kg; BMI 22.5 $kg \cdot m^{-2}$; fasting TAG 1.1 $mmol \cdot l^{-1}$	The volunteers completed a habitual training session lasting more than 30 min	Three oral fat tolerance tests: 15 h, 60 h and 6.5 days.	6 h total and incremental TAG AUC	Both the *t*AUC and *i*AUC were significantly greater at 60 h and 6.5 days, compared with 15 h ($P < 0.05$)

Table 1.1. continued

Lead author(s) and date	Participants	Exercise	Exercise-meal delay (hours)	Main measure(s)	% Change with exercise (vs. control)
Herd et al. (1998)	14 (6 male) recreationally active, young adults were split into two groups at baseline: Runners (those who would undertake a 13 week programme of running training) and Controls (maintain normal lifestyle). • Runners (N = 8): Age 22 y; BMI 24.6 $kg \cdot m^{-2}$; VO_2max 51.4 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 1.15 $mmol \cdot l^{-1}$ • Controls (N = 6): Age 24.5 y; BMI 23.3 $kg \cdot m^{-2}$; VO_2max 48.5 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 0.70 $mmol \cdot l^{-1}$	The last exercise session for the runners, after 13 weeks of training, was a 40 min run at a pace described as "somewhat hard" to "hard" on the Borg RPE scale. Controls did no exercise	Three oral fat tolerance tests: 15 h, 60 h and 9 days after last exercise session.	6 h total TAG AUC	For runners, *t*AUC was 37% higher at 60 h than 15 h, and 46% higher at 9 d than 15 h; these changes were significantly different from those in controls (group x time interaction; $P < 0.05$)
Zhang et al. (1998)	21 recreationally trained males. Age 27 y; body mass 82 kg; body fat 16%; VO_2max 48 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 0.97 $mmol \cdot l^{-1}$	60 min treadmill exercise at 60% VO_2max	Three trials: • -1 h • 1 h • 12 h	8 h incremental TAG AUC	• -5% (-1 h; $P > 0.05$) • -38% (1 h; $P < 0.05$) • -51% (12 h; $P < 0.05$)
Malkova et al. (1999)	12 healthy, physically active, normolipidaemic, male nonsmokers. Age 21 - 36 y; BMI 24.0 $kg \cdot m^{-2}$; VO_2max 58.6 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 0.92 $mmol \cdot l^{-1}$	• 90 min treadmill run at 60% VO_2max (after placebo) • 90 min treadmill run at 59% VO_2max (after acipimox)	16 h	6 h total and incremental TAG AUC	*t*AUC reduced by 20.8% and 22.3%, and *i*AUC lowered by 32.6% and 43.4%, for placebox and acipimox trials respectively ($P < 0.05$ for all)
Gill & Hardman (2000)	11 moderately active, postmenopausal women. Age 60.2 y; BMI 24.8 $kg \cdot m^{-2}$; VO_2max 30.7 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 1.00 $mmol \cdot l^{-1}$	90 min treadmill walk at 62.9% VO_2max	At least 12 h, but not specifically stated	6 h total and incremental TAG AUC	Exercise reduced both *t*AUC and *i*AUC significantly ($P < 0.05$), but the percentage shifts were not reported.
Malkova et al. (2000)	8 physically active/endurance-trained, normolipidaemic, nonsmoking men. Age 21 - 46 y; BMI 24.6 $kg \cdot m^{-2}$; body fat 17.6%; VO_2max 56.8 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 0.91 $mmol \cdot l^{-1}$	120 min treadmill run at 64% VO_2max	16 h	Time-averaged postprandial TAG concentration (averaged over 6 h)	34.0% reduction in postprandial TAG concentration ($P < 0.01$)
Murphy et al. (2000)	10 sedentary participants (3 men and 7 postmenopausal women). Fasting TAG 1.04 $mmol \cdot l^{-1}$ • Men: Age 46.0 y; body mass 98.6 kg; BMI 29.7 $kg \cdot m^{-2}$; VO_2max 40.3 $ml \cdot kg^{-1} \cdot min^{-1}$ • Women: Age 55.0 y; body mass 69.7 kg; BMI 27.2 $kg \cdot m^{-2}$; VO_2max 28.8 $ml \cdot kg^{-1} \cdot min^{-1}$	Two exercise trials: • 30 min walk at 60.4% VO_2max • Three 10 min walks at 59.6% VO_2max	30 min walk immediately prebreakfast, 10 min walks immediately before breakfast, lunch and dinner	11.5 h average postprandial TAG concentration following breakfast, lunch and dinner, with blood samples taken 1.5 h, 2.5 h and 3.5 h after each meal	Average postprandial TAG concentration was lower in walking trials than control (11.5% lower after 30-min walk, 12.0% lower after three 10-min walks) (main effect of trial, $P < 0.01$; no post hoc tests mentioned)

Table 1.1. continued

Lead author(s) and date	Participants	Exercise	Exercise-meal delay (hours)	Main measure(s)	% Change with exercise (vs. control)
Thomas et al. (2000)	6 sedentary, normolipidaemic men. Age 25.8 y; body mass 81.9 kg; body fat 25.9%; VO_2max 39.9 ml·kg^{-1}·min^{-1}; fasting TAG 1.55 mmol·l^{-1}	60 min treadmill jog at 63.0% VO_2max	0 h (Fat meal consumed immediately after exercise)	8 h incremental TAG AUC	No statistically significant difference between exercise and control (*P* not given)
Gill et al. (2001a)	8 active, normolipidaemic, nonsmoking men. Age 48.3 y; BMI 25.5 kg·m^{-2}; VO_2max 39.2 ml·kg^{-1}·min^{-1}; fasting TAG 1.37 mmol·l^{-1}	90 min treadmill walk at 59.4% VO_2max	Not stated (exercise on afternoon of day 1, test meal on day 2)	6 h total and incremental TAG AUC	17.7% lowering of *t*AUC (*P* = 0.014) and 17.1% reduction in *i*AUC (*P* = 0.011)
Gill et al. (2001b)	11 physically active, normolipidaemic, male nonsmokers. Age 51.7 y; body mass 74.7 kg; BMI 24.2 kg·m^{-2}; body fat 27.2 %; VO_2max 38.9 ml·kg^{-1}·min^{-1}; fasting TAG 1.03 mmol·l^{-1}	90 min treadmill walk at 64.9% VO_2max	Not stated (exercise ceased between 16:00 and 17:00 on day 1, and test meal was ingested on day 2)	Time-averaged postprandial TAG concentration (*t*AUC/8 h) and time-averaged postprandial TAG increase (*i*AUC/8 h)	Exercise reduced both time-averaged postprandial TAG concentration (-23.3%; *P* = 0.0002) and time-averaged postprandial TAG increase (-27.3%; *P* = 0.002) significantly, relative to control
Herd et al. (2001)	8 physically active, normolipidaemic, males. Age 27.0 y; BMI 24.5 kg·m^{-2}; VO_2max 3.95 L·min^{-1}; fasting TAG 0.76 mmol·l^{-1}	90 min cycle at 62.3% VO_2max	16 h	6 h total and incremental TAG AUC	27.7% lowering of *t*AUC and 41.8% reduction in *i*AUC (*P* < 0.05 for both)
Thomas et al. (2001)	25 (12 male) healthy, normolipidaemic nonsmokers. 13 trained and 12 sedentary. Age 30.4 y; body mass 68.8 kg; body fat 17.9%; VO_2max 42.8 ml·kg^{-1}·min^{-1}; fasting TAG 1.00 mmol·l^{-1}	60 min treadmill exercise at 59% VO_2max with meal containing medium chain triglycerides (MCT) vs. MCT meal control vs. long chain triglyceride (LCT) meal control	12 h	8 h incremental TAG AUC	Exercising before the MCT meal significantly lowered the 8h TAG *i*AUC when compared with control trials for both the MCT and LCT meal (*P* < 0.05)
Gill et al. (2002a)	11 apparently healthy, premenopausal women. Age 24.3 y; BMI 21.3 kg·m^{-2}; VO_2max 39.7ml·kg^{-1}·min^{-1}; fasting TAG 0.70 mmol·l^{-1}	• 60 min treadmill walk at 50.0% VO_2max • 120 min treadmill walk at 51.3% VO_2max	18 h	6 h total and incremental TAG AUC, with linear trend analysis used to determine if exercise-induced changes in a dose-dependent manner	*t*AUC was 9.3% and 22.8% lower than control after the 1 h and 2 h walks respectively (*P* = 0.001 for trend). *i*AUC was reduced by 10.6% after the 1 h walk and by 31.8% after the 2 h walk (*P* = 0.10 for trend)
Gill et al. (2003a)	9 healthy, normally active, nonsmoking, premenopausal women. Age 27.4 y; body mass 61.3 kg; BMI 22.2 kg·m^{-2}; body fat 25.7%; VO_2max 42.0 ml·kg^{-1}·min^{-1}; fasting TAG 0.67 mmol·l^{-1}	120 min treadmill walk at 50.9% VO_2max	18 h	6 h total and incremental TAG AUC	Exercise reduced *t*AUC by 23% (*P* < 0.01) and *i*AUC by 42.4% (*P* = 0.02), relative to control

Table 1.1. continued

Lead author(s) and date	Participants	Exercise	Exercise-meal delay (hours)	Main measure(s)	% Change with exercise (vs. control)
Gill et al. (2003b)	8 apparently healthy, endurance-trained men. Age 27.8 y; BMI 23.6 $kg \cdot m^{-2}$; body fat 16.9%	Participants performed an exercise session (as per their normal training) before a fat meal, then detrained for one week before the second fat tolerance test	Not stated (exercise was performed on the day before test meal ingestion)	Time-averaged postprandial TAG concentration (*t*AUC/6 h) and time-averaged postprandial TAG rise (*i*AUC/6 h)	Both the time-averaged postprandial TAG concentration (+52.5%; P = 0.002) and the time-averaged postprandial TAG rise (+69.4%; P = 0.004) were significantly higher when detrained (without exercise on the day prior to the fat tolerance test)
Petitt et al. (2003)	14 (10 male) apparently healthy, recreationally weight-trained caucasians. Age 24.3 y; body mass 73.6 kg; BMI 24.4 $kg \cdot m^{-2}$; body fat 19.5%; fasting TAG 1.01 $mmol \cdot l^{-1}$	88 min walk with a gross EE of 1.6 MJ and average heart rate of 99 $beats \cdot min^{-1}$	16 h	6 h incremental TAG AUC	*i*AUC was not significantly different between control and walking trials (P > 0.05)
Altena et al. (2004)	18 (7 male) inactive, normolipidaemic nonsmokers. Age 25.0 y; body mass 70.9 kg; BMI 24.0 $kg \cdot m^{-2}$; body fat 22.2%; VO_2max 39.6 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 1.04 $mmol \cdot l^{-1}$	• Continuous 30 min treadmill jog at 62.5% VO_2max (CON-EX) • 3 x 10 min treadmill jogs at 62.2% VO_2max (INT-EX)	12 h	8 h total and incremental TAG AUC	*i*AUC was 26.9% lower after INT-EX (P = 0.031), but only 15.9% after CON-EX (P not given, but declared nonsignificant). *t*AUC was not different between trials (P = 0.093).
Dalgaard et al. (2004)	12 men with type 2 diabetes, three of whom smoked cigarettes. Age 59.3 y; BMI 27.9 $kg \cdot m^{-2}$; VO_2max 28 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 2.8 $mmol \cdot l^{-1}$	Two separate exercise trials, each involving two 20 min bouts of cycling at 40%VO_2max, with a 20 min rest between bouts	• -3.5 h (after meal: POST) • 15 h (before meal: PRE)	8 h total and incremental TAG AUC	Compared with control, *t*AUC was 3.4% lower with POST and 7.2% higher with PRE (P = 0.72). *i*AUC was 8.9% higher than control after POST and 20.1% higher after PRE (P = 0.21)
Gill et al. (2004)	10 lean and 10 centrally obese men. All were nonsmokers and apparently healthy. • Lean men: Age 47.9 y; BMI 23.0 $kg \cdot m^{-2}$; VO_2max 43.9 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 0.85 mmol·l-1 • Obese men: Age 46.5 y; BMI 31.7 $kg \cdot m^{-2}$; VO_2max 40.6 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 1.74 $mmol \cdot l^{-1}$	90 min treadmill walk at 50.6% VO_2max for lean men and 51.1% VO_2max for obese men	16 - 18 h	Time-averaged postprandial TAG concentration (*t*AUC/8 h) and time-averaged postprandial TAG rise (*i*AUC/8 h)	Exercise lowered the postprandial TAG concentration by 25% (lean) and 23.9% (obese) (P < 0.0005 for main effect of trial), with lean men having lower concentrations (P = 0.002). Postprandial TAG rise was also reduced by exercise (-25.5% for lean, -22.1% for obese; P < 0.0005 for main effect of trial)

Table 1.1. continued

Lead author(s) and date	Participants	Exercise	Exercise-meal delay (hours)	Main measure(s)	% Change with exercise (vs. control)
Katsanos et al. (2004)	13 healthy, physically active, nonsmoking males. Age 23.8 y; body mass 77.9 kg; BMI 24.3 $kg \cdot m^{-2}$; body fat 11.9%; VO_2max 49.5 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 0.80 $mmol \cdot l^{-1}$	1100 kcal of energy were expended on a treadmill at two different exercise intensities • LOW = 25.1% VO_2max (mean time of 237.5 min) • MOD = 65.0% VO_2max (mean time of 90.8 min)	1 h	8 h incremental TAG AUC	After MOD, TAG *i*AUC was 39% lower than control ($P < 0.01$) and 34% lower than LOW ($P < 0.05$). LOW was not significantly different from control (8% lower; $P > 0.05$)
Katsanos and Moffatt (2004)	10 untrained, but recreationally active, healthy, nonsmoking males. Age 25.2 y; body mass 82.0 kg; BMI 24.5 $kg \cdot m^{-2}$; body fat 13.2%; VO_2max 46.6 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 1.14 $mmol \cdot l^{-1}$	90 min treadmill walk at ~50% VO_2max (actual intensities were 51.3% for PRE and 52.1% for POST)	Two trials: • PRE (finished 0.5 h before meal) • POST (starting 1.5 h after meal)	8 h incremental TAG AUC	Relative to control, TAG *i*AUC was 48.9% lower with PRE and 52.1% lower with POST ($P < 0.05$ for both).
Kolifa et al. (2004)	9 healthy, normolipidaemic, male nonsmokers. Age 20 - 25 y; body mass 84.9 kg; BMI 24.7 $kg \cdot m^{-2}$	60 min cycle at 74% HRmax	14 h	8 h total and incremental TAG AUC	-25.7% for *t*AUC ($P = 0.003$) and -18.5% for *i*AUC (*P* not given, but declared nonsignificant)
Petridou et al. (2004)	11 sedentary, nonsmoking men. Age 21.7 y; body mass 73.2 kg; BMI 22.5 $kg \cdot m^{-2}$	45 min cycle at 62% predicted HRmax. Power output of 102 W	0 h (Test meal immediately after exercise)	8 h total and incremental TAG AUC	Following exercise, *t*AUC was 8.4% lower than control and *i*AUC was 17.1% lower (both $P > 0.05$)
Smith et al. (2004)	10 recreationally active males. Age 25.0 y; body mass 76.6 kg; BMI 23.2 $kg \cdot m^{-2}$; body fat 9.8%; VO_2max 53.1 $ml \cdot kg^{-1} \cdot min^{-1}$	60 min treadmill jog at 60.7% VO_2max	12 h	8 h total and incremental TAG AUC	Exercise did not reduce *t*AUC significantly ($P > 0.05$), but the *i*AUC was significantly lower after exercise ($P < 0.05$)
Zhang et al. (2004)	10 sedentary, hypertriglyceridaemic males. Age 40.3 y; body mass 97.7 kg; BMI 30.3 $kg \cdot m^{-2}$; body fat 21.7%; VO_2max 36.7 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 3.28 $mmol \cdot l^{-1}$	60 min treadmill exercise at 60% VO_2max	Two exercise trials: • 12 h pre • 24 h pre	8 h incremental TAG AUC	Exercising 12 h before fat intake reduced TAG *i*AUC by 37% and 33% compared with control and 24h-pre trials respectively (both $P < 0.02$). Control and 24h-pre trials did not differ significantly ($P > 0.05$)
Kokalas et al. (2005)	8 high-level female rowers. All were normolipidaemic and eumenorrhoeic, and none smoked. Age 18 - 25 y; body mass 66.9 kg; BMI 22.9 $kg \cdot m^{-2}$; fasting TAG 0.26 - 0.80 $mmol \cdot l^{-1}$	80 min on a rowing ergometer at 55% Wmax (= 130 W)	14 h	8 h total and incremental TAG AUC	After exercise, *t*AUC was 35.2% lower ($P < 0.001$) and *i*AUC was 36.1% lower ($P = 0.13$) than control

Table 1.1. continued

Lead author(s) and date	Participants	Exercise	Exercise-meal delay (hours)	Main measure(s)	% Change with exercise (vs. control)
Pfeiffer et al. (2005)	16 healthy, sedentary, normotriglyceridaemic, male nonsmokers. Age 24.8 y; body mass 68.9 kg; BMI 21.1 $kg \cdot m^{-2}$; VO_2max 41.2 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 1.03 $mmol \cdot l^{-1}$	Three treadmill walks of different duration at ~50% VO_2max, with actual intensities in brackets: • 30 min (49.8% VO_2max) • 60 min (50.1% VO_2max) • 90 min (50.0% VO_2max)	0 h (Fat meals eaten immediately after exercise)	6 h incremental TAG AUC (2 meals, given 3 hours apart)	When compared with a control trial, none of the three exercise sessions were found to lower lipaemia: • +2% (0.5 h; $P = 1.00$) • -14% (1 h; $P = 0.24$) • -15% (1.5 h; $P = 0.23$)
Barrett et al. (2006)	12 healthy, recreationally active males. Age 21.1 y; BMI 23.0 $kg \cdot m^{-2}$; VO_2max 53.0 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 0.95 $mmol \cdot l^{-1}$	• 4 x 15 min treadmill walks at 62% VO_2max (CON-EX) • 4 x 16 min bouts of intermittent games activity at 72% VO_2max (INT-EX)	16 h	6 h total and incremental TAG AUC	Compared with control, *t*AUC was 24.8% lower with INT-EX ($P = 0.001$) and 18.6% lower with CON-EX ($P = 0.028$). *i*AUC was 24.7% lower after INT-EX ($P = 0.046$) and 20.8% lower after CON-EX (*P* not given)
Gill et al. (2006)	20 apparently healthy, nonsmoking men. Age 47.2 y; BMI 27.3 $kg \cdot m^{-2}$; VO_2max 42.2 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 1.31 $mmol \cdot l^{-1}$	90 min treadmill walk at 50.9% VO_2max	16 - 18 h	Time-averaged postprandial concentration, and rise in concentration, of remnant-like lipoprotein particle TAG (RLP-TAG). Blood samples were taken for 8 h	Relative to control, exercise lowered the time-averaged postprandial RLP-TAG concentration by 29.1% ($P < 0.01$) and induced a 24.7% smaller rise in RLP-TAG concentration after the test meal ($P < 0.01$)
Miyashita et al. (2006)	10 healthy, recreationally active, nonsmoking men. Age 25.0 y; body mass 80.2 kg; BMI 25.4 $kg \cdot m^{-2}$; body fat 9.4%; VO_2max 56.3 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 1.35 $mmol \cdot l^{-1}$	• Continuous 30 min treadmill run at 71.1% VO_2max (CON-EX) • 10 x 3 min treadmill runs at 69.6% VO_2max (ACC-EX)	17 h	7 h total and incremental TAG AUC (2 meals, given 3 hours apart)	Compared with control, *t*AUC was 21.9% and 24.4% lower with ACC-EX and CON-EX. *i*AUC was 30.8% lower after ACC-EX and 31.8% lower after CON-EX (all $P < 0.02$)
Pfeiffer et al. (2006)	12 healthy, normotriglyceridaemic men, who were not endurance trained. Age 24.7 y; body mass 74 kg; BMI 22.4 $kg \cdot m^{-2}$; VO_2max 39.6 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 1.08 $mmol \cdot l^{-1}$	3 separate exercise trials, involving 30 min of cycling, with a target EE of: • 420 kJ (actual EE of 455 kJ; intensity of ~ 26% VO_2max • 630 kJ (actual EE of 656 kJ; intensity of ~ 37% VO_2max • 840 kJ (actual EE of 851 kJ; intensity of ~ 48% VO_2max	0 h (Fat meals eaten immediately after exercise)	6 h total and incremental TAG AUC (2 meals, given 3 hours apart)	There were no significant differences between any of the trials regarding the TAG *t*AUC ($P = 0.93$) or *i*AUC ($P = 0.97$)

Table 1.1. continued

Lead author(s) and date	Participants	Exercise	Exercise-meal delay (hours)	Main measure(s)	% Change with exercise (vs. control)
Teixeira et al. (2006)	27 sedentary men in total. • 10 normotriglceridaemics: Age 41 y (median); fasting TAG 1.24 mmol·l^{-1} • 17 hypertriglceridaemics: Age 42 y (median); fasting TAG 2.93 mmol·l^{-1}	30 min treadmill walk, with no slope, at constant speed (anaerobic threshold)	-0 h (Fat meal eaten immediately before exercise)	6 h total TAG AUC	TAG *t*AUC was 15.8% lower with exercise, but this did not reflect a statistically significant difference from control (P = 0.113)
Zhang et al. (2006)	10 sedentary, hypertriglyceridaemic, insulin resistant males. Age 40.1 y; body mass 96.3 kg; BMI 31.3 kg·m^{-2}; body fat 21.7%; VO_2max 37.0 ml·kg^{-1}·min^{-1}; fasting TAG 2.97 mmol·l^{-1}	Three separate trials, involving 60 min of treadmill exercise, at different intensities: • 40% VO_2max • 60% VO_2max • 70% VO_2max	11 h	8 h incremental TAG AUC	*i*AUC was reduced by 30% (P = 0.002), 31% (P = 0.017) and 39% (P = 0.018) with exercise at 40% VO_2max, 60% VO_2max and 70% VO_2max respectively
Barrett et al. (2007)	19 healthy, recreationally active, male adolescents were randomly split into two groups. • Continuous exercise group (N = 10). Age 15.3 y; body mass 63.4 kg; BMI 20.3 kg·m^{-2}; VO_2peak 44.8 ml·kg^{-1}·min^{-1}; fasting TAG 1.09 mmol·l^{-1} • Intermittent games group (N = 9). Age 15.4 y; body mass 59.8 kg; BMI 19.0 kg·m^{-2}; VO_2peak 51.1 ml·kg^{-1}·min^{-1}; fasting TAG 1.11 mmol·l^{-1}	• CON-EX group: Four 15 min blocks of uphill walking at 58.9% VO_2max, with 3 min recoveries • INT-GAMES group: Four 18.5 min blocks of games activity at 68.8% VO_2max, with 3 min recoveries	16 h	6 h total and incremental TAG AUC (from capillary blood samples)	Groups were not compared as intensity and duration of sessions was different. For CON-EX, *t*AUC was 13.5% (P = 0.05) and iAUC was 4.9% lower (P = 0.519) than control. For INT-GAMES, exercise reduced *t*AUC by 26.2% (P = 0.002) and *i*AUC by 46.5% (P = 0.018)
Clegg et al. (2007)	8 recreationally trained, nonsmoking males. Age 22.9 y; body mass 83.4 kg; BMI 25.3 kg·m^{-2}; fasting TAG 0.68 mmol·l^{-1}	60 min cycle at 60% predicted HRmax	0 h (Fat meal eaten immediately after exercise)	Plasma TAG in the fasted state and at 2 h, 4 h and 6 h after a high-fat meal	A main effect of time was found for TAG (P < 0.05), with no time x trial interaction (P > 0.05). No mention is made of the data having been tested for a main effect of trial
Gill et al. (2007)	10 men with type 2 diabetes, who were otherwise healthy and did not smoke. Age 49.3 y; BMI 30.5 kg·m^{-2}; VO_2max 34.7 ml·kg^{-1}·min^{-1}; fasting TAG 1.75 mmol·l^{-1}	90 min treadmill walk at 51.2% VO_2max	16 - 18 h	8 h total TAG AUC	*t*AUC was 1.0% higher than control after exercise (P = 0.88)
James et al. (2007)	8 (5 male) healthy, normal-weight nonsmokers. Age 29.8 y; body mass 61.0 kg; BMI 20.8 kg·m^{-2}; fasting TAG 0.93 mmol·l^{-1}	Three 30 min treadmill exercise bouts at 65% HRmax, with 5 min recoveries between bouts	15 h	8 h total and incremental TAG AUC	*t*AUC was 23.3% lower than control after exercise (P = 0.053,) and iAUC was reduced by 36.8% (P not stated, but declared nonsignificant)

Table 1.1. continued

Lead author(s) and date	Participants	Exercise	Exercise-meal delay (hours)	Main measure(s)	% Change with exercise (vs. control)
McClean et al. (2007)	10 recreationally trained, nonsmoking men. Age 21.5 y; body mass 77.0 kg; BMI 23.6 $kg \cdot m^{-2}$; VO_2max 58.5 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 0.81 $mmol \cdot l^{-1}$	60 min bout of treadmill exercise at 60% HRmax	-2 h	Serum TAG concentrations in the fasted state, and at 2 h, 3 h and 4 h after a high-fat meal	No direct "exercise vs. control" comparison was made. In the exercise trial, TAG peaked at 2 h, and was significantly lower at 3 h and 4 h, than at 2 h ($P < 0.05$). With control, TAG increased until 4 h. With EX, TAG was 23.9% and 31.5% lower than control at 3 h and 4 h respectively
Zhang et al. (2007)	10 sedentary males with metabolic syndrome. Age 35.0 y; body mass 90.7 kg; BMI 30.0 $kg \cdot m^{-2}$; body fat 23.6%; VO_2peak 36.0 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 2.6 $mmol \cdot l^{-1}$	Three separate treadmill jogs at 60% VO_2max: • 30 min • 45 min • 60 min	12 h	8 h incremental TAG AUC	Exercise lowered *i*AUC, compared with control, after 45 min (31% lower; $P = 0.016$) and 60 min (33% lower; $P = 0.017$), but not 30 min (% change not given; $P > 0.05$)
Burton et al. (2008)	13 apparently healthy, nonsmoking men. Age 40 y; BMI 31.1 $kg \cdot m^{-2}$; VO_2max 39.3 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 1.34 $mmol \cdot l^{-1}$	Two separate treadmill walks at ~50% VO_2max, which lasted until a net EE of 27 $kJ \cdot kg^{-1}$ body mass had been induced • Energy deficit (DEF): 90.8 min walk at 48.6% VO_2max • Energy replacement (REP): 90.8 min walk at 49.1% VO_2max with replacement of net exercise EE	16 h	8.5 h total and incremental TAG AUC (2 moderate-fat meals given and 11 blood samples taken)	*t*AUC after DEF was significantly lower than CON (-13.9%) and REP (-8.9%) (both $P < 0.05$), but CON and REF did not differ (REF 5.5% lower; $P > 0.05$). DEF *i*AUC was 10.5% lower than CON and 13.1% lower than REP, while REP was 3.0% higher than CON ($P > 0.05$ for all)
Mestek et al. (2008)	14 physically inactive males with metabolic syndrome. Age 43 y; body mass 107.3 kg; BMI 34.3 $kg \cdot m^{-2}$; body fat 37%; VO_2peak 26.3 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 2.45 $mmol \cdot l^{-1}$	Three separate treadmill exercise trials, each expending 500 kcal of energy: • 102.8 min at 38.6% VO_2peak (LOW) • 60.2 min at 63.5% VO_2peak (MOD-1) • 2 bouts of 30.8 min at 63.1% VO_2peak, with 3-5 h between (MOD-2)	12 – 14 h	6 h incremental TAG AUC (blood samples at 2 h intervals)	*i*AUC was lower than control after LOW (-27%, $P = 0.02$), but not MOD-1 (-20%, $P > 0.05$) or MOD-2 (percentage change not stated, $P > 0.05$)
Mitchell et al. (2008)	• 10 sedentary normal-weight women. Age 18.9 y; body mass 61.10 kg; BMI 22.17 $kg \cdot m^{-2}$; body fat 22.71%; VO_2peak 32.2 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 0.69 $mmol \cdot l^{-1}$ • 10 sedentary overweight women. Age 21.2 y; body mass 85.08 kg; BMI 29.94 $kg \cdot m^{-2}$; body fat 36.10%; VO_2peak 25.5 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 1.06 $mmol \cdot l^{-1}$	60 min semirecumbent cycling at approximately 60% VO_2peak (actual intensity was 58.7% VO_2max for normal-weight women and 65.7% VO_2peak for overweight women)	Not clearly stated (exercise seemingly ceased 15 - 18 h before test meal)	6 h total TAG AUC	TAG *t*AUC was significantly lower with prior exercise than control ($P < 0.05$). The lack of an interaction with group suggests that normal-weight and overweight women both benefit

Table 1.1. continued

Lead author(s) and date	Participants	Exercise	Exercise-meal delay (hours)	Main measure(s)	% Change with exercise (vs. control)
Miyashita (2008)	8 sedentary, overweight men. Age 26.5 y; body mass 86.3 kg; BMI 28.9 $kg \cdot m^{-2}$; fasting TAG 1.85 $mmol \cdot l^{-1}$	Two separate exercise trials: • Continuous 30 min cycle at 61.3% HRmax (CON-EX) • 10 x 3 min bouts of cycling at 60.3% HRmax (ACC-EX) with 30 min recoveries	17 h	6 h total and incremental TAG AUC	Compared with control, *t*AUC was 18.2% lower with ACC-EX ($P = 0.042$) and 15.2% lower with CON-EX ($P = 0.032$). *i*AUC was 38.6% lower after ACC-EX ($P = 0.052$) and 30.9% lower after CON-EX ($P = 0.125$)
Miyashita et al. (2008)	15 healthy, recreationally active, nonsmoking males. Age 23.4 y; body mass 74.9 kg; BMI 23.4 $kg \cdot m^{-2}$; body fat 11.2%; VO_2max 56.3 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 0.87 $mmol \cdot l^{-1}$	Two separate exercise trials: • Continuous 30 min treadmill walk at 42.4% VO_2max (CON-EX) • 10 x 3 min bouts of treadmill walking at 41.4% VO_2max (ACC-EX), with 30 min recoveries	17 h	7 h total and incremental TAG AUC (breakfast and lunch given 3 hours apart)	Compared with control, *t*AUC was 16.1% lower with both ACC-EX ($P = 0.045$) and CON-EX ($P = 0.019$). *i*AUC was 17.9% lower after INT-EX and 29.1% lower after CON-EX ($P > 0.05$ for both)
Miyashita and Tokuyama (2008)	12 healthy, recreationally active, nonsmoking males. Age 23.7 y; body mass 71.2 kg; BMI 23.9 $kg \cdot m^{-2}$; fasting TAG 1.22 $mmol \cdot l^{-1}$	30 min cycle at 66.7% HRmax. Average power output of 82 W	13 h	6 h total and incremental TAG AUC	Exercise lowered *t*AUC by 30% ($P = 0.039$) and *i*AUC by 33% ($P = 0.012$)
Plaisance et al. (2008)	15 sedentary, hypertriglyceridaemic, nonsmoking males. Age 46 y; body mass 105.3 kg; BMI 34.0 $kg \cdot m^{-2}$; body fat 35%; VO_2max 27.7 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 3.23 $mmol \cdot l^{-1}$	Treadmill walk at 60 - 70% VO_2max expending 500 kcal (average time 51 min)	1 h	8 h total and incremental TAG AUC	-13.3% for *t*AUC ($P < 0.001$) and -31.6% for *i*AUC ($P < 0.05$)
Shannon et al. (2008)	• 6 African American female nonsmokers. Age 21.7 y; body mass 70.4 kg; BMI 25.8 $kg \cdot m^{-2}$; VO_2max 32.6 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 0.83 $mmol \cdot l^{-1}$ • 6 White female nonsmokers. Age 27.0 y; body mass 69.7 kg; BMI 25.0 $kg \cdot m^{-2}$; VO_2max 30.1 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 1.16 mmol·l-1	90 min treadmill walk at 60% VO_2max	13 h	6 h incremental TAG AUC	Exercise reduced TAG *i*AUC for African American women (61.8% lower than control; $P < 0.05$), but not White women (17.0% lower than control; $P > 0.05$)
Tobin et al. (2008)	• 8 sedentary men with type 2 diabetes. Age 59.0 y; body mass 94.2 kg; BMI 29.0 $kg \cdot m^{-2}$; VO_2max 24.7 $ml \cdot kg^{-1} \cdot min^{-1}$ • 7 sedentary male non-diabetic controls. Age 58.0 y; body mass 92.4 kg; BMI 28.0 $kg \cdot m^{-2}$; VO_2max 33.6 $ml \cdot kg^{-1} \cdot min^{-1}$	60 min cycle at 60% VO_2max (actual intensity was 61% VO_2max for diabetic patients and 53% VO_2max for non-diabetic controls)	-1.5 h	10 h incremental TAG AUC	Exercise reduced TAG *i*AUC for diabetic patients (34.8% lower than control; $P < 0.05$), but not for non-diabetic controls (24.6% higher with exercise; $P > 0.05$)

Table 1.1. continued

Lead author(s) and date	Participants	Exercise	Exercise-meal delay (hours)	Main measure(s)	% Change with exercise (vs. control)
Tolfrey et al. (2008)	8 healthy male adolescents. 4 were regular exercisers, while the other 4 were sedentary. Age 12.9 y; body mass 45 kg; BMI 17.8 $kg \cdot m^{-2}$; body fat 15%; VO_2peak 52 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 0.63 $mmol \cdot l^{-1}$	Two exercise trials in which six 10 min treadmill exercise bouts were performed, with 10 min recoveries. Intensities were: • 53% VO_2peak (MOD) • 75% VO_2peak (VIG)	14.7 h	6 h total and incremental TAG AUC (capillary blood samples)	Relative to control, *t*AUC was 24.1% lower with MOD and 19.8% lower with VIG (P = 0.04 for main effect of trial; no post hoc tests to determines where differences lay). *i*AUC was 45.9% and 35.1% lower than control after MOD and VIG respectively (P = 0.08 for main effect of trial)
Bloomer et al. (2009a)	20 normolipidaemic, nonsmoking, sedentary women in total • 10 African American (AA) women. Age 29 y; body mass 87 kg; BMI 31 $kg \cdot m^{-2}$; body fat 30% • 10 White (W) women. Age 30 y; body mass 82 kg; BMI 30 $kg \cdot m^{-2}$; body fat 32%	45 min cycle at 65% of heart rate reserve	0.25 h (exercise concluded 15 min before test meal ingestion)	6 h total TAG AUC	*t*AUC was significantly lower for AA than W women during exercise and control trials (P < 0.01); however exercise did not reduce *t*AUC for either group relative to control (-4.7% for AA, -3.5% for W; both P > 0.05)
Harrison et al. (2009)	8 recreationally active, normolipidaemic, nonsmoking males. Age 26.9 y; body mass 83.5 kg; BMI 26.0 $kg \cdot m^{-2}$; VO_2peak 46.8 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 0.95 $mmol \cdot l^{-1}$	90 min cycle at 70% VO_2peak, followed by ten 1 min full-effort sprints, with 1 min recoveries. The exercise was completed on two occasions: once without replacing the carbohydrate used (EX-DEF), and once with carbohydrate replacement (EX-BAL)	Not clearly stated, but more than 10 h between exercise cessation and test meal ingestion	Time-averaged postprandial TAG concentration (*t*AUC/time) and time-averaged postprandial TAG increment (*i*AUC/time). Blood samples were collected over a 6 h postprandial period	Following EX-DEF, the postprandial TAG concentration was 40.6% lower than control and 29.1% lower than EX-BAL (P < 0.05 for both); EX-BAL was 16.2% lower than CON (P = 0.29). Postprandial TAG increment was 47.1% lower than CON after EX-DEF (P < 0.05) and 23.5% lower than CON after EX-BAL (P > 0.05)
MacEneaney et al. (2009)	10 normal-weight and 8 overweight adolescent boys. All were moderately active and none smoked. • Normal-weight boys: Age 15.6 y; body mass 65.9 kg; BMI 20.9 $kg \cdot m^{-2}$; VO_2max 52.1 $ml \cdot kg^{-1} \cdot min^{-1}$ • Overweight boys: Age 15.9 y; body mass 84.1 kg; BMI 28.3 $kg \cdot m^{-2}$; VO_2max 41.8 $ml \cdot kg^{-1} \cdot min^{-1}$	600 kcal treadmill exercise bout at 65% VO_2max, taking 59 min for NW boys and 52 min for OW boys	12 - 14 h	6 h total and incremental TAG AUC	Exercise lowered *t*AUC by 16.9% for NW and 26.5% for OW (both P < 0.05), and reduced iAUC by 17.1% (NW) and 32.7% (OW) (both P > 0.05). TAG responses were not significantly different between NW and OW boys for either trial; pooled data found the exercise *i*AUC to be significantly lower than in the control trial

Table 1.1. continued

Lead author(s) and date	Participants	Exercise	Exercise-meal delay (hours)	Main measure(s)	% Change with exercise (vs. control)
Maraki et al. (2009)	8 healthy, normolipidaemic, nonsmoking, premenopausal women. Age 27.1 y; body mass 62.9 kg; BMI 21.8 $kg \cdot m^{-2}$; body fat 27.1%; VO_2peak 35.3 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 0.78 $mmol \cdot l^{-1}$	• Control trial: Rest while following prescribed diet • Exercise trial: 100 min treadmill walk at 30.2% VO_2peak (net EE of 1.04 MJ) and prescribed diet containing 1.39 MJ less energy than control trial	Not stated (exercise was between lunch and dinner on day 1, test meal was ~08:00 on day 2)	Time-averaged total and incremental TAG concentrations (for 6 h after a high-fat mixed meal)	Compared with control, total TAG was 19.1% lower (P = 0.003) and incremental TAG was 20.4% lower (P = 0.334) after exercise and energy restriction
Melton et al. (2009)	16 obese, prediabetic women. Age 30 y; body mass 86 kg; BMI 32 $kg \cdot m^{-2}$; body fat 31%; VO_2max 19 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG < 1.7 $mmol \cdot l^{-1}$	45 min cycle at 65% of heart rate reserve	0.25 h (exercise concluded 15 min before test meal ingestion)	TAG concentration in the fasted state and 1 h, 2 h, 4 h and 6 h after a high-fat mixed meal	No main effect of exercise on TAG and no time x trial interaction (both $P > 0.05$)
Tyldum et al. (2009)	8 healthy men. Age 42 y; body mass 89.8 kg; BMI 28.8 $kg \cdot m^{-2}$; VO_2max 52.6 $ml \cdot kg^{-1} \cdot min^{-1}$; fasting TAG 1.18 $mmol \cdot l^{-1}$	• High-intensity interval exercise (HIIE): 10 min treadmill run at 50 - 60% HRmax, followed by 4 x 4 min intervals at 85 - 95% HRmax, with 3 min "active recovery" at 50 - 60% HRmax between intervals • Continuous moderate exercise (CMD): 47 min treadmill walk at 60 - 70% HRmax	16 - 18 h	TAG concentration in the fasted state and 0.5 h, 2 h and 4 h after a fat-containing meal	No significant differences between trials (P values not stated)
Dekker et al. (in press)	9 hypertriglyceridaemic men. All men led sedentary or low activity lifestyles. Age 59 y; body mass 104.4 kg; BMI 33.8 $kg \cdot m^{-2}$; body fat 31.4%; fasting TAG 2.9 $mmol \cdot l^{-1}$	60 min treadmill walk at 55% VO_2peak	16 h	6 h total TAG AUC	Exercise lowered tAUC significantly ($P < 0.05$) compared with control
Maraki et al. (in press)	6 healthy, sedentary, nonsmoking, normolipidaemic, premenopausal women. Age 28.3 y; BMI 21.7 kgm^{-2}; VO_2peak 36.7 $ml \cdot kg^{-1} \cdot min^{-1}$	Three interventions designed to induce energy deficit of 2 MJ relative to control: • Calorie restriction of 2 MJ (CR) • 90 min treadmill walk at 60% VO_2peak with net EE of 2 MJ (EX) • Calorie restriction of 1 MJ, plus 101 min treadmill walk at 30% VO_2peak with net EE of 1 MJ (CR-EX)	Not stated (exercise or calorie restriction on day 1; test meal on morning of day 2)	6 h total TAG AUC	Relative to control, tAUC was 12% lower (CR), 23% lower (EX) and 19% lower (CR-EX) ($P < 0.05$ for all). tAUC for EX was significantly lower than for CR (P = 0.05)

Table 1.2. Studies investigating the effect of a single bout of resistance exercise on postprandial TAG concentrations

Lead authors(s) and date	Participants	Exercise	Exercise-meal delay (hours)	Main measure(s)	% Change with exercise (vs. control)
Petitt et al. (2003)	14 (10 male) apparently healthy, recreationally weight-trained Caucasians. Participants had performed weigh-lifting activities an average of 3 days/wk for 60 min/day over the previous 6 y. Age 24.3 y; body mass 73.6 kg; BMI 24.4 $kg \cdot m^{-2}$; body fat 19.5%; fasting TAG 1.01 $mmol \cdot l^{-1}$	3 sets of 10 repetitions of 10 exercises at 10 repetition maximum (10-RM). 2 min recoveries were given between sets and exercises. Total exercise time of 88 min	16 h	6 h incremental TAG AUC	*i*AUC was 14% lower than control ($P < 0.05$)
Burns et al. (2005)	11 healthy, normolipidaemic, nonsmoking men. Recreationally active, but not weight-trained. Age 23.5 y; body mass 84.3 kg; BMI 25.9 $kg \cdot m^{-2}$; body fat 14.4%; fasting TAG 1.03 $mmol \cdot l^{-1}$	4 sets of 10 repetitions of 11 different weight-lifting exercises at 80% of 10-RM. Participants had 2 min to complete each set, with the remainder of the time (after 10 reps) used for recovery. Total exercise time of 88 min	16 h	6 h total and incremental TAG AUC	Relative to control, *t*AUC was 4.7% lower ($P = 0.47$) and *i*AUC was 4.1% higher ($P = 0.63$) after exercise
Shannon et al. (2005)	10 (4 male) healthy, resistance-trained nonsmokers. Participants had been involved in at least 1 y of consistent (3 d/wk) resistance training. Age 24.4 y; body mass 66.0 kg; BMI 23.4 $kg \cdot m^{-2}$; body fat 23%; fasting TAG 0.89 $mmol \cdot l^{-1}$	All participants performed 3 trials. Trials had 1 set (~ 20 min), 3 sets (~ 48 min) or 5 sets (~ 90 min), each involving 10 repetitions of 8 resistance exercises at 75% of 1-RM. A rest of 1 min was given between sets and exercises. Energy expended during exercise was replenished within postexercise meals	13 h	6 h incremental TAG AUC	Compared with control, *i*AUC was 14.9% (1 set), 11.8% (3 sets) and 17.2% (5 sets) lower with resistance exercise (*P* values not specifically stated, but were greater than 0.05 for all)
Burns et al. (2006)	10 healthy, nonsmoking males who had performed at least two resistance exercise sessions a week for at least 6 months. Age 25.2 y; body mass 78.6 kg; BMI 25.8 $kg \cdot m^{-2}$; body fat 16.8%; fasting TAG 1.11 $mmol \cdot l^{-1}$	3 sets of 12 repetitions of 10 different weight-lifting exercises at 80% of 12-RM. Participants had 3 min to complete each set, then rested for the remainder of the time after 12 reps. Total exercise session lasted 90 min	1 h	5 h total and incremental TAG AUC	Relative to control, *t*AUC was 48.1% higher ($P < 0.008$) and *i*AUC was 88.8% higher ($P < 0.005$), after exercise
Burns et al. (2007)	24 nonsmoking males. All were recreationally active, with some participating in regular resistance training. Age 23.5 y; body mass 75.8 kg; BMI 23.8 $kg \cdot m^{-2}$; body fat 16.7%; fasting TAG 1.05 $mmol \cdot l^{-1}$	Five 45-min bouts of lifting. Each bout involved 4 sets of 15 repetitions of 5 different exercises. Upper body exercises at 30% of 1-RM, lower body exercises at 40% of 1-RM. 1-min recovery between exercises and 5-min rest between sets. Bouts were spread across the day	17 h	6 h total and incremental TAG AUC	Relative to control, *t*AUC was 11.9% lower ($P = 0.037$) and *i*AUC was 17.9% lower ($P = 0.043$), after exercise

Table 1.2. continued

Lead authors(s) and date	Participants	Exercise	Exercise-meal delay (hours)	Main measure(s)	% Change with exercise (vs. control)
Zafeiridis et al. (2007)	10 healthy young men with recreational experience in weight lifting (2-3 sessions/wk for more than 1 y). Age 24.6 y; body mass 77.8 kg; BMI 24.2 kg·m^{-2}; body fat 17.1%	Two exercise trials each involving 12 reps of eight different exercises at 12-RM. 1.5 min between sets and 2 min between exercises • Low volume resistance exercise (LVRE) included 2 sets (gross EE of 0.76 MJ; total exercise time of 39 min) • High volume resistance exercise (HVRE) included 4 sets (gross EE of 1.40 MJ; total exercise time of 79 min)	16 h	6 h total and incremental TAG AUC	Compared with control, *t*AUC was 19.9% lower with LVRE (P = 0.017) and 24.5% lower with HVRE (P = 0.004). *i*AUC was reduced by 30.8% with LVRE (P = 0.195) and by 45.0% with HVRE (P = 0.041), compared with control
Pafili et al. (2009)	9 nonsmoking males. No regular resistance exercise in the previous 12 months and less than 2 h/wk of recreational physical activity. Age 27.2 y; body mass 76.2 kg; BMI 23.7 kg·m^{-2}; body fat 12.9%; fasting TAG 0.98 mmol·l^{-1}	8 sets of 6 repetitions of leg press that emphasised the eccentric movement with a load equal to 6-RM. 3 min rest between sets. Total session time of 25.6 min; net exercise time of 4.6 min. Gross EE of 0.64 MJ; net EE of 0.46 MJ	An identical test meal (followed by 6 h of blood samples) was given 16 h and 40 h after the same exercise session	6 h total and incremental TAG AUC	Relative to control, *t*AUC was 12.1% lower 16 h after EX (P < 0.04) and 7.0% lower 40 h after EX (P = 0.27). *i*AUC was not significantly different between OFTTs (P = 0.25)
Singhal et al. (2009)	10 healthy, nonsmoking men. All had performed resistance exercise at least 2 days/week for the previous 3 y. Age 21 - 36 y; body mass 84.3 kg; BMI 26.0 kg·m^{-2}; body fat 15.2%; fasting TAG 1.04 mmol·l^{-1}	Two exercise trials; both involving 3 sets of 10 exercises. 3 min given to complete each set (remainder, after 10 reps, as rest); total session time of 90 min. Sessions were: • MOD: 16 repetitions at 50% of 8-RM • HI: 8 repetitions at 100% of 8-RM	15.5 h	3 h total and incremental TAG AUC	Compared with control, *t*AUC was 34.8% lower with HI (P = 0.014) and 26.0% lower with MOD (P = 0.052). *i*AUC was reduced by 25.7% with HI and by 23.4% with MOD, relative to control (P = 0.225 for main effect of trial)

Table 1.3. The one study known to have investigated the effect of a single session, combining aerobic and resistance exercise, on postprandial TAG concentrations

First author and date	Participants	Exercise	Exercise-meal delay (hours)	Main measure	% Change with exercise (vs. control)
Silvestre et al. (2008)	12 healthy, recreationally trained, nonsmoking men. Participants performed aerobic training at least 3 times a week and strength training twice a week. Age 21.8 y; body mass 80.7 kg; BMI 25.1 $kg \cdot m^{-2}$; body fat 13.8%; fasting TAG 0.70 $mmol \cdot l^{-1}$	3 sets of 10 repetitions of 5 resistance exercises at 95% of 10-RM, followed by 2 sets of sit-ups for 1 min. Recovery varied from 1 - 2 min depending on exercise performed. 30-min treadmill run, expending 450 kcal, followed the sit-ups. Total session time was 75 min; total EE of 3.7 MJ	Two trials: • 4 h (EX-4) • 16 h (EX-16)	6 h total TAG AUC	*t*AUC was 26% lower than control with EX-16 and 15% lower than control with EX-4 (both $P < 0.05$); *t*AUC was not significantly different between EX-4 and EX-16 ($P > 0.05$)

As seen in **Table 1.1**, the vast majority of early studies investigated the effect on plasma TAG concentration of exercising after ingestion of a fat-containing meal. However, research articles published during the past 15 years have focussed almost exclusively on studying whether exercise before meal intake alters postprandial lipaemia. Due to the fact that most research investigating the effect of single exercise bouts on postprandial lipaemia has been published post-1994, over 80% of the studies listed in **Table 1.1** involve aerobic exercise prior to meal ingestion, rather than postprandial aerobic exercise. The study of Aldred et al. (1994) was a departure from previous literature in that exercise was performed many hours before ingestion of a mixed meal, rather than consuming the meal immediately after exercise (Zauner et al., (1968) or having the meal prior to exercise (all previous articles). The experimental design of Aldred et al. involved two 2-day trials: an exercise bout or rest during the afternoon of day 1 and a high-fat meal followed by repeated blood samples on the morning of day 2. This study found that performing 2 h of treadmill walking at 30% VO_2max induced a significant lowering of both the fasting TAG concentration and the incremental lipaemic response (the increase in TAG over the 6 h period after the meal) when compared with the control (no exercise) trial. Since this experiment, many studies have investigated the effect of a single moderate-intensity aerobic exercise bout, performed before ingestion of a high-fat meal, on plasma TAG concentrations, with most using the 2-day protocol first devised by Aldred et al. The clear message from these studies is that aerobic exercise can substantially attenuate postprandial lipaemia, with the timing of the exercise (relative to consumption of the test meal), and the energy expended during the session, identified as factors which mediate the extent to which plasma TAG concentrations are altered postprandially.

1.6.2. The exercise-meal time delay and postprandial lipaemia

Exercising either 12 h or 1 h before an oral fat tolerance test (OFTT), but not 1 h after, was shown to bring about a significant lowering of lipaemia when compared with a control trial (Zhang et al., 1998). Both Katsanos et al. (2004) and Plaisance et al. (2008) confirmed the finding of Zhang et al. (1998) that moderate exercise completed 1 h before an OFTT reduces postprandial lipaemia. This 1 h delay before meal ingestion may be important, as exercise did not lower lipaemia significantly in studies where meals with moderate-fat contents were provided immediately after exercise (Petridou et al., 2004; Pfeiffer et al., 2005; Pfeiffer et al., 2006) or when high-fat meals were consumed 15 minutes after a 45 min moderate exercise bout (Bloomer et al., 2009a; Melton et al., 2009). However, Zauner et al. (1968) found postprandial serum turbidity (a surrogate measure of lipaemia) to be lower when either strenuous or mild exercise was performed immediately before a fat meal, and Katsanos & Moffatt (2004) reported a substantial and significant lowering of postprandial lipaemia when a 90 min treadmill walk was completed just 30 min prior to an oral fat tolerance test. A 24 h delay between exercise and fat intake appears to be too long to have a significant impact on postprandial lipaemia, with postprandial TAG concentrations 12 h after treadmill exercise shown to be significantly lower than both a control trial and trial where exercise was performed 24 h previously (Zhang et al., 2004). A recent investigation (detailed in **Table 1.3)** using resistance exercises followed by a 30-minute treadmill run found that the exercise protocol effectively lowered postprandial lipaemia when performed either 4 h or 16 h before an oral fat tolerance test, with no significant differences between exercise trials (Silvestre et al., 2008). Most studies (~70% of aerobic exercise trials) have included at least one trial with a delay of 11 – 18 h between exercise and test meal intake; more than 75% of such studies have found a beneficial effect of prior aerobic exercise on postprandial lipaemia (**Table 1.1**).

The time delay between cessation of exercise and meal ingestion, which would lower lipaemia most appreciably, is not yet established, but the above observation suggests that a delay of 11 – 18 h is effective. Little to no research has investigated the effect of performing aerobic exercise 2 – 10 h before an oral fat tolerance test, and the results from studies where an aerobic exercise session was completed 1 h or less before a fat-containing meal, have not been consistent regarding the effect on postprandial lipaemia The effect on lipaemia of exercising during the postprandial period has not been studied as extensively as for preprandial exercise, but at least 15 studies have been published. As mentioned earlier, Zhang et al. (1998) found no reduction in postprandial TAG concentrations, compared with a control trial, when 60 minutes of moderate-intensity treadmill exercise was performed 1 h after eating a fat meal. This finding is in agreement with six studies (Chinnici and Zauner, 1971; Dalgaard et al., 2004; Feldman and Nixon, 1982; Maruhama et al., 1977; Teixeira et al., 2006; Welle, 1984), but in contrast to six others (Cohen and Goldberg, 1960; Hardman and Aldred, 1995; Katsanos and Moffatt, 2004; Klein et al., 1992; Nikkilä and Konttinen, 1962; Schlierf et al., 1987) which found postprandial TAG to be lower after exercise. It must be noted, however, that the studies of Nikkilä & Konttinen and Schlierf et al. only reported TAG to be lower immediately after cessation of exercise, not across a broader section of the postprandial period, and the reduction in TAG reported by Cohen and Goldberg also related to a single postprandial time point. It is therefore not clear whether postprandial exercise is beneficial to lipid metabolism, but any TAG-lowering effect is likely to be brought about through a mechanism distinct from preprandial exercise. Indeed, there is some evidence to suggest that postprandial exercise increases intravascular lipolysis of TAG-rich lipoproteins, with greater muscle perfusion and increased lipoprotein lipase activity, immediately after exercise, both potentially involved (Schlierf et al., 1987).

1.6.3. Exercise intensity, energy expenditure and negative energy balance

In addition to the timing of an exercise bout, the energy expenditure (EE) of the prior exercise session is also believed to be an important determinant of the extent to which postprandial lipaemia will be lowered. Tsetsonis et al. (1996a) found that 90 minutes of moderate-, but not low-intensity treadmill exercise, performed 15 h before a high-fat mixed meal, reduced postprandial TAG concentrations. This implied an intrinsic effect of exercise intensity, but when the duration of the low-intensity exercise was doubled, such that EE was equal to that in the moderate-intensity bout, both exercise sessions were found to attenuate lipaemia significantly compared with a control trial, with no difference between the two exercise intensities (Tsetsonis et al., 1996b). In further support for the idea that EE is important, a dose-response relationship was noted between EE and postprandial lipaemia at 50% VO_2max, with 2 h of exercise found to reduce lipaemia by twice that of a 1 h bout (Gill et al., 2002a). A more recent study reported that moderate-, but not low-intensity exercise, reduced postprandial lipaemia when the EE of the sessions was matched (Katsanos et al., 2004). The test meal was however ingested 1 h after exercise in the study of Katsanos et al., as opposed to 15 h later in the study of Tsetsonis and Hardman (1996b), therefore an interaction between timing and exercise intensity may exist. Performing moderate exercise for 90 minutes or longer is thought to produce an energy deficit which leaves the body in a state of short-term negative energy balance. When this energy deficit is compensated for through additional energy intake, the lowering of lipaemia typically seen on the following day is essentially abolished (Burton et al., 2008). This suggests that the effect of moderate-intensity exercise on postprandial lipaemia is largely due to the existence of a negative energy state. However, as Burton et al. did not balance out the entire net EE of the exercise with energy given after the exercise bout, instead providing the majority (55%) of the extra energy *before* the exercise

was performed, the design of this study prohibits a firm conclusion being reached. Furthermore, although a transient negative energy state is thought important for exercise to reduce lipaemia, this is not to say that the entire effect of exercise is due to a negative energy balance, or that inducing a negative energy state by other means will mirror the effects of an exercise bout. A study in which energy intake was restricted to match the energy expended during a treadmill walk found that lipaemia was lowered after exercise but not after the energy restriction trial (Gill and Hardman, 2000). Simply restricting energy intake is not likely to deplete intramuscular TAG stores, whereas moderate-intensity aerobic exercise has been shown to do so (Kiens et al., 1999), therefore the two trials are not comparable in this sense. A more recent study (Maraki et al., in press) found that caloric restriction was successful in lowering lipaemia relative to a control trial, but inducing the same energy deficit (2 MJ) via moderate exercise reduced postprandial TAG concentrations significantly when compared with the caloric restriction trial. These studies suggest that the effects of exercise on lipaemia cannot be entirely mimicked by modifications to energy intake, and indeed, the impact of exercise on the intramyocellular lipid pool may be a factor in the lowering of postprandial TAG concentrations.

1.6.4. Continuous versus intermittent exercise bouts

As American College of Sports Medicine (ACSM) guidelines recommend the *accumulation* of 30 minutes of moderate to vigorous physical activity on most days of the week (ACSM, 1998), it does not appear necessary that exercise be performed in one continuous bout for health benefits to be accrued. Currently, a small number of studies have been published which sought to establish whether improvements in postprandial lipaemia are evident when exercise is performed intermittently, as opposed to in a single continuous bout. Undertaking

three 30-minute walks across the day before an OFTT was found to be just as beneficial as one 90-minute walk (Gill et al., 1998). Similarly, both a 30-minute walk and three 10-minute walks reduced the average postprandial TAG concentration (three meals given across the course of day) compared with a control trial, but there was no difference between exercise trials (Murphy et al., 2000). Another report found that three 10-min treadmill jogs significantly reduced the incremental lipaemic response to a high-fat meal, whereas a 30-min jog did not, suggesting intermittent exercise may be more than effective than a single continuous bout (Altena et al., 2004). Extending the idea of accumulating short bouts of exercise even further, ten 3-minute runs with 30 minutes recovery between each were reported to lower TAG concentrations during the postprandial period in line with a 30-minute run at 70% VO_2max (Miyashita et al., 2006). The findings of this study have since been confirmed in response to a high-fat meal after ten 3-minutes bouts of walking in young healthy men (Miyashita et al., 2008) and in response to a moderate-fat meal after ten 3-minute bouts of cycling in obese men (Miyashita, 2008). Performing four sets of intermittent games activity lasting 16 minutes per set was also shown to reduce postprandial lipaemia to the same extent as four 15-minute uphill treadmill walks (Barrett et al., 2006). In contrast, performing moderate treadmill exercise in two 30-min bouts (with 3 – 5 h between bouts) was not found to lower postprandial lipaemia, whereas a single 100-min bout at a low intensity produced a significant reduction (Mestek et al., 2008). The energy expenditure of the two sessions was matched (500 kcal for both), but the same study found no benefit of a single 60-min bout at moderate intensity, suggesting that intensity may have been a factor, not just continuity/intermittency of exercise. Tyldum et al. (2009) reported that neither high-intensity interval exercise on a treadmill, nor continuous moderate exercise in the form of a 47 min brisk walk, reduced postprandial TAG concentrations. While the latter two studies remind us

that exercise does not reduce lipaemia 100% of the time, the overall balance of evidence thus far suggests that prior aerobic exercise lowers postprandial lipaemia significantly, regardless of whether the exercise is performed as a single continuous session or is broken down into multiple smaller bouts.

1.6.5. Exercise mode

Studies investigating the effect of prior exercise on postprandial lipaemia have tended to use aerobic exercise as the physical stimulus, and within the sub-category of aerobic exercise, by far the most common mode used is treadmill exercise. Treadmill exercise, whether walking or running, is used in the vast majority of studies, and has been shown to reduce the lipaemic response to a fat-containing meal on numerous occasions (examples include: Gill et al., 1998; Gill and Hardman, 2000; Gill et al., 2001a; Gill et al., 2001b; Gill et al., 2002a; Gill et al., 2003a; Gill et al., 2004; Katsanos et al., 2004; Malkova et al., 1999; Malkova et al., 2000; Miyashita et al., 2006; Tsetsonis and Hardman, 1996a; Tsetsonis and Hardman, 1996b; Tsetsonis et al., 1997; Zhang et al., 1998; Zhang et al., 2004; Zhang et al., 2006; Zhang et al., 2007). However, other studies have reported no benefit of treadmill exercise, performed 11 – 18 h prior to an oral fat tolerance test, on postprandial lipaemia (Altena et al., 2004 {30-min continuous bout}; Burton et al., 2008 {net exercise EE replacement trial}; Gill et al., 2007 {patients with type 2 diabetes}; James et al., 2007 {borderline significant improvement}; Petitt et al., 2003; Shannon et al., 2008 {6 participants}; Tsetsonis and Hardman, 1996a {low intensity bout}; Tyldum et al., 2009). Exercising on a cycle ergometer has been shown to be effective in lowering lipaemia significantly for healthy populations (Herd et al., 2001; Kolifa et al., 2004; Miyashita, 2008; Miyashita and Tokuyama, 2008), but not in men with type 2 diabetes (Dalgaard et al. (2004). The only study known to have had participants exercise on a

rowing ergometer found postprandial lipid metabolism to be improved the next day (Kokalas et al., 2005). The results from investigations using resistance exercise protocols have been far more equivocal than for aerobic exercise studies, with 5 studies reporting a beneficial effect of resistance exercise (Burns et al., 2007; Pafili et al., 2009; Petitt et al., 2003;; Singhal et al., 2009; Zafeiridis et al., 2007), two reporting no benefit (Burns et al., 2005 and Shannon et al., 2005) and one finding postprandial lipaemia to be increased (Burns et al., 2006). The only report of a protocol using resistance in conjunction with aerobic exercise (Silvestre et al., 2008) found postprandial TAG concentrations to be lower on the day following exercise than in a control trial.

1.6.6. Acute versus chronic benefits of exercise

Postprandial lipaemia has been shown to be higher for sedentary individuals than for those who are endurance-trained (Cohen et al., 1989; Hartung et al., 1993; Merrill et al., 1989; Ziogas et al., 1997) or physically active (Dixon et al. 2009), however, the notion that reduced lipaemia after exercise training is due to a chronic training adaptation, rather than the effect of a single recent exercise session, remains unproven. If the acute effect of exercise is removed (by leaving at least 24 h between the last exercise bout and an oral fat tolerance test), most studies appear to show no significant difference in postprandial lipaemia between trained and untrained individuals, whether nonsmokers (Bloomer et al., 2009b; Herd et al., 2000) or smokers (Bloomer & Fisher-Wellman, 2009) are tested. However, at least one article has found endurance-trained individuals to have a lower incremental lipaemic response to a fat meal than sedentary individuals when no exercise was taken on the day before the fat tolerance test (Merrill et al., 1989). Studies where inactive individuals have undergone a programme of exercise training do not show an improvement in postprandial lipaemia, post-

training, when a washout period of 48 h (Aldred et al., 1995; Herd et al., 1998; Ribeiro et al., 2008) or 24 – 36 h (Paton et al., 2006) from the last exercise session is left before an OFTT, but one study reported a reduction in postprandial TAG-rich lipoprotein concentrations post-training, having waited 36 h after the last exercise bout before test meal ingestion (Weintraub et al., 1989). When OFTTs were conducted 15 h, 60 h and 6.5 days after endurance-trained people performed their last exercise bout, postprandial TAG concentrations were significantly higher for the OFTTs at 60 h and 6.5 days than for the 15 h OFTT (Hardman et al, 1998). Similarly, when young adults underwent 13 weeks of running training, followed by 9 days of detraining, postprandial lipaemia was significantly higher for OFTTs conducted at 60 h and 9 d after the last exercise session, than at 15 h after (Herd et al., 1998). The available evidence suggests that the reduced postprandial lipaemia seen in endurance athletes is most likely to be due to the influence of acute exercise, and in the absence of a recent exercise bout, there is no substantial lipaemia-lowering effect of chronic exercise training. Therefore regular (daily) exercise must be undertaken if long-term improvements to postprandial lipid metabolism are to be maintained.

1.6.7. Mechanisms

While negative energy balance on a whole-body level is thought to contribute towards the lipaemia-lowering effect induced by prior exercise, the specific physiological/biochemical pathways through which the effect is mediated have not been firmly established. The clearance of TAG from the circulation is mediated by the enzyme lipoprotein lipase (LPL), which is most abundant in adipose tissue, and skeletal and cardiac muscle. To hydrolyse TAG, LPL must translocate to its active site on the luminal surface of the vascular endothelium, where it cleaves the first and third fatty acids of the TAG molecule, leaving 2-

monoacylglycerol (Frayn, 2003). Studies measuring postheparin plasma lipoprotein lipase activity after prolonged exercise have found significant increases in the activity of the enzyme compared with pre-exercise values (Kantor et al., 1984; Sady et al., 1986). If an exercise-induced increase in the activity of lipoprotein lipase were to increase clearance of plasma TAG – a not unreasonable suggestion given that the LPL:TAG ratio would be greater – then such a mechanism could perhaps explain the reduction in postprandial lipaemia after moderate exercise. Exercise has been shown to selectively increase LPL in skeletal muscle, not adipose tissue (Seip et al., 1995), and the timeframe over which skeletal muscle LPL increases after exercise would appear to fit with the delayed effect of exercise on postprandial lipaemia, as peak LPL mRNA expression occurs approximately 4 h postexercise, LPL protein mass peaks at around 8 h postexercise and peak activity of the actual enzyme occurs at some point beyond 8 h (Seip and Semenkovich, 1998). Early studies in the field of exercise and postprandial lipaemia implied that enhanced clearance of plasma TAG due to increased skeletal muscle LPL activity was likely to be responsible for reductions in postprandial lipaemia (Aldred et al., 1994; Tsetsonis and Hardman, 1996a; Tsetsonis and Hardman, 1996b). However, as TAG clearance rates were not calculated, and LPL activity was not measured during these investigations, firm conclusions could not be drawn. Studies in which prior moderate exercise reduced postprandial lipaemia have tended to find no significant increase in either postheparin plasma LPL activity (Ferguson et al., 1998; Gill et al., 2003a; Katsanos et al., 2004) or skeletal muscle LPL activity (Herd et al., 2001). Furthermore, increases in LPL activity due to low- (Katsanos et al., 2004) and moderate-intensity exercise (Zhang et al., 2002) have been observed without an accompanying reduction in postprandial lipaemia (Katsanos et al., 2004) or non-fasting TAG concentration (Zhang et al., 2002), and the rate of TAG clearance has been shown to be unaffected by prior moderate exercise (Gill et

al., 2001a). Despite the increase in LPL activity being non-significant in the studies of Gill et al. (2003a) and Herd et al. (2001), both studies did find that the difference in lipaemia between exercise and control trials correlated with the difference in LPL activity. Cumulatively, these observations suggest that increases in LPL activity are not common after moderate-intensity exercise, and, if reported, enhanced LPL activity does not necessarily translate to attenuated lipaemia; therefore prior moderate exercise is likely to mediate postprandial lipaemia, at least in part, through an alternative mechanism. The main alternative to LPL-mediated increases in TAG clearance is a lowering of VLDL-TAG due to a reduced secretion rate of VLDL particles from the liver. Initial evidence that exercise may affect postprandial came from training studies using rats. Chronic exercise was shown to reduce triacylglycerols, primarily through a reduction of the VLDL secretion rate, and the extent to which VLDL-TAG was lowered was very comparable with the overall decrease in total plasma TAG (Mondon et al., 1984; Simonelli and Eaton, 1978). In 1991, a third study confirmed these findings in rats and proposed that exercise training alters hepatic fatty acid partitioning, with increased oxidation of fatty acids in the liver and reduced esterification, therefore lowering the rate of secretion of VLDL-TAG into the plasma (Fukuda et al., 1991). This mechanism has some support in human studies, with reports that serum/plasma 3-hydroxybutyrate concentration, an indirect marker of hepatic fatty acid oxidation, is elevated on the day after moderate exercise, in both the fasting and postprandial states (Burton et al., 2008; Malkova et al., 2000; Gill et al., 2001a). Negative correlations have also been observed between the exercise-induced change in lipaemia (exercise trial value minus control) and the exercise-induced change in fasting (Gill et al., 2007) and postprandial 3-hydroxybutyrate concentration (Burton et al., 2008; Gill et al., 2007), implying that alterations to hepatic fat oxidation may be responsible for changes in postprandial lipaemia after exercise. These

reports are promising and suggest that increased hepatic fatty acid oxidation may be the main mechanism through which prior moderate exercise lowers postprandial TAG concentrations, however the indirect nature of the measures taken in these studies encourages caution when attempting to draw definitive conclusions. As a third option, at least one study (Kolifa et al., 2004) has concluded that reductions in postprandial lipaemia after exercise are partially due to a slowing of the rate at which dietary fat is released from the intestine. That such a mechanism would affect postprandial TAG concentrations to any great extent is generally thought to be unlikely, however, due to the length of time between exercise cessation and intake of a fat-containing test meal in most studies (usually 11 h or more).

1.7. Prior exercise and postprandial lipaemia: Some unanswered questions

It is now 15 years since the first widely acknowledged study into the effects of a single preprandial aerobic exercise bout on postprandial lipaemia was published (Aldred et al., 1994). In the intervening decade-and-a-half, knowledge has advanced considerably regarding many different aspects of when, how and by how much, prior exercise will reduce postprandial TAG concentrations, but several interesting questions remain unanswered or only partially answered. One such question relates to the optimum time delay between exercise and meal intake. As mentioned earlier in this introduction, a window of time exists after an exercise bout, during which the lipaemic response to a fat-containing meal will be decreased, compared with ingesting the same meal without prior exercise, provided that the energy expended during exercise is sufficient. Current evidence suggests that the total TAG response to a fat meal will be lower if ingestion occurs 1 – 18 h after a single exercise bout, but within this 17 h range, there has been little investigation of whether one length of delay produces a larger reduction in lipaemia than another. Knowledge of the time delay between

cessation of exercise and meal ingestion which induces the largest, or most consistent beneficial effect on postprandial TAG concentrations is of practical value if individuals at risk of CHD are to schedule exercise sessions into their day for maximum health benefit. The fact that the optimum time delay has not been investigated to any great extent may be because such a study is logistically difficult to conduct. Firstly, participants would be required to take part in a minimum of three trials (two exercise sessions and a control trial) if two time delays were to be compared. Secondly, it is not known whether the lipaemic response to the same meal is different when the meal is ingested at different times of day, e.g. intake in the morning vs. early afternoon vs. evening. Therefore, if the exercise session were to be performed at the same time in each trial, it would first be necessary to determine whether the postprandial TAG response to the same meal is equivalent at different times of day. If time of day did have an effect on lipaemic response, then exercise would need to be performed at awkward/unusual times of the day, or night, to accommodate the meal being ingested at the same time of day for each trial. Due to these logistical concerns, such a study was not undertaken as part of this thesis. Another question to which a satisfactory scientific answer would be of obvious practical value is "What is the lowest volume of exercise which will reduce lipaemia significantly?" With the majority of U.K. adults not meeting ACSM guidelines set out regarding the minimum amount of physical activity which should be taken if health is to benefit, it is unrealistic to expect that most adults will gradually become more active and will eventually undertake 90-minute exercise bouts on a regular basis. Therefore, by knowing the lowest volume of exercise (particularly with attention to duration) which will induce a significant lowering of lipaemia, guidelines can be created which relay this information and clinicians will be in a better position to advise individuals at risk of CHD, of the exercise volume they should be working towards achieving. The question of "significance" can be

interpreted in at least two different ways and strikes at the heart of the problem in this instance. To find a "statistically significant" effect of a low-volume aerobic exercise session on postprandial lipaemia, it may just be a case of recruiting enough participants to make a small effect size significant. Indeed, the observed dose-response effect on lipaemia when exercising at 50% VO_2max suggests this is the case (Gill et al., 2002a). However, it is likely a threshold will exist under which no consistent reduction in lipaemia is measured, despite a large participant group, due to the effect size being too small in comparison with the test-retest variability. Regarding clinical significance being met, the picture is far less clear, as a "clinically significant" reduction in lipaemia has not been described and may differ between individuals based on their existing TAG concentrations and their genetic predisposition towards development of CHD. Several studies have emerged in recent years which address the question of how short a preprandial exercise bout can become before the benefits to postprandial lipid metabolism are lost. A comparison of three treadmill jogs at 60% VO_2max, performed 12 h before a high-fat meal, found that both 45 min and 60 min of exercise reduced postprandial lipaemia significantly, but 30 min did not (Zhang et al., 2007). Two other groups reported that a continuous 30 min treadmill jog (Altena et al., 2004) and two 20 min bouts of cycling at a low intensity (Dalgaard et al., 2004) were insufficient to lower lipaemia. In contrast, four studies have found the total lipaemic response to be lower than control on the day after 30 min of exercise (Miyashita et al., 2006; Miyashita, 2008; Miyashita et al., 2008; Miyashita & Tokuyama, 2008). Two of the four also reported a reduction in the incremental TAG response (Miyashita et al., 2006; Miyashita & Tokuyama, 2008), with the other two investigations finding no significant decrease from control for this measure (Miyashita, 2008; Miyashita et al., 2008). While Altena et al. (2004) found no reduction in lipaemia with 30 min continuous exercise, the same study did report a significant lowering of the incremental

lipaemic response following three 10-min treadmill jogs. At this point, slightly more studies report a statistically significant lowering of lipaemia after an exercise session lasting only 30 min than do not, but further research is required to determine whether 30 min of exercise is enough to induce a clinically meaningful reduction in postprandial TAG concentrations for individuals possessing established cardiovascular risk factors. Research addressing this specific issue does not feature in the experimental chapters of this thesis. One of the other main questions still not answered satisfactorily relates to the pathway through which exercise reduces TAG concentrations postprandially. Obvious candidates include enhanced clearance of TAG within TAG-rich lipoproteins by LPL or reduced VLDL-TAG secretion by the liver; however, to arrive at something approaching a definitive answer to this question, complex and expensive methodology must be employed. To trace the fate of ingested fat through the body, fatty acids labelled with a stable isotope, e.g. [U-^{13}C]palmitate, could be incorporated within a test meal, with blood samples, and muscle and adipose biopsies taken before and after the meal to determine the enrichment of the tracer (by tracer/tracee ratio) within these tissues. Intravenous infusion of fatty acids containing a different tracer, e.g. [$^{2}H_2$]palmitate could then be used to provide a source of labelled plasma non-esterified fatty acids (NEFA), thus allowing endogenous (VLDL) TAG as well as exogenous (chylomicron) TAG to be traced. This dual-tracer approach has been used previously in combination with arteriovenous difference measurements (Bickerton et al., 2007), and by comparing trials with and without prior exercise, the major mechanism through which exercise lowers postprandial TAG concentrations should be examinable. Elucidation of the primary mechanism through which prior exercise reduces lipaemia is important, as our current inability to explain how this change in plasma TAG concentration occurs, makes it difficult to present a convincing argument that a single walk is beneficial for fat metabolism. Identification of the main

mechanism responsible would highlight the potential benefit of exercise for those affected by specific chronic diseases, e.g. those with type 2 diabetes may benefit if aerobic exercise is shown to increase skeletal muscle TAG cycling, whereas patients with fatty liver disease may benefit from preprandial exercise if it is found to increase postprandial hepatic fat oxidation. Unfortunately, stable isotope tracers are expensive to purchase, and once analysis of samples to be collected is added into the estimate, the financial outlay is often multiplied several fold. Without financial assistance from external funding bodies, such studies are difficult to subsidise. Additionally, the level of expertise required to perform such a study is not inconsiderable and the difficulty of setting up the necessary methods for the first time, must not be underestimated. Therefore, despite the fact that stable isotope methodologies are ideal for metabolic investigations of this kind, that combination with biopsy sampling of tissues has not been used previously to investigate the mechanisms of exercise-induced lipaemic moderation, and that the perceived value of undertaking such an investigation is high, it was not possible to conduct such an investigation during the course of the Ph.D. studies in this thesis.

With respect to the current thesis, four separate investigations were undertaken, each addressing a different question related to the effect of prior exercise on postprandial lipaemia. The first study, presented here in chapter three, was concerned with examining whether 90 minutes of brisk walking, performed approximately 13 h before eating a moderate-fat meal, would induce the same percentage reduction in postprandial TAG concentrations, compared with a control trial, as when the same exercise bout was before a high-fat meal. A positive correlation has been found between dietary fat load and postprandial lipaemia (Cohen et al., 1988a; Dubois et al., 1994; Dubois et al., 1998), and most previous studies showing a

reduction in postprandial lipaemia after a single moderate-intensity exercise session have used test meals wherein the percentage of energy from fat was far higher than that habitually ingested as part of the average U.K. diet. Therefore, this study tackled the important and practical concern that exercise may only have lowered postprandial lipaemia in previous investigations because the fat load given was exceptionally high. To ensure that the research question could be answered directly by this one study, all participants took part in four investigative trials, i.e. control and exercise trials with both moderate- and high-fat meals. The meals given within the studies of Cohen et al. (1988a), Dubois et al. (1994) and Dubois et al. (1998) were not isoenergetic and the greater energy intake with the higher-fat meals may be partially responsible for the more pronounced TAG response. Indeed, in studies where fat load differed, but energy intake was the same (Chen et al., 1992; Pedrini et al., 2006; Whitley et al., 1997), a significant difference in postprandial lipaemia was not seen. Therefore, a secondary aim of this study was to determine whether fat load affected postprandial lipaemia, with and/or without prior exercise, when the total energy present in the meals was identical. The second investigation (chapter four) attempted to discern whether the knowledge that preprandial aerobic exercise consistently lowers postprandial lipaemia, could be used to design a resistance exercise protocol which also improves postprandial lipid metabolism. The relevant literature on this topic is divided, with evidence for a beneficial effect (Burns et al., 2007; Pafili et al., 2009; Petitt et al., 2003; Singhal et al., 2009; Zafeiridis et al., 2007), no effect (Burns et al., 2005; Shannon et al., 2005) and a detrimental effect (Burns et al., 2006) of resistance exercise on postprandial lipaemia. Most of these studies had had participants lift heavy weights before eating a high-fat meal, with conflicting results. The high reproducibility of reductions in lipaemia, across studies, after aerobic exercise suggested that a circuit-type resistance exercise session which evoked a substantial aerobic component, may

be more effective in attenuating postprandial lipaemia than high-intensity resistance exercise, which is largely anaerobic. This premise was tested by comparing the TAG response of the study participants after a high-fat meal without prior exercise, with the response to the same meal after performing both low- and high-intensity resistance on separate occasions. The third experiment (chapter five) focussed on another very practical issue: whether individuals would choose to consume more energy on the morning after aerobic exercise if the option to eat and drink more was available, and whether this would abolish the exercise-induced lipaemic diminution seen in studies where participants ingested a test meal containing exactly the same quantity of energy during both exercise and control trials. The study was designed such that all participants had an *ad libitum* breakfast following two different preconditions: an evening of rest in the lab or an evening of exercise on the treadmill. The fact that energy intake during the *ad libitum* breakfasts was being recorded, was not revealed to the participants, therefore the effect of exercise on energy intake and TAG concentrations after the breakfast could be determined with minimal influence from behavioural and psychological factors which may have been apparent had the participants known that their food and drink intake was being monitored. The fourth and final study (chapter six) used ultrasonography to explore whether the same exercise bout used successfully in previous research to lower postprandial lipaemia (a brisk 90-minute treadmill walk) would also increase blood flow to the liver and/or the recently exercised skeletal muscle (specifically, the legs) during the postprandial period. At least one study (Malkova et al., 2000) has been published wherein postprandial leg blood flow was measured on the morning after moderate exercise. This study found postprandial calf blood flow to be significantly increased on the day after aerobic exercise, relative to a control trial. As the only documented evidence that skeletal muscle blood flow is elevated postprandially on the day following moderate exercise,

this study requires confirmation. Furthermore, as the participants in the study of Malkova et al. were young and active, it is yet to be tested whether a population with known risk factors for CVD (middle-aged, overweight and sedentary) would react in the same manner. The idea that blood flow to the liver may be elevated on the day after exercise, and could therefore contribute to the lipaemia-lowering effect, does not appear to have been investigated previously. The investigation was made possible by employing the same basic experimental design used in most previous studies, but including ultrasound measurements of flow through the aorta, the hepatic portal vein and the femoral artery at the same time-points when venous blood samples were collected.

Therefore, the four main questions addressed by the studies were:

1. **Is the beneficial effect of prior exercise on postprandial lipaemia the same for a moderate fat meal as it is for a high-fat meal?**
2. **Is the reduction in postprandial lipaemia greater when a resistance exercise session is made more aerobic?**
3. **Is the beneficial effect of prior exercise on postprandial lipaemia lost when the meal is consumed *ad libitum*?**
4. **Is the beneficial effect of prior exercise on postprandial lipaemia partly due to redistribution of blood flow?**

CHAPTER TWO

GENERAL METHODS

2.1. Ethical approval and informed consent

2.1.1. Ethical approval

The studies conducted in chapters three and four of this thesis were granted ethical approval by The School of Sport and Exercise Sciences Local Ethics Sub-Committee, University of Birmingham. The Black Country Research Ethics Committee, a local branch of the National Research Ethics Service (NRES), granted ethical permission for the research in chapters five and six to be undertaken.

2.1.2. Informed consent

All potential participants met with the investigators to go through the information presented in the participant information sheet and to ensure that each man fully understood what his involvement in the research would be. If, after this discussion, the individual still wanted to participate in the study, then a general health questionnaire was provided, to gather information on medical history and current health status. All men for whom the study was judged not to pose a significant danger to health signed a consent form to demonstrate their understanding of what would be involved and their willingness to take part voluntarily.

2.2. Study design

2.2.1. General design

All four studies followed a two-day approach, with an exercise session or rest during the evening of day 1, and an oral fat tolerance test (OFTT) on the morning of day 2. Details particular to the design of each specific study are outlined in the relevant experimental chapter.

2.2.2. Control of diet and physical activity

Participants were asked to keep a diary of all food and drink consumed on the day prior to, and the day of, their first trial; this diet was then replicated before all subsequent trials. Participants agreed not to perform any physical activity beyond that required for activities of daily living during the two days leading up to an oral fat tolerance test, and ingestion of alcohol and caffeine was prohibited for 24 hours prior to each exercise bout/evening rest.

2.3. Preliminary exercise testing – sub-maximal incremental treadmill test

After completion of a general health questionnaire and consent form, participants were shown to the laboratory to perform a sub-maximal incremental treadmill test. Whilst in the lab, measurements of height and body mass were recorded, and participants were fitted with a heart rate monitor (Polar Vantage NV, Polar Electro Oy, Kempele, Finland). Following five minutes of seated rest, resting heart rate was measured and expired air was collected into Douglas bags for subsequent analysis. Specifically, oxygen content of the breath was measured using a paramagnetic transducer and carbon dioxide using an infrared transducer. Both transducers were housed within the same unit (1440D Gas Analyser, Servomex Group Limited, England), allowing simultaneous measurement of the different gases. Expired air volume was also measured (Dry Gas Meter, Harvard Apparatus, USA) and oxygen uptake was calculated using these values. After completion of these measurements, participants were

asked to step onto the treadmill. The men were given five minutes to become familiarised with walking on the treadmill and were asked to select a speed which they felt confident of maintaining for 90 minutes, but which would represent a moderate effort for them. Once a speed was agreed between participant and experimenters (with heart rate generally in the 100 - 110 beats·min^{-1} range), the first five-minute walking stage began with the treadmill at 0% gradient. Heart rate and expired gas measurements were made during the fifth minute of each five-minute stage and participants were asked to supply a rating of perceived exertion (RPE) from a Borg RPE Scale (Borg, 1982) at the conclusion of each stage. The treadmill gradient was increased by 2-3% at the end of each five-minute stage, with the test being terminated upon subjects reaching 85% of their individual predicted maximal heart rate. Maximal heart rates were predicted using the equation of Tanaka et al., 2001 (i.e. maximal heart rate = 208 – 0.7 x age). Heart rate was subsequently plotted against VO_2, and a prediction of individual VO_2max was made via extrapolation of a linear plot (trendline). VO_2 vs. treadmill gradient plots were then made and the equation of the slope was used to determine the gradient required to elicit 60% of VO_2max for each man.

2.4. Main trials

2.4.1. Aerobic exercise trials

Participants arrived at the laboratory at 18:00. They then walked for 90 minutes at the speed selected in the preliminary test, and at a treadmill gradient calculated to evoke 60% of individual predicted VO_2max. To confirm the VO_2 at which the men were actually working, expired breath samples were collected during the last two minutes of each sixth of the walk, i.e. spaced at 15-minute intervals. On occasion, subtle alterations to the treadmill gradient were made, to ensure that oxygen uptake remained as close to 60% VO_2max as possible.

Heart rate was monitored continuously throughout the trial, with values stored electronically, at 5-second intervals, for subsequent download.

2.4.2. Control trials

During the control trial, all participants remained seated or supine for a period equivalent in length to the exercise trial. The men were allowed to perform paperwork, watch television/DVDs, work on a computer or read a book, but no physical activity above that required to perform these actions was permitted.

2.5. Evening (post-intervention) meal

After exercising or resting in the laboratory, all participants were provided with an evening meal. This meal contained 36% of energy as fat, 46% as carbohydrate and 18% as protein, with the fat content guided by governmental guidelines for a balanced diet (Department of Health, 1994). The total energy content of the meal provided for the men was calculated based on each individual's habitual evening energy intake. Participants were asked to keep a detailed food diary for three consecutive evenings; total energy counts for each evening were made using the nutrition information available on the packaging of the foods eaten, and the nutrition software package "Comp-eat" (Comp-eat version 5.0.1). A mean of the three evenings was taken and this value represented the total quantity of energy given to the participant. Evening meals were consumed within one hour of finishing an exercise session/rest period, after which the men were asked to fast until the following morning.

2.6. Oral fat tolerance tests

Participants arrived at the laboratory at 08:00 after an overnight fast. A cannula was inserted into an antecubital or forearm vein and a fasting blood sample (5 ml) was drawn. To allow for determination of oxygen consumption and whole-body substrate utilisation, a fasting expired breath sample was collected for five minutes into a Douglas bag, with further breath samples taken at 2 h and 5 h postprandially (chapter three), or 2.5 h and 5.5 h postprandially (chapters four, five and six). Participants were then taken to the research kitchen where they were presented with the test meal.

Venous blood samples were drawn 0.5, 1, 2, 3, 4, 5 and 6 hours after consumption of the test meal, with the first 2 ml of blood drawn at each time-point being discarded as waste. The cannula was kept patent by flushing every 20 minutes with non-heparinised saline. Participants were given no further food and were permitted to drink only water during the postprandial period. Water was provided *ad libitum* during the first trial, with the volume being recorded and replicated in remaining trials.

Blood samples were dispensed into precooled K_3-EDTA vacutainers for recovery of plasma, and into plain vacutainers for obtainment of serum. Blood contained within K_3-EDTA tubes was centrifuged immediately, for 15 minutes, at 4^oC and 3500 RPM. Plasma was then aspirated and divided into aliquots within Cobas cups for immediate storage at -80^oC. Uncoated vacutainers were left on the bench top for 30 minutes to allow clotting to occur before centrifugation, after which, serum was dispensed into Eppendorf cups.

2.7. Body fat estimations

For the studies pertaining to chapters three and four, all participants had skinfold measures taken during their final trial to estimate body fat percentage. Measurements were made at the bicep, tricep, subscapular and suprailiac sites by an ISAK-accredited kinathropometrist; log values were then entered into the age-specific four-site skinfold equations of Durnin and Womersley (1974) to calculate body density. Body density values were converted to body fat percentages using the equation of Siri (1956). For the investigations described in chapters five and six, participants underwent a dual-energy x-ray absorptiometry (DXA) scan immediately prior to their control trial to assess body fat percentage.

2.8. Analytical procedures

Plasma was analysed by enzymatic colourimetric methods using a 96-well plate reader (Multiskan MS, Labsystems, Finland) for triacylglycerol (Sigma-Aldrich, Poole, UK) and using a centrifugal analyser (Cobas Mira Plus, Roche, Switzerland) for NEFA (Wako, Germany) and glucose (ABX Diagnostics, Montpellier, France). Serum was analysed for insulin by ELISA (IDS Ltd, Germany). Owing to the nature of the TAG assay, plasma glycerol concentrations were also measured to allow for correction of TAG concentrations. Samples were stored at -80^{o}C until immediately prior to analysis, with all samples for each participant being analysed in the same run.

2.9. Calculations and statistics

2.9.1. Sample size calculations

To estimate the number of participants required to reject the null hypothesis (where the null hypothesis states that exercise does not lower the area under plasma TAG versus time curve,

compared with a control trial), an online power and sample size calculator (http://www.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3/download-and-register) was used. The specific calculator downloaded is described in the paper of Faul et al. (2007). The data used for the sample size calculation were from a study by Gill et al. (2001b) which reported a 23% reduction in time-averaged postprandial TAG concentration after a 90-min treadmill walk, compared with a control trial. Using a paired samples t-test, sample size calculations indicated that a minimum of 5 participants would be necessary to reject the null hypothesis with power set at 0.9, an alpha level of 0.05 and a correlation of 0.5 between the two measures (the correlation for TAG AUC between exercise and control trials was higher than 0.5 for all experiments in this thesis). To ensure differences between exercise and controls trials would be detected if present, eight participants were recruited for each of the four studies within this thesis. After completion of the investigations, retrospective calculations were conducted to determine how powerful each study was, and when the difference between trials for a main measure was not statistically significant, sample size calculations were run to assess how many participants would be required to find a statistically significant difference (with power of 0.9, alpha level of 0.05 and correlation as calculated between the variables being tested). The results of these power and sample size calculations are presented in the specific experimental chapters to which they apply.

2.9.2. Substrate oxidation rates and energy expenditure

The total energy expended during 90-minute treadmill walks was calculated using indirect calorimetry, with the assumption that 1g of fat and carbohydrate yield 9.75 kcal and 4.07 kcal of energy respectively when exercising at a moderate intensity (Jeukendrup and Wallis, 2005), and assuming that no protein was oxidised during the exercise. Substrate oxidation

rates during exercise were calculated using the equations for moderate to high intensity exercise (50-75 % VO_2max) proposed by Jeukendrup and Wallis (2005). The equations of Peronnet and Massicotte (1991) were used for calculation of resting substrate oxidation rates with the assumption that resting carbohydrate oxidation was exclusively from glucose.

2.9.3. Normality testing

Data sets were tested for a normal distribution using the Shapiro-Wilk test. TAG measures were found to be normally distributed in chapters three, four and six, but not in chapter five. TAG data were therefore logarithmically transformed in chapter five before statistical analysis was performed.

2.9.4. Main effects and trend analysis

Analysis of variance (ANOVA) for repeated measures was used to determine main effects for all postprandial measures, as well as any interactions between the main effects. The first two orthogonal polynomial contrasts (linear and quadratic components) were computed simultaneously to further explain the differences between conditions across time. These analyses were performed using SYSTAT 11 for Windows. The majority of previous studies have used repeated measures ANOVA followed by post hoc Tukey tests to determine where the differences lie when the omnibus F-test has shown that statistically significant differences exist. However, this approach has substantial limitations for investigations with a within-subjects design. Tukey's Honestly Significant Difference (HSD) test assumes that all differences between pairs of means have the same population variance, however, as violation of sphericity is the rule rather than the exception in repeated measures designs (Field, 1996), and even small departures from sphericity have been shown to seriously affect test size and

power, post hoc tests which pool the error terms of all pairwise comparisons (e.g. Tukey's HSD) are advised against (Boik, 1981). Monte Carlo methods have shown that Tukey's Wholly Significant Difference (WSD; now more commonly known as "HSD") test can allow the alpha rate to inflate to twice the nominal value (from 0.05 to 0.10), under conditions where sphericity is violated (Maxwell, 1980), therefore doubling the chance of committing a type 1 error. This led the author to the conclusion that "… Tukey's WSD is, in general, unsatisfactory for performing pairwise comparisons in a repeated measures design". By contrast, even in cases where the departure from sphericity is large, Bonferroni's adjustment successfully maintained alpha at or below the nominal level; a finding confirmed by Baker and Lew (1987). Most studies using repeated measures suffer from some degree of nonsphericity; however, the severity of the departure from sphericity is frequently overlooked, and a nonsignificant *P* value, as assessed by Mauchly's test, is often taken as evidence that the data do not depart from sphericity significantly. Stevens (1986) asserts (in agreement with Maxwell (1980)) that when ε (sphericity) is less than 0.70 (where 1.00 reflects a data set in which the variances of the differences between treatments levels are equal and sphericity holds), Bonferroni's adjustment, not the Tukey HSD test, should be used to conduct pairwise comparisons. Therefore, in the experimental chapters of this thesis, Bonferroni's adjustment was used when it was necessary (chapter 4) to determine which pairs differed after a significant main effect was observed by omnibus F-test. Due to its conservativeness, Bonferroni's adjustment is not practicable when assessing differences between conditions across several time points; in this situation, orthogonal polynomial contrasts provide an elegant solution. Orthogonal polynomials provide single degree of freedom comparisons of the waveform over time and hence do not violate the assumption of independence between means required for use of post hoc Tukey tests. Linear components

reflect the overall increases or decreases over time whereas the quadratic components reflect any non-linear peak or trough that might occur within the time window. As trend analysis via the use of orthogonal polynomial contrasts expects symmetry of the waveform across the time axis, the natural logarithm (base *e*) of time was used to improve symmetry of the metabolite curves. Log transformed time was only used where logarithmic transformation was found to improve symmetry across time. The metric used for statistical analysis was decided on a case-by-case basis following scrutinisation of each curve. Graphical depictions of the data plotted against both real time and log transformed time can be seen in the **Appendices**.

2.9.5. Area under the curve scores and other comparisons

In order to allow comparisons with previous work, areas under the curve representing the total response for each metabolite are also reported. The area under the curve (AUC) score is a conventional measure of a metabolite's concentration in the plasma over time, and was calculated as the 6 h area under the plasma/serum metabolite concentration versus time curve, using the trapezoidal rule. As the "total response" includes, and is influenced by, the fasting concentration of each metabolite, it is also of interest to calculate the "incremental response" of each metabolite. The incremental area under the curve (iAUC) score was calculated as the area under the curve normalised to the 0 h value; thus it is a true postprandial measure reflecting only the change in concentration after a meal. Two-way analysis of variance (exercise x meal) was conducted on the AUC scores, iAUC scores, fasting values, peak values and time to peak values in chapter three, whereas one-way analysis of variance was used for chapter four, and paired samples t-tests were used for chapters five and six, to examine the effect of exercise on the same variables. In studies with more than one exercise trial (chapters three and four), variables measured during exercise were compared using paired samples t-

tests. SPSS 16 for Mac was used for all such analysis. Relationships between variables were examined using Pearson's product-moment correlation coefficient.

2.9.6. Homeostasis model assessment of insulin resistance

Fasting concentrations of glucose and insulin were used to derive a validated surrogate measure of insulin resistance (Levy et al., 1998). Specifically, fasting glucose and insulin concentrations were entered into a "HOMA2 calculator" downloaded from the Diabetes Trials Unit, University of Oxford website (http://www.dtu.ox.ac.uk/index.php?maindoc=/4-T/). This calculator produces an insulin resistance score representing the reciprocal of % insulin sensitivity. The calculator is an updated version of the mathematical feedback model first developed by Matthews et al (1985), however, unlike the original equations, the new model is appropriate for use with currently available insulin assays.

2.9.7. Statistical significance

Statistical significance was accepted at the level of $P < 0.05$. All data are presented as mean values ± standard error of the mean (S.E.M.) unless otherwise stated.

CHAPTER THREE

Is the beneficial effect of prior exercise on postprandial lipaemia the same for a moderate fat meal as it is for a high fat meal?

ABSTRACT

Background: Plasma triacylglycerol (TAG) concentrations are elevated for many hours after consumption of a fat-containing meal; a situation which can promote atherosclerosis. Moderate intensity exercise can lower the TAG response to a high-fat meal, however, the British diet is moderate in fat and no study to date has compared the effect of such exercise on responses to high and moderate fat meals.

Aim: The aim of the study was to investigate the effect of brisk walking, performed 13 h before intake of both high fat and moderate fat meals, on postprandial plasma TAG concentrations.

Participants: Eight inactive, overweight men, aged 47.0 ± 9.1 years (mean ± SD) completed the study; none participated in more than one hour of structured physical activity per week.

Design: The study followed a two-day approach with an exercise session (Ex) or rest (Con) during the evening of day 1, and an oral fat tolerance test (OFTT) on the morning of day 2. To compare the effect of walking versus control on the response to both high-fat and moderate-fat meals, participants completed four trials: a high-fat exercise trial (High Ex), a high-fat control trial (High Con), a moderate-fat exercise trial (Mod Ex) and a moderate-fat control trial (Mod Con). High-fat meals contained 66% of total energy as fat, while the percentage was 35% for moderate-fat meals; both meals were however isoenergetic. Exercise trials involved 90 minutes of brisk walking at 60% of individual VO_2max; subjects rested for the same duration during control trials. Venous blood was sampled on the morning of day 2

in the fasted state, 30 minutes after ingesting the test meal, 1h post-meal and then hourly until 6 hours after meal completion.

Results: 90 minutes of brisk walking reduced the total TAG response to a high fat meal by 29% (relative to High Con); the same bout of exercise performed before a moderate fat meal lowered total TAG by 26% (compared with Mod Con).

Conclusions: The beneficial effect of a moderate intensity aerobic exercise bout on postprandial lipid metabolism is just as great, in percentage terms, when the meal eaten is of a moderate, rather than a high fat content.

3.1. INTRODUCTION

Evidence suggests that an exaggerated TAG response to a fat meal is a risk factor for CHD (Karpe et al., 1998). Performing aerobic exercise 11 – 18 h prior to ingesting a fat-containing meal has been shown on many occasions to lower the extent of the subsequent lipaemia, as outlined in the introduction to this thesis, however, the fat content of the test meal used may not be relevant in the context of meals consumed as part of the habitual diet.

World Health Organisation (WHO) guidelines recommend that fat should contribute 15-30% of an individual's daily energy intake, although it is acknowledged that highly active individuals consuming diets rich in fruit and vegetables will not necessarily risk unhealthy weight gain (and associated chronic disease risks) when fat represents 35% of energy intake (Joint WHO/FAO Expert Consultation on Diet, Nutrition and the Prevention of Chronic Diseases, 2003). A government commissioned survey of British men, aged 19-64, found dietary fat consistently accounted for 35-36% of energy intake across age groups (Henderson

et al., 2003), however, almost all studies investigating the effects of exercise on postprandial lipaemia have used oral fat loads which are greatly in excess of this percentage (average of 65-66%: Gill et al., 1998; Gill and Hardman, 2000; Gill et al., 2001a; Gill et al., 2001b; Gill et al., 2002a; Gill et al., 2003a; Gill et al., 2003b; Gill et al., 2006; Gill et al., 2007; Kokalas et al., 2005; Kolifa et al., 2004; Tsetsonis and Hardman, 1996a; Tsetsonis and Hardman, 1996b; Zhang et al., 1998; Zhang et al., 2004; Zhang et al., 2006; Zhang et al., 2007). Therefore, it could be argued that the use of test meals containing far higher fat percentages than seen on average in the British diet has artificially magnified any differences between exercise and control trials in such studies. Studies where exercise was performed 14 -17 h prior to ingesting a test meal containing 35 – 37% of energy as fat, have found a reduction in the total lipaemic response, relative to control, but no significant lowering of the incremental lipaemic response has been observed (Burton et al., 2008; Kokalas et al., 2005; Kolifa et al., 2004; Miyashita, 2008). James et al. (2007) found that exercising on the day before a 45% fat meal reduced postprandial lipaemia by 23% (a borderline significant reduction); however, as with papers which found a statistically significant reduction in total TAG response with prior exercise, a lowering of the fasting TAG concentration was largely responsible for this change. Another report, in which healthy, active men performed a 30 min cycle, 13 h before a meal containing 45% of energy as fat, found that both the total and incremental lipaemic responses were significantly attenuated after exercise (Miyashita and Tokuyama, 2008). Three further studies failed to find a benefit of either cycling (Petridou et al., 2004; Pfeiffer et al., 2006) or walking (Pfeiffer et al., 2005) on postprandial TAG response to a moderate-fat meal, but as the meals were eaten immediately after exercise in these studies, this makes it difficult to draw comparisons with other studies where subjects ingested the meal 11 h or more after the exercise bout. Due to the discrepant nature of findings from studies using moderate-fat meals,

it is possible that the beneficial effects of physical activity in reducing postprandial TAG concentrations have been overstated. While a cross-sectional study could compare the effect of exercise on the response to a moderate-fat meal in one group and a high-fat meal in another, the question of whether exercise is as potent in lowering lipaemia when the fat content of the meal is reduced cannot truly be answered unless the responses to both meals are considered in the same individuals. No such work has been published. The current study therefore investigated the effect of 90 minutes of prior treadmill walking on postprandial lipaemia after meals of both high and moderate fat content in the same participants. Elevated postprandial lipaemia has been shown to be more common amongst certain population sub-groups, with maleness (Heller et al., 1993; Kolovou et al., 2006), ageing (Jackson et al., 2003), inactivity (Dixon et al., 2009; Hardman et al., 1998) and obesity (Lewis et al., 1990; Gill et al., 2004; Bartual et al., 2006) being particular risks; therefore it is particularly relevant to recruit people with several of these risk factors as a subject population in which to investigate potential interventions. Middle-aged, inactive, overweight men were therefore recruited, due to their increased risk of developing cardiovascular disease.

3.2. METHODS

3.2.1. Participants

Eight, non-smoking, inactive, overweight men {age 47.0 ± 9.1 years, height 179 ± 7 cm, body mass 92.7 ± 4.8 kg, BMI 29.2 ± 2.3 $kg \cdot m^{-2}$, body fat 32.9 ± 6.1 % and estimated VO_2max 37.5 ± 5.3 $ml \cdot kg^{-1} \cdot min^{-1}$ (mean ± S.D.)} participated in the study. Inactivity was defined as participation in one hour or less of structured physical activity per week, and overweight was classed as a body mass index (BMI) in excess of 25 $kg \cdot m^{-2}$. None of the men were known to have cardiovascular disease or diabetes, and each potential participant's general practitioner

was contacted to ensure that the study did not present a particular danger to the patient's health based on his medical history. Participation in the study was not permitted until written GP approval was received. All men known to be taking medication affecting lipoprotein metabolism were excluded from participating in the study.

3.2.2. Study design

The study was designed as outlined in **General Methods** (section 2.2.) with all participants completing four trials: a high-fat exercise trial (High Ex), a high-fat control trial (High Con), a moderate-fat exercise trial (Mod Ex) and a moderate-fat control trial (Mod Con). Oral fat tolerance tests were separated by a minimum of 4 days. Trial order was counterbalanced to control for the effect of serial position in which the trial occurred, with each subject following a different test order.

3.2.3. Preliminary exercise testing

All participants completed a sub-maximal incremental treadmill test as described in **General Methods** (section 2.3).

3.2.4. Exercise and control trials

Exercise sessions and control trials were conducted as outlined in **General Methods** (section 2.4)

3.2.5. Evening (post-intervention) meal

All participants were provided with an evening meal following each walk/rest, as described in the **General Methods** (section 2.5). The mean energy (± S.D.) provided by the meal was 4.41 ± 0.50 MJ (1055 ± 120 kcal).

3.2.6. Oral fat tolerance tests

Oral fat tolerance tests were conducted as described in the **General Methods** (section 2.6). The high-fat meal provided 1.2g fat/kg body mass, 1.2g carbohydrate/kg body mass and 0.2g protein/kg body mass (66% fat, 29% carbohydrate, 5% protein as percentage of total energy), and consisted of a liquid component containing whole milk, double cream and sugar, along with a solid component of hazelnut cereal bars. The moderate fat meal provided 0.64g fat/kg body mass, 1.93g carbohydrate/kg body mass and 0.74g protein/kg body mass (35% fat, 47% carbohydrate, 18% protein as percentage of total energy) and comprised of a drink containing whole milk, double cream, sugar and skimmed milk powder, along with apricot cereal bars. The meals were isoenergetic (both providing 68.6 kJ of energy per kg body mass) and were constructed such that the mass of cereal bar given in the different meals would be the same. The meals provided 6.36 ± 0.12 MJ (1520 ± 28 kcal) of energy, with fat loads of 111 ± 2 and 59 ± 1 g (mean ± S.E.M.) for the high-fat and moderate-fat meals respectively. The percentage of fat accounted for by saturates was 54.7% for the high-fat meal and 54.8% for the moderate-fat meal. As a greater volume of liquid was present in the moderate fat meal (due to it being less energy dense than the high fat liquid), a small volume of water (calculated on an individual basis) was provided for the subjects along with the high-fat meal to ensure the total volume of liquid consumed in each test meal was equal.

3.2.7. Body fat estimation

See **General Methods** (section 2.7).

3.2.8. Analytical procedures

The analytical procedures used are described in the **General Methods** chapter (section 2.8). Within-batch coefficients of variation were 2.2% for TAG (after correction for plasma glycerol), 1.0% for NEFA, 1.8% for glucose and 5.2% for insulin.

3.2.9. Calculations and statistics

All calculations and statistical analyses were performed as described in **General Methods** (section 2.9). For orthogonal polynomial contrast analysis, logarithmic transformation of time was found to improve symmetry for NEFA, glucose and insulin, but not for TAG; therefore, only the NEFA, glucose and insulin analyses were performed with time logged to the base *e*.

3.3. RESULTS

3.3.1. Responses during brisk walking

During both walks, participants walked at a speed of 5.7 ± 0.2 $km \cdot h^{-1}$ up a gradient of 4.2 ± 0.5 %. Mean oxygen uptake was 21.7 ± 1.1 $ml \cdot kg^{-1} \cdot min^{-1}$ (58.1 ± 1.1 % VO_2max) during the walk before the high-fat meal (HF walk) and 22.0 ± 1.0 $ml \cdot kg^{-1} \cdot min^{-1}$ (58.8 ± 0.7 % VO_2max) during the walk before the moderate-fat meal (MF walk). The gross energy expenditure during the walks was 3.75 ± 0.19 and 3.80 ± 0.18 MJ, with 59.0 ± 4.0 and 60.0 ± 2.9 % of energy from carbohydrate, and 41.0 ± 4.0 and 40.0 ± 2.9 % from fat, for the HF and MF walks respectively. Mean heart rate was 127 ± 5 $beats \cdot min^{-1}$ during the HF walk and 128 ± 3

beats·min^{-1} during the MF walk. Ratings of perceived exertion from the Borg RPE Scale (Borg, 1982) were 14.3 ± 0.4 (HF) and 13.7 ± 0.7 (MF) for the walks; the exercise was therefore perceived to lie between the descriptors "somewhat hard" and "hard". No significant differences were found between the two walks for any of the responses recorded.

3.3.2. Plasma and serum concentrations in the fasted state

Fasting plasma concentrations of triacylglycerol, NEFA and glucose, and serum concentrations of insulin, are presented in **Table 3.1**. Plasma TAG concentrations were reduced by 28.1% and 22.1%, compared to their respective controls, when walking was performed before ingestion of a high-fat meal and a moderate-fat meal (effect of exercise, P = 0.001). Plasma NEFA concentrations, serum insulin concentrations and HOMA scores were not found to be significantly different between exercise and control conditions prior to either meal, however an effect of meal about to be eaten was found for insulin (P = 0.048) and HOMA (P = 0.048). Plasma glucose concentrations were significantly lower following exercise (P = 0.001), with reductions of 8.0% prior to the high-fat meal and 3.5% prior to the moderate-fat meal.

Table 3.1.
Plasma and serum concentrations in the fasted state for both walking and control trials before ingestion of either a high-fat or moderate-fat meal (mean ± S.E.M.)

	Con High	Con Mod	Walk High	Walk Mod
TAG (μmol·l^{-1})	1207 ± 226	1142 ± 205	868 ± 231**	890 ± 146**
NEFA (μmol·l^{-1})	326 ± 29	319 ± 28	373 ± 32	325 ± 25
Glucose (mmol·l^{-1})	5.84 ± 0.32	5.90 ± 0.32	5.38 ± 0.25**	5.69 ± 0.28**
Insulin (μIU·ml^{-1})	11.9 ± 2.6	14.5 ± 2.8$^{\#}$	12.0 ± 2.8	14.9 ± 2.9$^{\#}$
HOMA	1.56 ± 0.35	1.94 ± 0.38$^{\#}$	1.59 ± 0.36	1.94 ± 0.38$^{\#}$

**Significantly different from control trials (main effect of exercise; $P \leq 0.001$).
$^{\#}$Significantly different from high-fat meal trials (main effect of meal to be eaten; $P < 0.05$)

3.3.3. Postprandial plasma and serum concentrations

3.3.3.1. Triacylglycerol

Main effects and trend analysis

Plasma triacylglycerol concentrations for 6 h after intake of either a high-fat or moderate-fat meal, with walking and control trials for each meal, are shown in **Figure 3.1**. Plasma TAG concentrations were lower after walking trials than controls, thus reflecting a main effect of exercise ($P < 0.001$). A main effect of time ($P < 0.001$) was evident, with TAG concentration increasing during the postprandial period for all trials, however no significant effect of meal was apparent ($P = 0.295$). Significant exercise x time ($P < 0.001$) and meal x time ($P = 0.037$) interactions existed, however there was no interaction between exercise and meal ($P = 0.459$). For both significant interactions, the first two orthogonal polynomial contrasts were computed to describe the time course of the effects. For the exercise x time interaction, 92% of the total variance was explained by a linear function ($P = 0.001$), with the slope of the increase in TAG over time being steeper for control trials than for walking trials. As the difference in TAG concentration between exercise and control trials at the end of the postprandial period was greater than the difference before the meal, it can be interpreted that TAG increased significantly more quickly in the control trials. For the meal x time interaction, 81% of the total variance was explained by a quadratic function ($P = 0.009$). **Figure 3.2** shows the change in plasma TAG concentration from baseline across time, therefore allowing for clearer discernment of differences between trials due to meal intake. When considered alongside the observation that there was no main effect of meal, the significant meal x time interaction, largely explained by a quadratic function, suggests that

while TAG concentrations are not significantly lower after a moderate-fat meal than a high-fat meal, the TAG response to a moderate-fat meal is different across time from that to a high-fat meal, i.e. mean TAG concentration can be seen to peak at 3 h in the Walk High trial and at 5 h in the Con High trial, before falling, whereas TAG concentrations continued to rise until 6 h in the moderate-fat meal trials. Whilst orthogonal polynomial contrasts revealed a difference in the pattern of TAG concentration change across time with the different meals, there was not a statistically significant difference with respect to the time at which peak TAG concentration occurred in the four conditions. A non-significant trend was found for TAG to peak earlier after ingestion of a high-fat meal (effect of meal: $P = 0.068$); with greater participant numbers this finding may have reached statistical significance, as sample size calculations based on our data indicated that with ten more participants, P would have been less than 0.05. Peak TAG values were substantially reduced with exercise (30.0% lower for high-fat meal and 26.9% lower for moderate-fat meal; $P = 0.001$ for effect of exercise), but meal fat concentration did not impact on the peak values observed (effect of meal; $P = 0.245$). There was not a significant interaction between the three conditions of the study (exercise x meal x time interaction: $P = 0.203$), however the quadratic component of the main three-way interaction term showed a nonsignificant trend ($P = 0.08$) and the linear component had a P value of 0.106. Together, these components accounted for 76% of the variation within the main interaction, and, as the shape of the TAG curves was either linear (moderate-fat) or quadratic (high-fat), a greater sample size may have resulted in a significant difference being seen with respect to the effect of exercise on TAG concentrations across the 6 h postprandial period for the two meals.

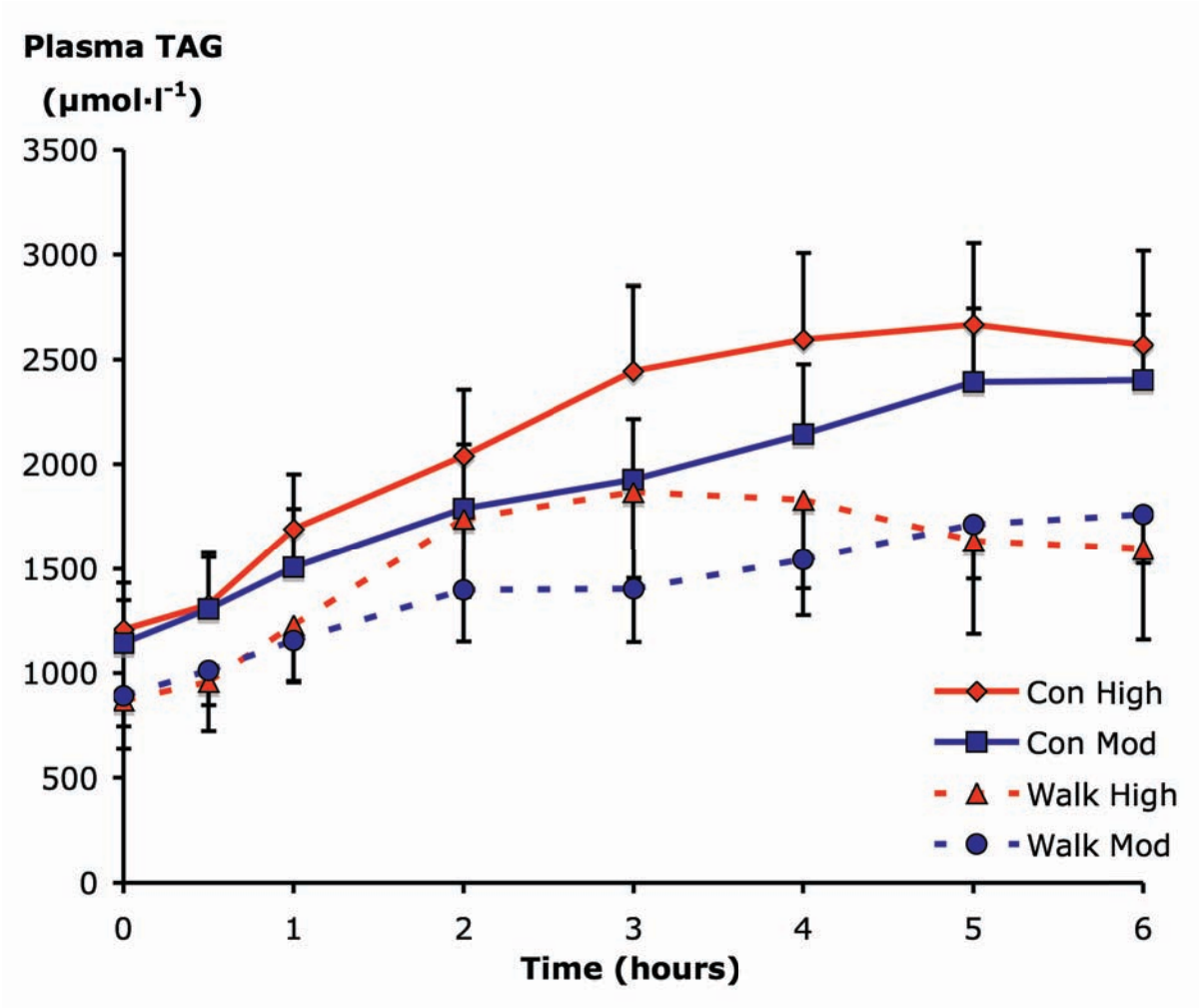


Figure 3.1. Mean (± SEM) plasma triacylglycerol concentrations in the fasted state and for 6 h after consumption of a high fat meal (High; red lines) or moderate fat meal (Mod; blue lines) with either prior exercise (Walk; dashed lines) or prior rest (Con; solid lines).

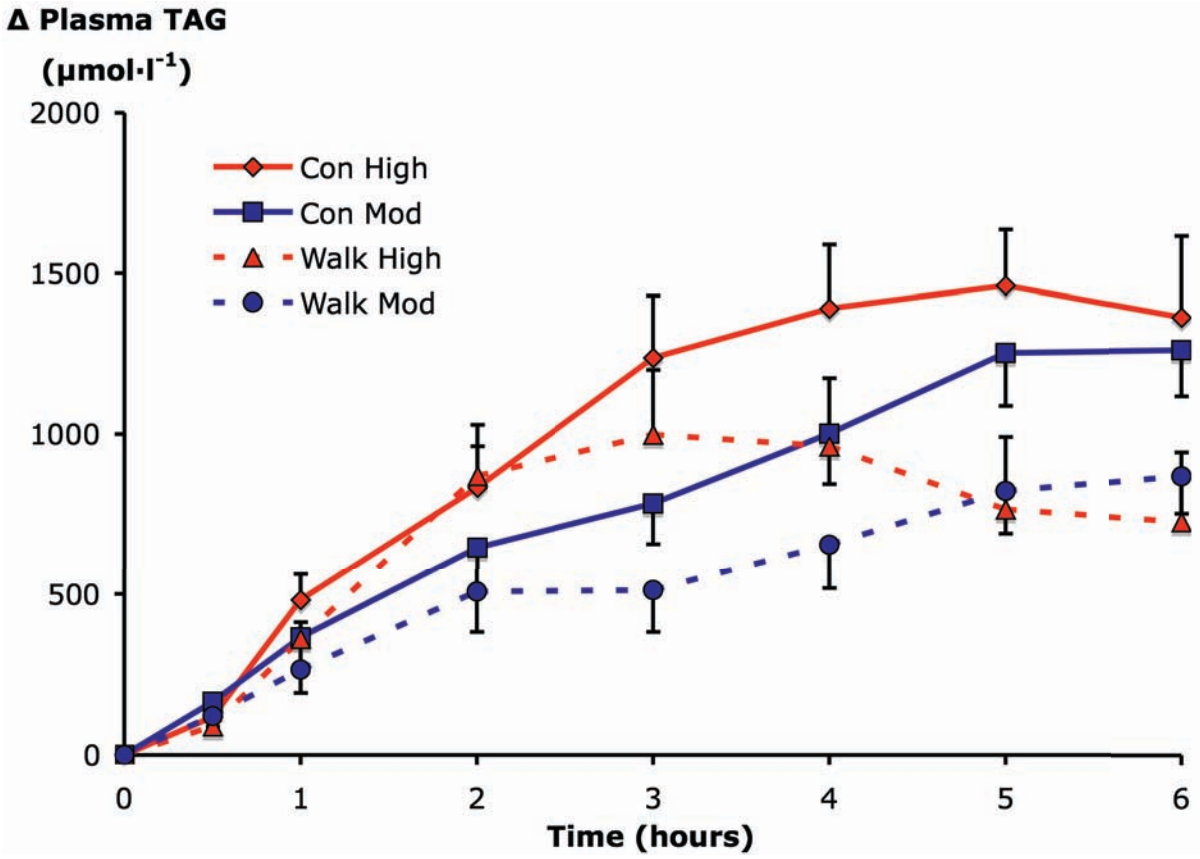


Figure 3.2. Mean (± SEM) change in plasma TAG concentrations in the 6 h after consumption of a high fat meal (High; red lines) or moderate fat meal (Mod; blue lines) with either prior exercise (Walk; dashed lines) or prior rest (Con; solid lines).

AUC scores

Total and incremental lipaemic responses are shown in **Figure 3.3**. Total lipaemic responses to the high-fat and moderate-fat meals were reduced with exercise by 28.5% and 26.0% respectively ($P < 0.001$). Based on the sample size within this study (16 paired comparisons of TAG AUC between exercise and control trials), retrospective power calculations indicated that the power to reject the null hypothesis was 1.000. When this was broken down further to determine the power for each meal, with $N = 8$ for each power calculation, the power to reject the null hypothesis for the high-fat meal trials was 0.995 and for the moderate-fat meal trials was 0.902. The total lipaemic response to the moderate-fat meals was not significantly lower

than the response to the high-fat meals (P = 0.226 for effect of meal), but there was a non-significant trend for the incremental response to be lower following moderate-fat meal intake (effect of meal; P = 0.075). Retrospective sample size calculations predicted that for a significant effect of meal to be found for total lipaemic response (lower response to moderate-fat meal), 31 participants would be needed (each doing exercise and control trials for both meals). For the incremental lipaemic response to differ significantly between meals, it was calculated that 14 participants consuming both meals on two occasions would be necessary. As with the total responses, the incremental lipaemic responses to both meals were suppressed with prior walking (suppression of 29.1% for HF meal and 31.7% for MF meal; P = 0.001 for effect of exercise).

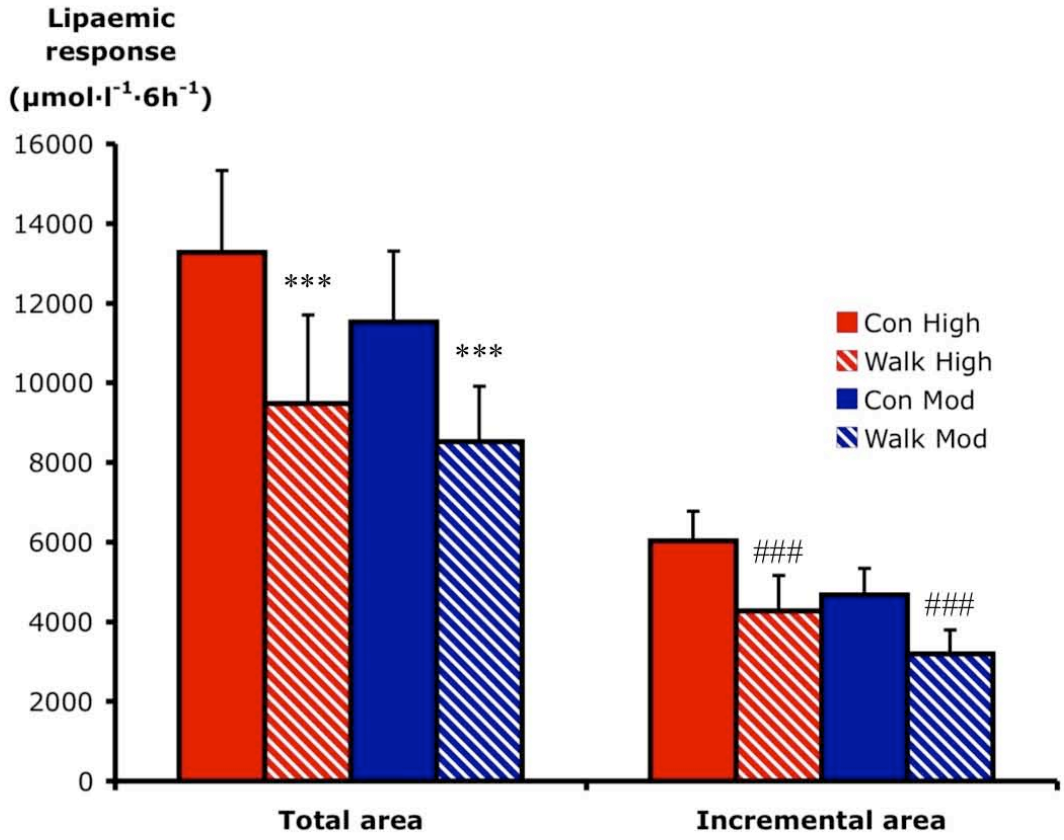


Figure 3.3. Area under the curve scores showing the total and incremental lipaemic responses. High-fat meals are coloured red and moderate-fat meals are coloured blue. Control trials are solid bars, whereas walking trials are diagonally hatched.
*** Main effect of exercise for total AUC, $P < 0.001$. ### Main effect of exercise for incremental AUC, $P \leq 0.001$.

Correlations

Fasting TAG concentrations in each of the four trials were positively correlated with TAG AUC (r = 0.957 – 0.990, all $P < 0.001$), iAUC (r = 0.745 – 0.924; all $P < 0.05$) and peak TAG values (r = 0.930 – 0.974, all $P \leq 0.001$). Age, weight, BMI and body fat % were not found to correlate with any of the TAG measures, however, estimated VO_2max correlated with several. VO_2max showed an inverse correlation with fasting TAG concentrations and TAG AUC for all four trials (r values ranged from -0.713 to -0.943, all $P < 0.05$). Despite these significant correlations between measures of TAG and VO_2max, no relationship was found between

VO_2max and the magnitude of the reduction in TAG iAUC with walking, expressed in either absolute or percentage terms, for either meal ($P > 0.05$ for all). The difference between exercise and control responses for high-fat and moderate-fat meals was not correlated for either total TAG AUC ($P = 0.506$) or incremental TAG AUC ($P = 0.713$), indicating that the change in TAG AUC with exercise was not consistent for individuals across meals.

3.3.3.2. NEFA

Main effects and trend analysis

Plasma NEFA concentrations are depicted in **Figure 3.4**. A trend was observed for NEFA to be higher in the exercise conditions than in the control trials ($P = 0.078$), however this did not reach significance. Plasma NEFA concentration changed considerably during the postprandial period and a main effect of time was manifest ($P < 0.001$). A significant effect of meal was clearly visible ($P < 0.001$), with NEFA concentrations substantially higher after a high-fat meal than a moderate-fat meal. Additional to this, the shape of the NEFA curves was different between the high-fat and moderate-fat meals and a strong meal x time interaction was found ($P < 0.001$). All conditions saw an initial reduction in concentration compared to fasting values, however, NEFA concentrations reached a nadir after 1 h in the high-fat trials before rebounding to above baseline by 4 h, whereas NEFA concentrations continued to decline until 3 h following the moderate-fat meals and did not return to baseline values within 6 h. The slope of the increase in concentration (after the early fall) was much steeper for the high-fat trials than the moderate-fat trials and 97% of the variation within the meal x time interaction was attributable to a linear component ($P = 0.001$). Whilst only slightly elevated, NEFA concentrations remained higher for walk than control, after the moderate-fat meal, at all time-points. NEFA concentrations were raised until 4 h after the high-fat meal in the

exercise condition, relative to control, but this situation was reversed at the 5 h and 6 h time-points. The greater rate of increase in plasma NEFA during the late postprandial period in the control high-fat trial, compared to the walk high-fat trial (culminating in a crossing over of the lines on the NEFA vs. time graph), can explain the finding of a significant three-way interaction (exercise x meal x time; $P = 0.045$).

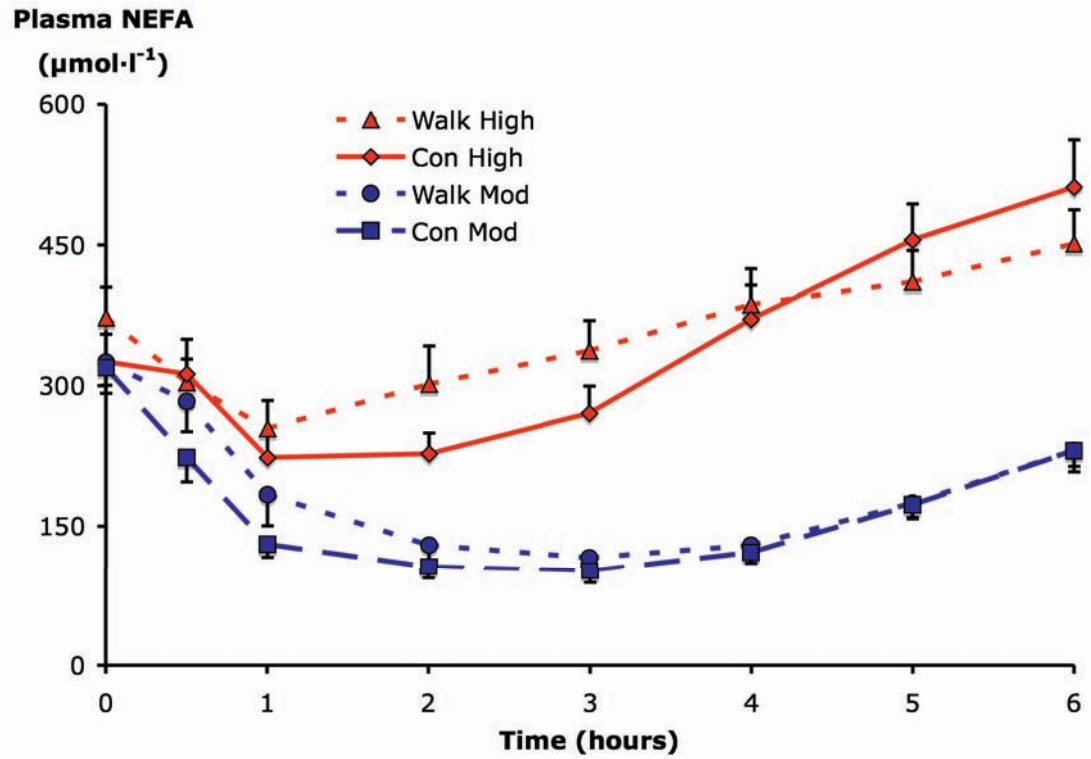


Figure 3.4. Mean (± SEM) plasma NEFA concentrations in the fasted state and for 6 h after consumption of a high fat meal (High; red lines) or moderate fat meal (Mod; blue lines) with either prior exercise (Walk; dashed lines) or prior rest (Con; solid lines).

3.3.3.3. Glucose

Main effects and trend analysis

Plasma glucose concentrations are illustrated in **Figure 3.5**. Whilst plasma glucose concentrations did change significantly during the postprandial period (effect of time; P = 0.015), no significant main effects of exercise or meal were found, nor were there any significant interactions between main effects (P > 0.05 for all). As 78% of the variation in glucose concentration across time was explained by linear (33%; P = 0.034) and quadratic factors (45%; P = 0.010), this suggests a curvilinear shape for the change in plasma glucose over the postprandial period.

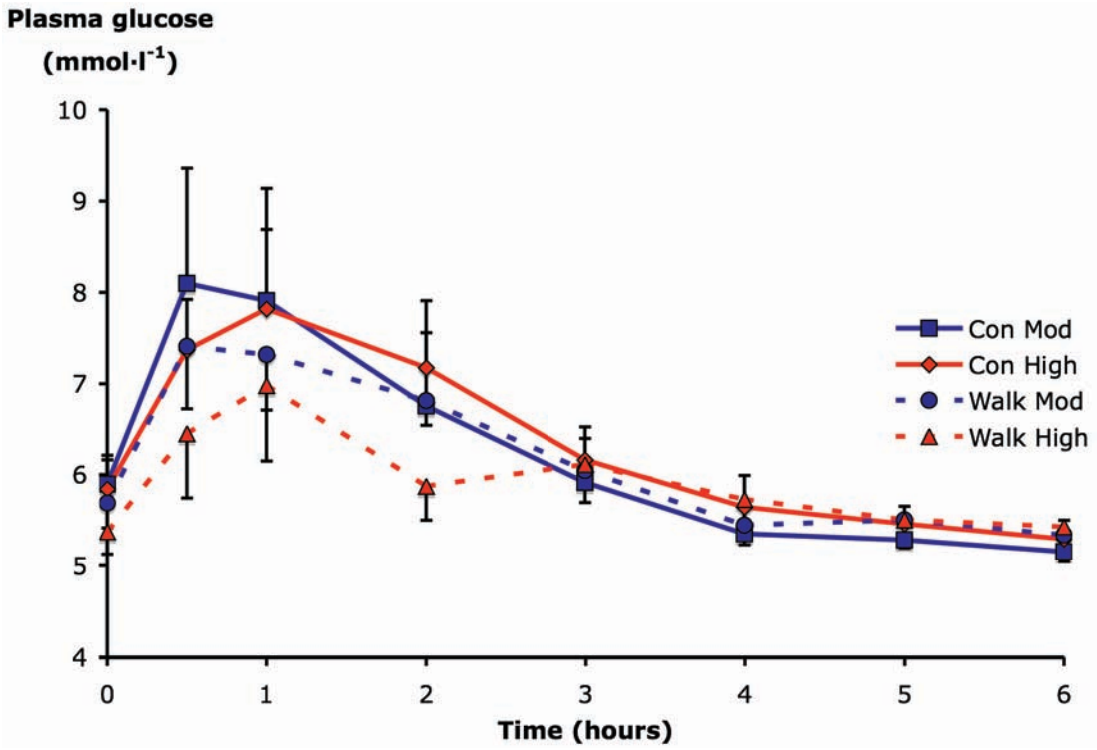


Figure 3.5. Mean (± SEM) plasma glucose concentrations in the fasted state and for 6 h after consumption of a high fat meal (High; red lines) or moderate fat meal (Mod; blue lines) with either prior exercise (Walk; dashed lines) or prior rest (Con; solid lines).

3.3.3.4. Insulin

Main effects and trend analysis

Serum insulin concentrations are displayed in **Figure 3.6**. Main effects of exercise (P = 0.019), meal (P = 0.032) and time (P = 0.003) were found for postprandial insulin concentrations. Insulin concentrations were lower with walking than control trials and were lower with high-fat than moderate-fat meals. Insulin concentrations rose sharply in the early postprandial period for all four conditions then steadily decreased towards baseline by 6 h; a quadratic function accounted for 78% of the variation in insulin concentration across time (P = 0.004). A significant exercise x time interaction was present (P = 0.023), with insulin concentrations peaking earlier in walking trials than control trials (1 h vs. 2 h). Hence for this interaction, 32% of the variation was explained by a quadratic component (P = 0.023). Although no significant interaction was found between the main effects of meal and time (meal x time interaction; P = 0.093), a significant quadratic component of the interaction (P = 0.029), explained 65% of the total variation. This quadratic meal x time interaction relates to the much shallower arc of the insulin concentration curves seen for the high-fat meals relative to the moderate-fat meals.

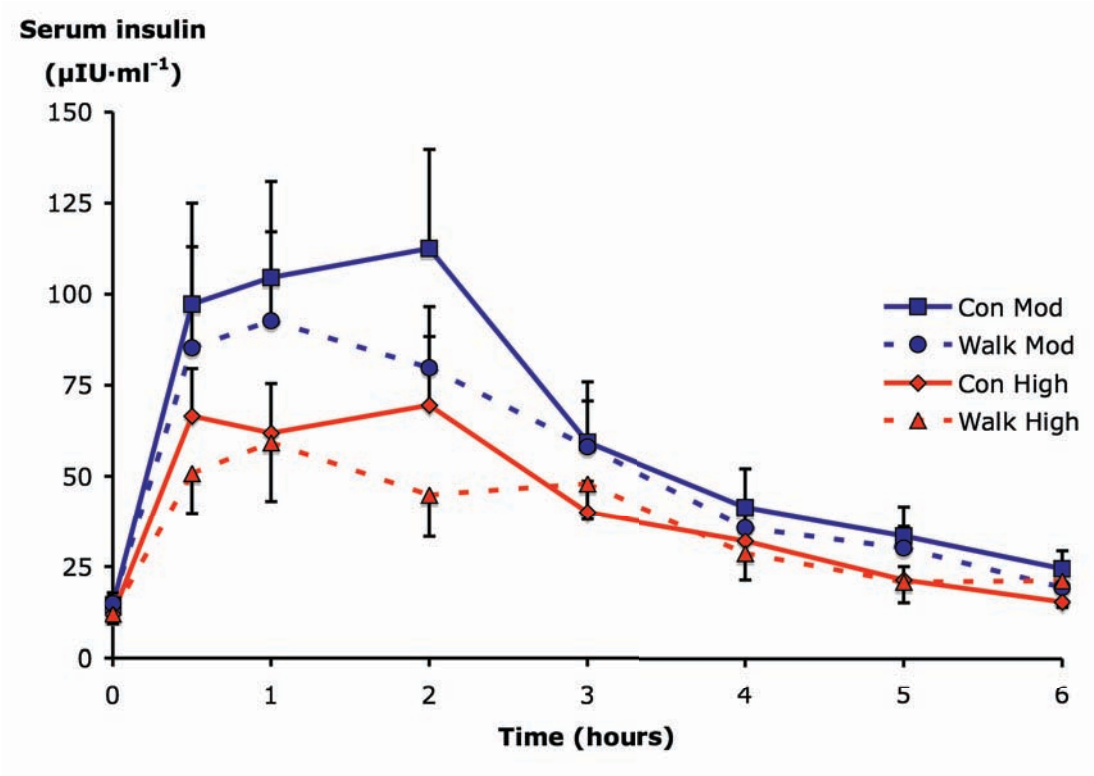


Figure 3.6. Mean (± SEM) serum insulin concentrations in the fasted state and for 6 h after consumption of a high fat meal (High; red lines) or moderate fat meal (Mod; blue lines) with either prior exercise (Walk; dashed lines) or prior rest (Con; solid lines).

3.3.3.5. Resting fat oxidation

Two of the eight study participants were consistently found to have respiratory exchange ratios (RERs) > 1 while at rest, both before and after meal intake. If stoichiometric equations are to accurately calculate resting rates of fat oxidation from indirect calorimetry measures, RER must reflect the respiratory quotient (RQ) during the period when expired gases are collected. The observed RER values > 1 suggest that this was not the case for these two individuals, with doubt therefore cast on the accuracy of resting expired gas measures for all the study participants.

The mouthpiece through which participants must breathe is relatively large and can be somewhat difficult to fit in the mouth. As the apparatus used to collect samples of expired air is not widely used outside of research settings, the men in the study are unlikely to have come across it previously. The awkwardness of the equipment, combined with the novelty of the situation, may have combined to cause hyperventilation; a possibility which appears likely given the very large expired air volumes produced by the two participants with RERs > 1. For the reasons outlined above, measures of fat oxidation, carbohydrate oxidation and resting energy expenditure were not assessed for statistical significant differences between trials. Resting RER values > 1 were found in all four studies, therefore subsequent experimental chapters do not include results for resting expired gas measures.

3.4. DISCUSSION

The main finding of this study was that a brisk 90-minute treadmill walk reduced the postprandial lipaemia seen after meals of both high and moderate fat content to very similar extents in percentage terms, relative to a control trial for each meal. We believe ours is the first study to report such findings within the same group of subjects. Additionally, we believe this is the first study to demonstrate that isoenergetic meals of high and moderate fat content exert distinct effects upon the plasma TAG concentration observed at different stages during the postprandial period, and that the differential time courses of postprandial lipaemia after these meals can be seen to diverge further when the added stimulus of prior exercise is present. Thirdly, this study has shown that in a group of eight middle-aged, overweight, inactive men, the total and incremental lipaemic responses to a moderate-fat meal are not significantly different from those seen after ingestion of a high-fat meal. This finding was consistent regardless of whether prior exercise was performed or not.

The current study was designed such that a conclusive answer could be reached regarding the question of whether prior moderate-intensity exercise lowers the postprandial lipaemia brought on by a moderate-fat meal as effectively as the postprandial lipaemia seen after a high-fat meal. Our data support the idea that a single brisk walk, performed on the evening before a meal containing either a moderate or high percentage of energy as fat, will attenuate fasting and postprandial TAG concentrations. The lack of an exercise x meal interaction, and the very similar percentage reductions in total and incremental lipaemic response for the two meals (relative to their respective control trials), suggests that the effect of exercise is not diminished when the test meal which is used to induce postprandial hypertriacylglycerolaemia is moderate, rather than high, in fat-derived energy. The fact that the incremental lipaemic response was substantially and significantly reduced after walking (with no exercise x meal interaction), is evidence that prior exercise exerts a true postprandial effect in limiting the increase in plasma TAG concentrations which was seen after both moderate- and high-fat meals.

With respect to the 6 h period after ingesting the test meals, TAG concentrations increased over time in walking trials, but the increase was not as large as in control trials. The smaller increase after exercise could reflect an increased clearance rate of TAG from the plasma, a decrease in secretion of VLDL-TAG from the liver, or even a reduced rate of appearance of chylomicron-TAG into the circulation. Based on our data, it is not possible to elucidate which of these possible mechanisms was of greatest importance in mediating the exercise-derived lowering of plasma TAG, although the 13 h delay between the end of the treadmill walk and the ingestion of the test meals would perhaps make it less likely for an attenuated

rate of appearance of chylomicron-TAG to be a substantial factor. The available evidence suggests that the lowering of postprandial TAG concentrations after moderate exercise is most likely to result from a reduced secretion of VLDL-TAG into the plasma (Gill, 2004).

While ingestion of moderate-fat meals did not result in lower postprandial lipaemia than seen with high-fat meals, there were differences in the shape of the TAG curves with the two meals. Essentially, the high-fat response showed an increase, peak and decline within the 6 h period of blood sampling, whereas the moderate-fat response displayed a near linear increase. Although individual subjects may produce two or three TAG peaks in response to a meal (Cohn et al., 1988; Kashyap et al., 1983; Olefsky et al., 1976), and one study has shown a biphasic response of mean plasma TAG concentration to a single mixed meal (Zampelas et al., 1994), studies where samples have been collected for more than 6 h, after exercise and control trials, have not shown peak TAG concentrations occurring after 6 h for a high-fat meal (Gill et al., 2001b; Gill et al., 2004; Gill et al., 2007; Miyashita et al., 2006; Zhang et al., 1998; Zhang et al., 2004; Zhang et al., 2006; Zhang et al., 2007). Therefore it appears sensible to conclude that TAG concentrations would continue to fall at time-points beyond 6 h of high-fat meal intake in this study. The rate of TAG increase appears to be reduced in the moderate-fat meal trials towards the end of the 6 h postprandial period, however, as peak values were recorded at 6 h, it cannot be said with certainty that TAG concentration would not have continued to increase if further samples had been collected. Studies in which blood samples have been collected beyond 6 h after moderate-fat meal ingestion have shown peak TAG values at 5 – 6 h (Kokalas et al., 2005; Kolifa et al., 2004); TAG concentration was lower at 8 h than 6 h in both studies. The TAG concentration in the late postprandial period may be of pathophysiological importance due its positive correlation with a surrogate measure

of early atherosclerosis, the intima-media thickness of the common carotid artery (Karpe et al., 1998). Therefore, the observation made within this study of an elevated TAG concentration late in the postprandial period after a moderate-fat meal, could have clinical relevance. Addition of carbohydrate to a fat load has previously been shown to slow gastric emptying and produce a later peak in plasma TAG concentration (Cohen and Berger, 1990; Westphal et al., 2002); our data illustrate that when two isoenergetic meals with different carbohydrate, fat and protein contents are consumed, peak TAG concentration is delayed after the meal with the higher carbohydrate content.

Although exercise reduced the rate of TAG increase, and meal type appeared to impact the time to reach peak values, it is unclear whether exercise affected the response to one meal more than the other with respect to the TAG time course. When TAG concentrations across the postprandial period are viewed in graphical form, exercise can be seen to affect the shape of the TAG curves differently. Incremental TAG concentrations (**Figure 3.2**) initially show a very similar increase after a high-fat meal in the walk and control conditions; it is not until 3 h post-meal that the curves begin to diverge. Therefore the difference in the incremental lipaemic response to a high-fat meal is almost exclusively due to differences occurring in the late postprandial period. In contrast, exercise did not alter the time at which TAG peaked after a moderate-fat meal, and the incremental TAG curves do not branch sharply from one another at any point; instead, they separate steadily across the 6 h postprandial period. In the high-fat meal trial, NEFA concentrations increased more sharply from 3 h to 6 h in the control than the exercise trial (the curves cross over resulting in the 3-way interaction seen in **Figure 3.4**). This may be indicative of an increase in fatty acid oxidation, or a reduction in spillover of LPL-derived fatty acids, both of which could result in a lowering of plasma TAG

concentrations through a reduction in VLDL-TAG secretion. The same pattern is not seen in NEFA concentrations over time for the moderate-fat meal trials; therefore this could represent a branch point between the meals regarding the effect of prior exercise. It should be noted, however, that only a nonsignificant trend for a three-way interaction was found; the speculations made here require investigation with a larger group of participants (and, ideally, measures of fatty acid/TAG kinetics in adipose tissue and skeletal muscle) before they can be considered deserving of wider recognition.

The greater NEFA response in the high-fat meal trials agrees with previous data regarding the response to isoenergetic meals containing different quantities of carbohydrate and fat (Whitley et al., 1997). Regarding possible mechanisms, the lower NEFA response after the moderate-fat meals may be due to reduced adipose tissue lipolysis. Insulin is known to inhibit adipose tissue lipolysis (Coppack et al., 1994) and as the insulin response in our study was greater for moderate-fat meals than high-fat meals, the degree of suppression of adipose tissue lipolysis is also likely to have been greater. Additionally, infusing insulin along with a high-fat mixed meal has been shown to increase reesterification of LPL-derived fatty acids in adipose tissue compared to when the meal was ingested alone (Frayn et al., 1994), therefore offering a second mechanism through which the moderate-fat meals could result in lower postprandial NEFA concentrations. Insulin may also slightly lower the activity of lipoprotein lipase (LPL) within skeletal muscle (Farese Jr., 1991), therefore reducing hydrolysis of circulating TAG. A reduction in the breakdown of TAG passing through skeletal muscle capillaries could theoretically reduce spillover of exogenous fatty acids. This may not occur in practice, however, as trapping of fatty acids released when TAG is cleaved by LPL within the vasculature of skeletal muscle, has been reported as close to 100% in the 6 h period after

mixed-meal ingestion (Evans et al., 2002). The fat load of this meal was lower than either of the meals given in the current study; therefore it is not clear whether fatty acid spillover from skeletal muscle would be increased if a greater fat load were present. Due to the lack of kinetic data in our study, we are unable to estimate the contribution of these three mechanisms to the lowered plasma NEFA response after moderate-fat meal intake.

Fasting glucose concentrations were modestly, but significantly, reduced for walking trials, however this did not translate into an improvement in insulin sensitivity as calculated from fasting glucose and insulin values using the updated homeostasis model assessment (Levy et al., 1998). Fasting insulin concentrations and HOMA scores were significantly higher for trials where a moderate-fat meal was about to be ingested (both $P = 0.048$), however, as no test meal had actually been eaten at this point, the reason for this difference is unclear. Some participants will have been aware that they were about to eat a moderate-fat meal (containing more carbohydrate) on a particular day, due to the fact that the trial order was counter-balanced and half of the men would therefore have to eat a moderate-fat meal during their last trial. This in itself should not have affected fasting insulin concentrations however, as there does not appear to be a cephalic insulin response brought on when anticipating food intake (Karhunen et al., 1997) and the magnitude of the insulin response immediately after eating (before glucose has entered the blood) is similar for carbohydrate- and fat-rich meals (LeBlanc et al., 1996). An alternative explanation concerns the pulsatility of insulin in the fasted state. Insulin is released in a pulsatile manner, with short-term high-frequency pulses occurring every 6 - 15 minutes (Goodner et al., 1977; Hunter et al., 1996; Juhl et al., 2002; Lang et al., 1979). As only a single blood sample was taken in the fasted state for each subject during each trial in our study, it is possible that the fasting serum insulin concentration

could be at the top of a peak before a moderate-fat meal (following a recent pulse of insulin), but at the bottom of a trough before high-fat meal intake. Drawing three fasting blood samples (spaced 5 minutes apart) in which to measure insulin concentration, may have prevented what we believe to be a chance finding.

Postprandial glucose concentrations were not different between walking and control, but, as postprandial insulin concentrations were significantly lower for the walking trials (for both meals), it is quite possible that postprandial insulin sensitivity was favourably altered in the exercise trials. However, we found no relationship between any measures of insulin and TAG (all correlations, $P > 0.05$), and when retrospective analysis of data sets from several studies was conducted, the exercise-induced changes in TAG and insulin (in both the fasted and postprandial states) were not found to be related (Gill et al., 2002b). As might be expected (due to the greater carbohydrate content), postprandial insulin concentrations were higher after the moderate-fat than the high-fat meals, a finding supported by Whitley et al. (1997) who found step-wise increases in the 5 h incremental AUC for insulin with increasing carbohydrate content.

Differences in incremental lipaemic response between meals

As alluded to earlier in this discussion, the observation that intake of high-fat meals did not result in larger incremental TAG responses than ingestion of moderate-fat meals, may have been due to the low sample size in the study (with 14 participants the response was predicted to be different between meals). However, while the moderate fat meal contained only ~53% of the fat-derived energy of the high fat meal, it was found that without prior exercise, the rise in plasma TAG concentration (across the 6 h postprandial period, relative to baseline) after

the moderate fat meal, was 77.5% of the rise seen with the high fat meal. When prior exercise was performed, a near identical situation was observed, with the increase in TAG after the moderate-fat meal being 74.6% of the high-fat meal rise. Our finding that the incremental TAG response to the moderate-fat meals appeared to be proportionally greater than the response to the high-fat meals (relative to the dietary fat load given) is supported by data from Whitley et al. (1997) which showed no difference in incremental TAG response to isoenergetic meals containing 44.5, 65.2 and 73.9 % of energy as fat. Similarly, when the incremental TAG AUC was calculated after virtually isoenergetic meals with 91.2% (meal 1) and 55.6% (meal 2) of energy as fat, TAG concentrations were significantly increased after both meals, but there was not a statistically significant difference between trials (Pedrini et al., 2006). An earlier study, in type 2 diabetics, reported that the postprandial TAG response to a mixed meal containing 25% of energy as fat was only marginally, and non-significantly, less than that to an isoenergetic meal containing 45% fat (Chen et al., 1992). The protein content of the two meals given by Chen et al. was kept constant (15% of energy), therefore suggesting that the higher carbohydrate content of the lower fat meal may have been a factor in preventing an attenuated lipaemic response. Other authors have found positive correlations between the quantity of fat ingested and either the resulting postprandial lipaemia (Cohen et al., 1988a; Dubois et al., 1994; Dubois et al., 1998) or the peak TAG concentration, (Murphy et al., 1995), however, the meals given in these studies were not isoenergetic and therefore the impact of the greater energy intake with the higher-fat meals cannot be discounted as an influencing factor in the more pronounced lipaemic response. If the energy content of two meals is kept balanced, the available evidence suggests that postprandial lipaemia will not be different between the two meals, even if the fat content of one is higher. Such findings would probably not extend to an investigation where the lipaemic response to isoenergetic meals

with radically different fat contents was compared, e.g. 90% fat vs. 10% fat, however, as most free-living individuals do not eat substantial meals containing as little as 10% fat or as much as 90% fat on a regular basis, the worth of performing such a study would be extremely questionable.

The lack of kinetic data in the present study precludes in-depth explanation of the mechanisms responsible for an increase in postprandial TAG concentration out of proportion with the intake of dietary fat, however we can draw on evidence from other authors to outline potential reasons for our finding. One possible mechanism, which could explain why plasma TAG concentrations after the moderate-fat meals were higher than expected relative to the fat load given, is reesterification of NEFA. Plasma NEFA concentrations were markedly reduced after both moderate-fat meals compared with high-fat meals. While this may largely be explained by the observation of a greater insulin response after the moderate-fat meals, thereby inhibiting hormone sensitive lipase and blocking lipolysis from adipose tissue to a greater extent (Coppack et al., 1994), it is also conceivable that reesterification of NEFA by the liver was increased after the moderate-fat meals. Insulin acutely increases mitochondrial GPAT activity in perfused rat livers (Bates et al., 1977), creating a scenario which would directly favour esterification. Insulin also increases concentrations of acetyl-CoA carboxylase (ACC), which in turn enhances production of malonyl-CoA (Zammit, 1996). Malonyl-CoA inhibits carnitine palmitoyl transferase 1 (CPT 1), with CPT 1 responsible for the conversion of acyl-CoA to acylcarnitine and the latter's subsequent entry into the mitochondrial matrix to undergo ß-oxidation. Pathways therefore exist through which insulin can directly and indirectly (secondary to reducing hepatic fatty acid oxidation) increase reesterification of TAG within the liver, although the extent to which this occurs *in vivo* is not known. Whole-

body substrate oxidation rates are known to be substantially affected by the type of macronutrient that has recently been ingested, with step-wise reductions in whole-body fat oxidation as carbohydrate content of a mixed meal is increased (Whitley et al., 1997; Stiegler et al., 2008). If findings seen on a whole-body level reflect fat oxidation in the liver, then it is possible that fatty acids taken up by the liver after a moderate-fat meal may be partitioned into reesterification pathways rather than oxidised. Studies in type 2 diabetic individuals have compared the effect of 2-week (Chen et al., 1993) and 6-week diets (Chen et al., 1995), with differing macronutrient compositions, on postprandial lipaemia, using a randomised crossover design. A diet in which fat contributed 30% of total energy (55% carbohydrate and 15% protein) was found to increase postprandial lipaemia when compared with a diet containing 45% of energy as fat (40% carbohydrate and 15% protein) (Chen et al., 1993). Similarly, 6-week diets containing the same ratios of macronutrients showed that the higher carbohydrate diet led to greater postprandial accumulation of intestinally-derived lipoproteins and an increased production of VLDL-TAG (Chen et al., 1995). These studies provide support for the notion that dietary carbohydrate increases postprandial TAG concentrations. As an alternative mechanism, clearance of plasma TAG into skeletal muscle may have been down-regulated after the moderate-fat meals, relative to the high-fat meals. Clearance of plasma TAG within lipoprotein particles is reliant on hydrolysis by the enzyme lipoprotein lipase (LPL). LPL is present in skeletal muscle, adipose tissue and the heart, and must translocate to the luminal surface of the capillary in order to allow TAG to be broken down and enter the cell. Contrary to observations in adipose tissue, skeletal muscle LPL activity has been shown to be lower in humans during the postprandial period than when fasting (Lithell et al., 1978). Infusion of insulin exerts a suppressive effect on the activity of lipoprotein lipase within skeletal muscle in humans (Farese Jr., 1991) and data from studies in rats have shown that

feeding a high-carbohydrate meal causes the same response, with the effect also directly attributable to the increase in insulin (Picard et al., 1999). As the insulin response to the moderate-fat meals was greater than the response to the high-fat meals, it is not beyond the realms of possibility to suggest that the increased insulin concentrations may have lowered skeletal muscle LPL activity and therefore reduced the uptake of TAG from the plasma pool by skeletal muscle. This potential mechanism requires much greater investigation, however, as human skeletal muscle LPL activity has also been shown to increase after a meal (Yost et al., 1998) and was unchanged after an insulin infusion in another study (Perreault et al., 2004). Furthermore, the activity of LPL within adipose tissue is consistently increased after insulin infusion or feeding (Lithell et al., 1978; Farese Jr., 1991; Yost et al., 1998), therefore any reduction in skeletal muscle LPL activity after the moderate-fat meals may be offset. A third potential mechanism through which postprandial TAG concentrations could be increased out of proportion with the intake of dietary fat, i.e. after a moderate-fat load, is by *de novo* lipogenesis (DNL). As the majority of the energy in the moderate-fat meal came from carbohydrate (47% of total energy), it is possible that hepatic synthesis of fatty acids from plasma glucose was upregulated, with the newly synthesised fatty acids becoming incorporated into VLDL-TAG. Quantitative determination of TAG concentration within different lipoprotein species did not take place during this investigation (only measurement of total plasma TAG concentration), however, previous literature has documented that the absolute rise in plasma TAG concentration after high-fat meals (Malkova et al., 2000; Gill et al., 2001a) and moderate-fat meals (Potts et al., 1994) is as attributable to an increase in VLDL-TAG as it is to an increase in chylomicron-TAG. Theoretically, therefore, it is possible that a meal rich in carbohydrate could increase plasma TAG concentrations out of proportion to the fat content of the meal, due to increased *de novo* lipogenesis bringing about

an increase in VLDL-TAG concentrations. While this may have been the case in the present study, the extent to which any rise in DNL after a carbohydrate-rich meal would increase plasma TAG concentrations is likely to be very minor. Indeed, even when mean rates of DNL are increased 45-fold, as seen after 4 days of carbohydrate hyperalimentation (Aarsland et al., 1996), the percentage of de novo synthesised fatty acids within plasma VLDL-TAG is still not more than 20 - 25% of the total from all sources. There is evidence to suggest that overweight men have higher fasting rates of DNL than lean men, and that DNL is significantly increased compared to baseline in the hours after a carbohydrate-rich meal (Marques-Lopes et al., 2001), however the carbohydrate load given in the study of Marques-Lopes et al. was far in excess of that given in the moderate-fat meal of this study.

Correlational data showed that VO_2max was inversely related to several TAG measures. The idea that subjects with higher cardiorespiratory fitness would have lower fasting and postprandial TAG concentrations is certainly logical, however this relationship may not be as clear-cut as it seems. To ensure the exercise bouts within the study were of a moderate intensity for all subjects, the treadmill walks were performed at 60% of *individual* VO_2max. This meant that subjects with a higher VO_2max tended to work at a higher absolute intensity and gross energy expenditure was greater for these individuals. Significant positive correlations were observed between VO_2max and the energy expenditure of the two walks ($P < 0.05$ for both), and as the energy expenditure of the exercise session is known to influence the magnitude of the reduction in lipaemia (Gill et al., 2002a; Tsetsonis and Hardman, 1996b) it is possible that the greater energy expenditure of the fitter participants is partially responsible for their lower lipaemia in the walking trials. The lack of a correlation between VO_2max and the extent of the reduction in lipaemia after walking, for either meal, is

encouraging from a public health perspective however, as it suggests that even the participants with very poor fitness were able to lower their TAG concentrations after a single walk. The positive correlation between VO_2max and the fasting/postprandial TAG measures in the control conditions suggests that overall fitness does however exert some influence on lipid metabolism.

The energy content of the high-fat meal in this study was guided by work previously published in the area of exercise and postprandial lipaemia, with energy from each macronutrient provided per kg of body mass. However, as the subjects used in this study were heavier than in most previous work, the energy provided by the meals was also greater. Indeed, the meals provided approximately 61% of the recommended daily energy intake for an average man. We acknowledge that the calorific content of the meals in this study was high and this should be considered when drawing comparisons with other work, however it should also be noted that, regardless of the high energy counts, none of the men failed to finish the meals. Whilst we believe the percentage of energy from fat used in the lower-fat meal in this study (35%) is justifiably moderate, the actual quantity of fat present in this meal was higher than in previous studies using moderate-fat meals, due to the greater overall energy content. Theoretically, it could be argued that a threshold intake of dietary fat exists, above which, additional fat intake makes little impact on plasma TAG concentrations over a 6 h period, due to limitations in the ability of the gastrointestinal tract to absorb the extra TAG. Although rate of appearance of dietary TAG was not measured in this study, our data offer little support for this theory, as the time at which peak TAG values were observed was not delayed with the high-fat meal, rather, the peak occurred earlier than with the moderate-fat meal. As there were 24 possible orders in which the four investigative trials could be

completed, and only 8 participants, we were not able to totally counterbalance the study. It was decided not to entirely randomise the order in which the participants completed their four trials, as this could have resulted in several participants following the exact same order of trials, which would have made it difficult to separate the effect of exercise/meal from the effect of trial order. Instead, we ensured that each of the four trials was completed in each of the four serial positions, i.e. there were always two participants completing each of the four trials first, second, third and fourth in time order. Our design was not that of a perfect latin square, and, with the benefit of hindsight, a latin square would perhaps have been the best design to use with respect to deciding the order of trials. The fact that trial order was not completely counterbalanced is a potential limitation of this study.

In conclusion, a 90-minute brisk walk was found to reduce the postprandial lipaemia associated with meals of both high and moderate fat content. The percentage reduction in postprandial lipaemia with walking was very similar for the two different meals when compared with control trials. Surprisingly, TAG concentrations were not significantly lower after a moderate-fat meal was ingested instead of a high-fat meal. The time course of changes in plasma TAG was, however, different between meals, with high-fat meals tending to prompt an earlier TAG peak. These findings could be interpreted as evidence that, when a meal contains a substantial quantity of other macronutrients (particularly carbohydrate), it does not require a substantial dietary fat load to elevate plasma TAG concentrations in a group of middle-aged, overweight, inactive men. In addition, exercising before a meal may be a more promising intervention to offset postprandial lipaemia than reducing the fat content of the diet.

CHAPTER FOUR

The effect of two different resistance exercise sessions on postprandial lipaemia

ABSTRACT

Elevated plasma triacylglycerol (TAG) concentrations during the postprandial period correlate positively with development of atherosclerosis and coronary heart disease. Performing aerobic exercise prior to intake of a high-fat meal has been shown to reproducibly lower postprandial lipaemia, however contradictory findings exist regarding the effectiveness of resistance exercise in reducing postprandial TAG concentrations. This study aimed to investigate whether performing low-intensity, high-repetition resistance exercise with short recovery (Light Ex; designed to evoke a more substantial aerobic component than previous studies) would reduce postprandial lipaemia to a greater extent than high-intensity, low-repetition resistance exercise with near-full recovery (Heavy Ex). Eight, inactive, males, aged 42.4 ± 11.9 years, with BMI 29.5 ± 3.5 (mean ± S.D.) completed three separate oral fat tolerance tests (66% of energy given as fat) following either Heavy Ex, Light Ex or seated rest (Control) the previous evening. Both exercise protocols comprised 3 sets of 12 different resistance exercises, but differed in load lifted per repetition, recovery time and number of repetitions performed per set. Heavy Ex involved 8 repetitions per set, at 100% of predetermined 10-repetition maximum (10-RM), with 2-minute recoveries between sets and exercises; during Light Ex, 20 repetitions were performed per set, at 40% of 10-RM, with 40-second recoveries. Protocols were matched for total weight lifted during the session. Fasting venous blood samples were drawn on the morning after exercise or rest, with additional samples collected hourly for 6 h following meal ingestion. Fasting plasma TAG concentrations (mean ± S.E.M.) were not different between conditions (Heavy Ex, 1.05 ±

0.13 mmol·l^{-1}; Light Ex, 1.08 ± 0.11 mmol·l^{-1}; Control, 1.08 ± 0.16 mmol·l^{-1}) and total area under the TAG versus time curve was not significantly lowered after either exercise session relative to Control (Heavy Ex, 11.2 ± 1.4 mmol·l^{-1}·6 h^{-1}; Light Ex, 11.6 ± 1.3 mmol·l^{-1}·6 h^{-1}; Control, 12.1 ± 1.6 mmol·l^{-1}·6 h^{-1}). Performance of resistance exercise 14 - 15 h prior to intake of a fat-rich meal did not lower postprandial TAG concentrations in untrained subjects regardless of the intensity of the preceding exercise bout.

4.1 INTRODUCTION

Increased non-fasting measures of TAG are associated with greater risk of heart attack and death (Nordestgaard et al., 2007), and elevated concentrations of TAG late in the postprandial period after a fat load have been shown to correlate with a marker of early atherosclerosis (Karpe et al., 1998). If progression from early atherosclerosis to coronary heart disease (CHD) is to be avoided, approaches which tackle the threat exerted by repeated episodes of exaggerated postprandial lipaemia are required. Therefore, interventions which are low in cost, pose minimal risk of side effects from their implementation, and are both readily available and appealing to the general public, are sought.

One such intervention that has received growing attention over the past 15 years is aerobic exercise. Many studies have now investigated the effect of a single moderate-intensity aerobic exercise bout, performed before ingestion of a high-fat meal, on plasma TAG concentrations. The clear message from these studies is that aerobic exercise can substantially attenuate postprandial lipaemia, with the timing of the exercise (relative to consumption of the test meal), and the energy expended during the session, identified as

factors which mediate the extent to which plasma TAG concentrations are altered postprandially (Gill & Hardman, 2003; Gill, 2004; Petitt & Cureton, 2003).

Although brisk walking is demonstrably very effective in reducing postprandial lipaemia, it is unlikely that it will appeal to all sections of the population as a form of exercise and some will prefer resistance exercise. Furthermore, there will be a proportion of the general public who are unable to perform a brisk walk or who are advised against doing so due to contraindications with an existing medical condition. For these people, it is important to determine whether alternative physical activity interventions, such as resistance exercise, are also capable of improving postprandial lipid metabolism. In contrast with studies using aerobic exercise, which overwhelmingly show a beneficial effect on postprandial lipaemia, studies using resistance exercise have produced conflicting findings. Of the eight published studies, five report resistance exercise to be beneficial (Burns et al., 2007; Pafili et al., 2009; Petitt et al., 2003; Singhal et al., 2009; Zafeiridis et al., 2007), two report no effect of a resistance exercise session on postprandial lipaemia (Burns et al., 2005; Shannon et al., 2005) and one study found postprandial TAG concentrations to be significantly higher than a control trial after performing resistance exercise (Burns et al., 2006). The first known study to investigate the effect of resistance exercise on postprandial lipaemia found that baseline and postprandial TAG concentrations were lowered, and resting fat oxidation was increased, 16 hours post-exercise (Petitt et al., 2003). The study of Petitt et al. was unusual however, as a 90-minute moderate-intensity aerobic exercise bout conducted as part of the research, with equivalent EE to the resistance exercise session, was found to have no effect on postprandial lipaemia. This finding goes against a large body of literature which has shown a reproducible beneficial effect of aerobic exercise on postprandial TAG concentrations. Burns

et al. (2005) conducted a study which largely replicated the protocol of Petitt et al., but with the EE of the session increased slightly to ensure an effect would be detected if really present. Burns et al. (2005) found that a single session of resistance exercise did not significantly reduce postprandial lipaemia. The authors suggested that because their participants were unaccustomed to resistance exercise (unlike the trained weightlifters used by Petitt et al.) this would lead to skeletal muscle damage, in turn leading to temporary insulin resistance and resulting in potential counteraction of the beneficial effect of exercise on lipaemia through a down-regulation of skeletal muscle lipoprotein lipase (LPL) activity. However, if anything, the data of Burns et al. (2005) suggest that insulin sensitivity was greater after exercise than control and another study by the same group, which found that postprandial TAG concentrations were increased after exercise compared with a control trial, used resistance-trained individuals who would presumably have experienced much less muscle damage (Burns et al., 2006). In contrast, a recent study which used an eccentric resistance exercise protocol previously shown to cause muscle damage, found that postprandial lipaemia was significantly reduced by exercise when using a participant population of men who had not taken regular resistance exercise for at least 12 months (Pafili et al., 2009). The study of Pafili et al. does not mention whether the men recruited had a background in resistance exercise training (i.e. whether they had been weightlifters in the past, but had not weight trained during the previous year), and when this study is excluded, there appears to be no published account of a single bout of resistance exercise significantly lowering postprandial lipaemia in a group of untrained individuals. Unlike aerobic exercise, where evidence suggests the EE of the session is somewhat predictive of whether postprandial lipid metabolism will be improved, resistance exercise does not seem to show a strong relationship between EE and postprandial lipaemia. Burns et al. (2005) found no benefit to postprandial

lipaemia of performing resistance exercise, despite the gross EE of their session (~ 2.3 MJ) being greater than that of Petitt et al. (2003) (~1.7 MJ), in which lipaemia was lower after exercise. Zafeiridis et al. (2007) found the total area under the TAG versus time curve to be significantly lower than a control trial on the day after either 2 or 4 sets of 8 different resistance exercises; at just 0.76 MJ, the EE during the 2-set session was lower than any aerobic exercise bout which has been found to reduce postprandial lipaemia significantly. Of the eight studies that have investigated the effect of resistance exercise on postprandial lipaemia, six have used a load which represents a high relative intensity of effort. Petitt et al. (2003) used 10 repetitions (reps) at 10-repetition maximum (10-RM); Burns et al. (2005) used 10 reps at 80% of 10-RM; Shannon et al. (2005) used 10 reps at 75% of 1-RM; Burns et al. (2006) used 12 reps at 80% of 12-RM; Zafeiridis et al. (2007) used 12 reps at 12-RM; and Pafili et al. (2009) used 6 reps at 6-RM. Two studies have opted for extra repetitions at a more moderate intensity: Burns et al. (2007) used 15 reps at 30% of 1-RM for upper body parts and 40% of 1-RM for the lower body, whereas Singhal et al. (2009) had one session where 16 reps were performed at 50% of 8-RM. The session used by Singhal et al. is unlikely to have provided a substantial aerobic workout, owing to the large recovery which participants would have had after completing each set, and while the protocol of Burns et al. (2007) may have been more aerobic in nature, the long duration and large lifting volume of the protocol makes it unlikely to be performed by untrained individuals. Furthermore, despite the protocol of Burns et al. (2007) taking almost 4 hours to complete, with an estimated gross EE of 5.1 MJ, the mean reduction in postprandial lipaemia was modest (12%) and the clinical relevance of a change of this magnitude has yet to be determined. Resistance exercise may not be as reproducible as aerobic exercise in reducing postprandial lipaemia due to the fact that most resistance exercise protocols have been extremely anaerobic in nature. We

hypothesise a circuit type session, which evokes a large aerobic component, is more likely to lower postprandial lipaemia than a standard resistance exercise session with heavy weights and long recoveries, because studies using aerobic exercise have consistently shown positive results. Our study therefore used a low-intensity bout (light load/ high reps/ short rests) as well as a high-intensity bout (heavy load/ low reps/ long rests) of resistance exercise.

In previous investigations of postprandial lipaemia after resistance exercise, the participants used have not been those who are at particular risk of having an exaggerated lipaemic response to a meal. Seven of the eight published studies have used young participants (mean age in all studies between 23 and 28 years), while one study (Singhal et al., 2009) stated that participants were 21 – 36 years but did not provide the mean age. As older people are known to exhibit a greater postprandial TAG response than young people (Jackson et al., 2003), the relevance of using young participants in these studies is not clear when the overall intention of such research is to determine whether CHD risk is ameliorated. All previous work (excluding Pafili et al., 2009) has used individuals who either have a history of resistance exercise training or are recreationally active, but it is those people not taking regular exercise who are at greatest risk of postprandial hypertriacylglycerolaemia. Also, almost all previous research has used populations whose weight-to-height ratio was in the normal range, and the few studies where mean BMI was in the overweight category had participants with body fat percentages in the lean or normal range, suggesting that their classification as overweight was due to extra muscle mass rather than fat. Overweight and obese individuals have been repeatedly shown to have higher TAG concentrations in the postprandial period than lean individuals (Bartual et al., 2006; Gill et al., 2004; Lewis et al., 1990), therefore studies using lean individuals are not likely to examine the effect of resistance exercise under conditions

whereby postprandial lipaemia is a health risk. Furthermore, while young lean active individuals may be able to lower their postprandial TAG response by performing resistance exercise, this does not necessarily mean that older, fatter, more sedentary individuals will derive the same benefit. The well established ability of a brisk 90-minute treadmill walk to lower postprandial lipaemia does not extend to patients with type 2 diabetes (Gill et al., 2007), therefore highlighting the importance of ensuring that exercise interventions are tested on the population groups to whom the highest risk of future disease, and greatest potential benefit of the intervention, applies. As past studies have not contained individuals with several risk factors known to increase their likelihood of displaying a large and prolonged elevation of TAG after intake of a fat-containing meal, this study aimed to only recruit men who met at least two of these three defined risk factors (middle-aged or older, overweight/obese and sedentary).

In summary, this study investigated whether the intensity of resistance exercise affects the ability of men at risk of exaggerated postprandial lipaemia to lower their postprandial TAG response, with particular attention focussed on ensuring that one resistance exercise session was more aerobic than the other. Eight human volunteers underwent three different treatments: a low-intensity resistance exercise session 14 - 15 hours before a high fat (66% fat) meal, a high-intensity resistance exercise session 14 - 15 hours before a high fat meal and a control trial involving a high fat meal without prior resistance exercise. The changes in plasma TAG during the postprandial period were investigated, with concentrations of non-esterified fatty acids (NEFA), glucose and insulin also measured, as changes in the metabolism of these factors postprandially are likely to have an impact, both directly and indirectly, on TAG concentrations.

4.2 METHODS

4.2.1. Participants

Eight, healthy, non-smoking, sedentary men volunteered to take part in the study; **Table 4.1** shows the participants' physical characteristics. None of the men performed more than one hour of structured physical activity per week and resistance exercise was an unfamiliar form of exercise for all. Additionally, none of the participants were suffering from a chronic cardiovascular or metabolic disease (coronary heart disease, stroke, diabetes, hypertension, dyslipidaemia, etc.), nor were they taking any medication known to affect substrate metabolism. Age ranged from 26 – 57 years, although most men were older than 45 years of age. Seven of the eight men were classified as overweight by body mass index (BMI in excess of 25 $kg \cdot m^{-2}$), with one man at the high end of the normal weight range (BMI = 23.9). Body fatness was high within the study group, with all men having body fat percentages above 25%.

Table 4.1.
Characteristics of the participants (n = 8)

	Mean ± S.D.
Age (years)	42.4 ± 11.9
Height (m)	1.72 ± 0.07
Body mass (kg)	88.1 ± 15.0
BMI ($kg \cdot m^{-2}$)	29.5 ± 3.5
Body fat (%)	29.8 ± 3.9

4.2.2. Study design

To investigate the effects of two different resistance exercise sessions on postprandial lipaemia, all participants took part in three distinct 2-day trials. On day 1, the participants performed either: a high-intensity, low repetition resistance exercise session (Heavy Ex); a

low-intensity, high repetition resistance session (Light Ex); or no resistance exercise (Control). On day 2, the participants returned to the laboratory to provide blood samples in the fasted state and during the 6 h period after consuming a high-fat mixed meal. While the format of day 1 was different for the three trials, the protocol on day 2 was identical. With three different trials, there were six possible orders in which the men could complete the study. The trial order was selected at random for participants during the early part of the study, but the order was later counter-balanced to ensure that the results obtained from each treatment were not influenced by the order in which participants completed each trial. All six possible trial orders were used (two of the six orders were necessarily used by two different participants) and no more than three participants ever started the study by taking part in the same trial. Individual trials for each participant were separated by a minimum period of 5 days. **General Methods** (section 2.2.2) explains the restrictions on diet and physical activity prior to the trials.

4.2.3. Preliminary 10-repetition maximum testing

In order to calculate the load to be lifted for each exercise, participants performed a preliminary resistance exercise session to attain a 10-repetition maximum (10-RM) for each of the 12 resistance exercises. 10-RM was defined as the weight which could be lifted exactly 10 times, demonstrating a full range of movement for the given exercise with each repetition, before volitional exhaustion was reached. Trial and error was used to arrive at a 10-RM for each exercise, with weight being added if 10 reps were surpassed, and subtracted if 10 reps were not achieved. Subjects were allowed complete recovery between attempts, and vocal encouragement was used as a motivational stimulus to engender true maximal performances for each exercise.

4.2.4. Exercise trials

Participants arrived at the laboratory at 17:00. The men were fitted with a Polar heart rate monitor (Vantage NV, Polar Electro Oy, Kempele, Finland) and a standard warm-up was completed before each of the two different resistance exercise sessions. As the two sessions were designed to evoke different aerobic components, the warm-up deliberately avoided aerobic exercise. Instead, the participants performed 10 - 12 lunges in the corridor outside the Strength Training Room, then alternated back and forth between the first two exercises used in the main protocol (reclined leg press and chest press), performing 3 sets of 10 reps of each exercise with the load set at less than 40% of 10-RM. This warm-up was designed to produce a modest elevation in heart rate, thus priming the body for the exertions of the session proper, but with minimal effect on aerobic metabolism. After completing the warm-up, participants started the heart rate watch, then immediately began the relevant resistance exercise session, with heart rate being automatically recorded every 5 seconds throughout the sessions. Three sets of 12 different resistance exercises were performed in each of the two exercise sessions, but load, number of reps lifted and recovery time were different, either: 8 reps at 100% of 10-RM, with 2 minutes recovery between sets and exercises (Heavy Ex) or 20 reps at 40% of 10-RM with 40 seconds recovery between sets and exercises (Light Ex), with the sessions matched for the total weight lifted. The twelve stations alternated between working upper and lower body parts, and the specific exercises, in the order they were performed, were: reclined leg press, chest press, leg curl, lat pull-down, calf raise, shoulder press, leg extension, bicep curl, upright leg press, tricep extension, back pull-down, and semi-reclined leg press. The order in which the exercises were completed was always kept the same, with 3 sets performed back-to-back before moving onto a new exercise. The load to be lifted for exercises during Light Ex (corresponding to 40% of 10-RM values determined in the preliminary session) was

occasionally calculated to be a whole number plus a fraction of a pound, e.g. 35.2 lb. As the resistance exercise machines used only allowed loading to be differentiated in one-pound increments, the load was rounded to the nearest whole pound in these circumstances. Following completion of the third set of each exercise, participants were presented with a Borg CR10 scale (Borg, 1982) and were asked to rate the degree of exertion placed upon the working muscles during the exercise they had just finished. Participants were also asked to provide a value from the Borg RPE Scale (Borg, 1982) to give an indication of the overall exertion they experienced during the exercise. Immediately after finishing each session, participants gave a further value from the Borg RPE Scale to provide feedback on how they perceived the exertion required to complete each session in its entirety.

4.2.5. Control trial

Control trials were as described in **General Methods** (section 2.4.2.).

4.2.6. Evening (post-intervention) meal

Participants were provided with an evening meal as described in the **General Methods** (section 2.5). The mean energy (± S.D.) provided by the meal was 4.48 ± 0.62 MJ (1070 ± 147 kcal).

4.2.7. Oral fat tolerance tests

The OFTT protocol is described in the **General Methods** (section 2.6). The test meal contained 1.20g fat/kg body mass, 1.20g CHO/kg body mass and 0.20g protein/kg body mass (68.6 kJ per kg body mass; 66% fat, 29% CHO and 5% protein as percentage of total energy). The test meal provided 6.05 ± 0.36 MJ (~ 1445 kcal) of energy on average and contained 106

± 6 g of fat, of which 54.7% was from saturates. The meal consisted of a drink containing whole milk, double cream and sugar, along with a solid component of hazelnut cereal bars.

4.2.8. Body fat estimation

Percentage body fat was estimated as per **General Methods** (section 2.7)

4.2.9. Analytical procedures

The analytical procedures used are described in the **General Methods** (section 2.8). Within-batch coefficients of variation were 2.6% for TAG (after correction for plasma glycerol), 1.0% for NEFA, 1.7% for glucose and 6.1% for insulin.

4.2.10. Calculations and statistics

All calculations and statistical analyses were performed as described in **General Methods** (section 2.9). For orthogonal polynomial contrast analysis, the NEFA, glucose and insulin curves were noticeably more symmetrical after logarithmic transformation of time, whereas the opposite was true for TAG; therefore, time was logged to the base *e* for NEFA, glucose and insulin, while TAG was analysed without any transformation.

4.3. RESULTS

4.3.1. Resistance exercise sessions

The mean total weight lifted across the sessions was 49968 ± 3468 lbs (22665 ± 1573 kg) for Heavy Ex and 49973 ± 3469 lbs (22667 ± 1574 kg) for Light Ex. During the Light Ex trial for one participant, the heart rate monitor failed to record; therefore neither the mean heart rate during this session, nor the exact duration taken to complete the trial are known. For this

reason, the mean heart rate during, and the total duration of, this participant's Heavy Ex trial were excluded from the data set, and values for mean heart rate and session duration were analysed with $N = 7$. As expected, the mean duration of Heavy Ex was significantly longer than Light Ex (83 ± 3 min vs. 49 ± 4 min; $P < 0.001$). Mean heart rate tended to be higher during Light Ex than Heavy Ex (107 ± 8 beats·min^{-1} vs. 101 ± 7 beats·min^{-1}; $P = 0.063$), but the difference was not significant with seven participants. Owing to the fact that different participants took different lengths of time to perform a set of repetitions, the fluctuations in heart rate across time were unique to each man and therefore no "mean heart rate response" existed which could be graphically depicted. However, sample heart rate traces during the two different resistance exercise protocols for two participants can be seen in **Figure 4.1**. The mean muscle RPE of the 12 exercises from the Borg CR10 Scale was significantly higher during Heavy Ex than Light Ex (5.82 ± 0.40 vs. 3.93 ± 0.26; $P = 0.001$). The degree of exertion placed upon the working muscles in order to complete the exercises was therefore judged to be "moderate" to "strong" for Light Ex, and "strong" to "very strong" for Heavy Ex. Overall ratings of perceived exertion from the Borg RPE Scale for the 12 exercises were also higher for Heavy Ex than Light Ex (14.2 ± 0.5 vs. 12.5 ± 0.4; $P = 0.006$). The overall RPE for Light Ex suggests that the effort required to complete the 12 exercises was between " light" and "somewhat hard", whereas the required effort was between "somewhat hard" and "hard" for the Heavy Ex trial. When asked, immediately after finishing each session, to select a score from the Borg RPE Scale which best represented the exertion required to complete the entire protocol, participants did not rate the sessions differently (Heavy Ex: 14.4 ± 0.5; Light Ex: 13.5 ± 0.5; $P = 0.247$).

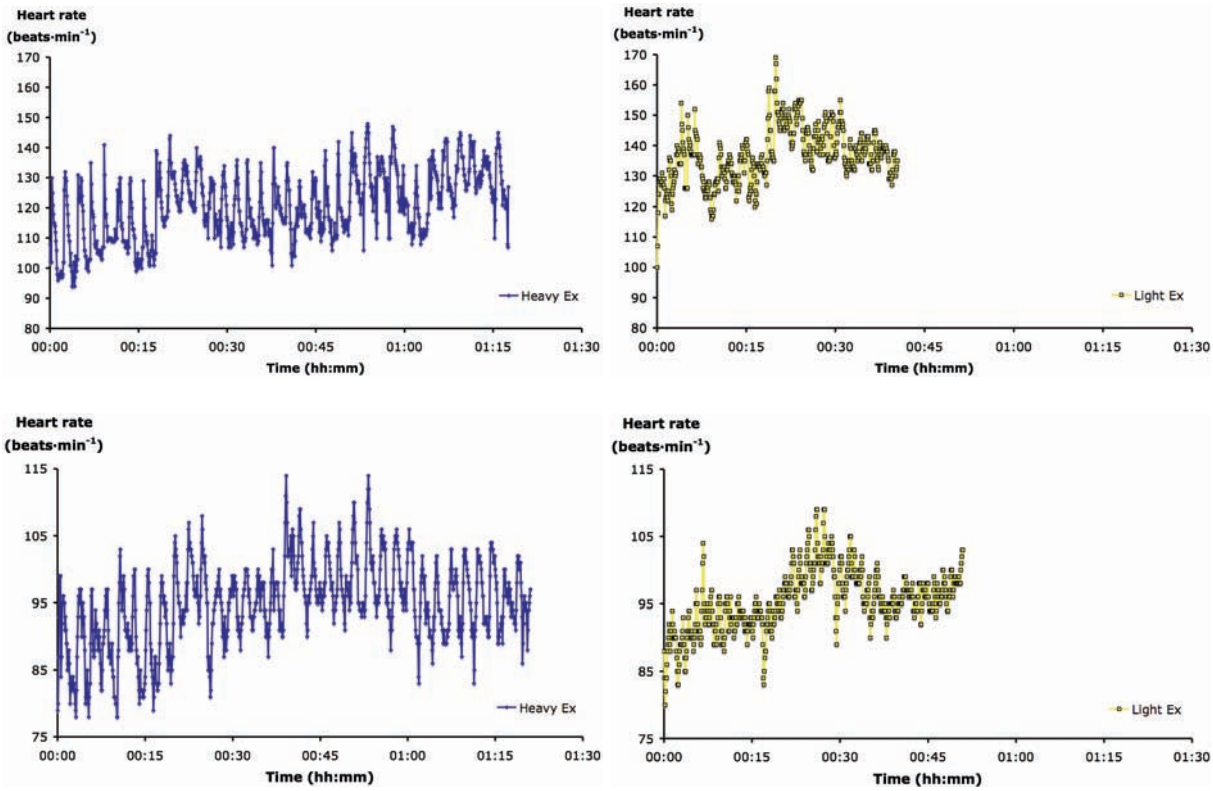


Figure 4.1. Individual heart rate traces for two participants during high-intensity (Heavy Ex; blue lines) and low-intensity (Light Ex; yellow lines) resistance exercise.

4.3.2. Plasma and serum concentrations in the fasted state

Fasting plasma concentrations of triacylglycerol, NEFA and glucose, serum concentrations of insulin, and HOMA scores are presented in **Table 4.2**. None of the metabolites measured in the fasted state showed a statistically significant difference in concentration between trials ($P > 0.05$ for all).

Table 4.2.
Plasma and serum concentrations in the fasted state for control and exercise trials (mean ± S.E.M.)

	Control	Light Ex	Heavy Ex
TAG ($\mu mol \cdot l^{-1}$)	1084 ± 161	1081 ± 109	1048 ± 132
NEFA ($\mu mol \cdot l^{-1}$)	318 ± 33	377 ± 33	370 ± 22
Glucose ($mmol \cdot l^{-1}$)	5.36 ± 0.15	5.49 ± 0.29	5.50 ± 0.36
Insulin ($\mu IU \cdot ml^{-1}$)	12.1 ± 2.3	12.4 ± 3.0	10.5 ± 2.3
HOMA	1.59 ± 0.30	1.64 ± 0.39	1.41 ± 0.32

4.3.3. Plasma and serum metabolites during the postprandial period

4.3.3.1. Triacylglycerol

Plasma triacylglycerol concentrations during the 6 h postprandial period can be seen in **Figure 4.2**. Triacylglycerol concentrations increased appreciably during the first 3 h of the postprandial period in all three conditions. Mean concentrations peaked between 4 and 5 h in all trials, but there was considerable variation between individuals, with peak values noted as early as 2 h, and as late as 6 h, after the test breakfast. The effect of time on TAG was significant ($P < 0.001$), with linear (73.2%; $P = 0.002$) and quadratic factors (24.8%; $P = 0.009$) accounting for almost all variation in TAG concentration across the postprandial period. TAG concentrations were not different between trials ($P = 0.649$), nor were the shapes of the TAG curves different over time (trial x time interaction; $P = 0.568$).

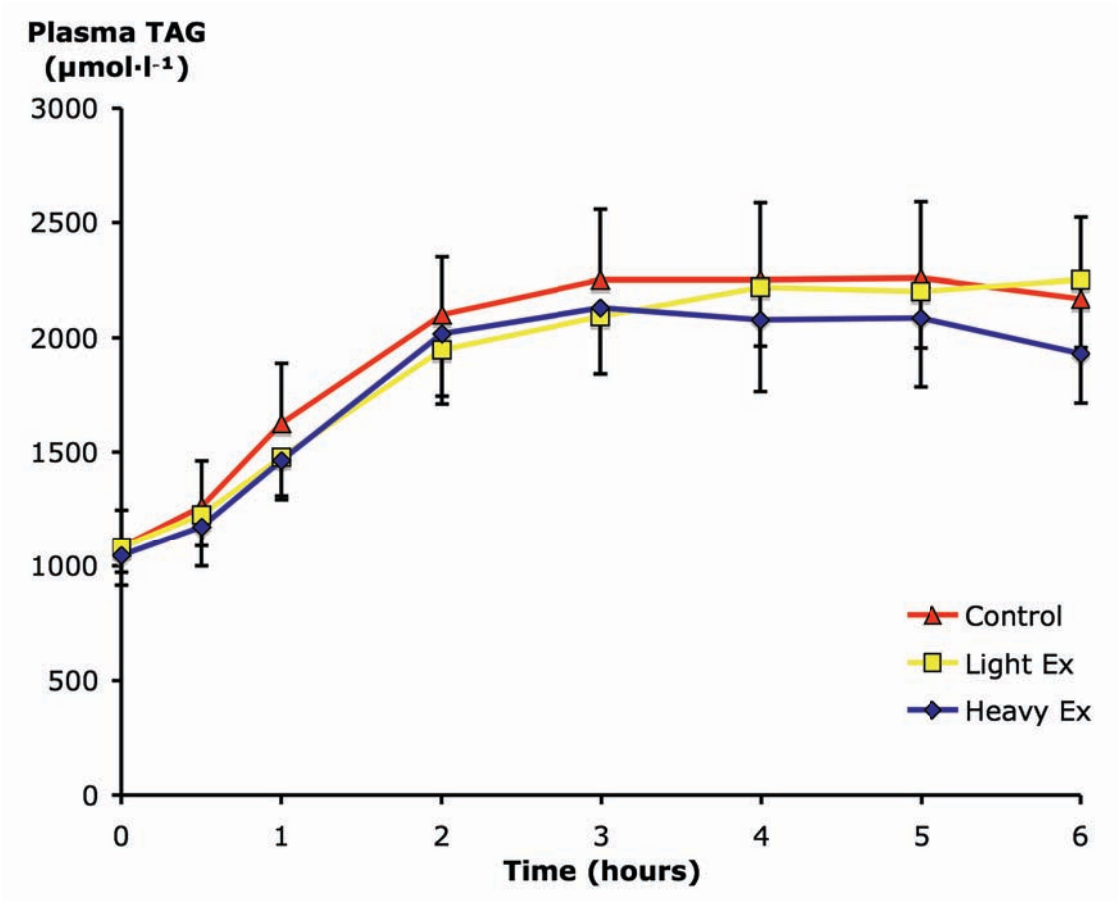


Figure 4.2. Plasma triacylglycerol concentrations in the fasted state and for 6 h after ingestion of a high-fat mixed meal.

Differences in mean total and incremental TAG responses between trials were small (see **figure 4.3**) and did not approach statistical significance ($P > 0.05$ for all). The individual changes in TAG response with exercise did not correlate with heart rate during the sessions, total weight lifted or any of the RPE scores ($P > 0.05$ for all). Retrospective power calculations were conducted to examine the power of the current study to reject the null hypothesis. Based on the sample size of 8 participants, the power within this study to reject the null hypothesis was 0.107 for Heavy Ex versus Control and was 0.065 for Light Ex. vs Control. To reject the null hypothesis (that TAG AUC was not different between exercise and

control trials), 71 and 241 participants would need to be recruited in total for the Heavy Ex and Light Ex trials respectively.

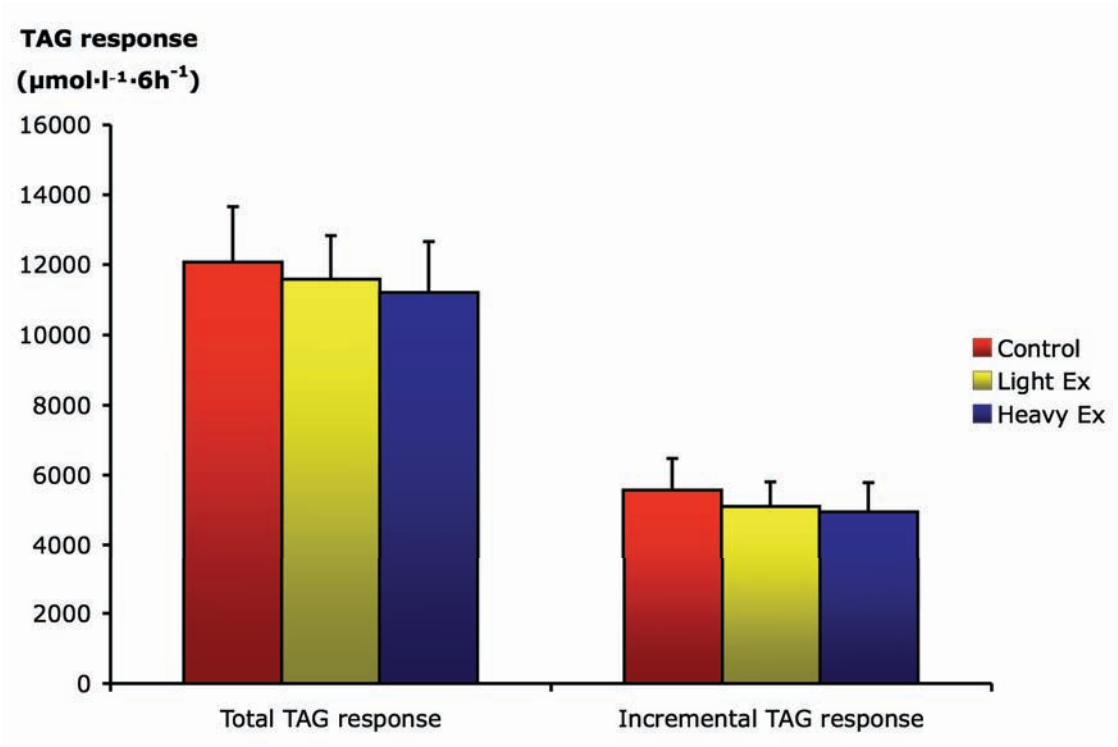


Figure 4.3. Total and incremental 6 h triacylglycerol versus time area under the curve scores.

4.3.3.2. NEFA

Figure 4.4 shows plasma NEFA concentrations during the postprandial period. NEFA concentrations dropped below baseline in the first hour after ingestion of the breakfast, but then rebounded and continued to increase for the remainder of the observation period. Mean NEFA values were above baseline by 4 h in all trials, with peak values recorded at 6 h. The change in concentration with time was significant ($P < 0.001$). A linear component accounted for 54.1% of the total variation ($P = 0.002$), whereas 44.4% was explained by a quadratic factor ($P < 0.001$); the shape of the NEFA curves could therefore be described as curvilinear.

The initial ANOVA revealed a significant main effect of trial (P = 0.033); when investigated further, NEFA concentrations were found to be significantly greater during the Heavy Ex trial than Control (P = 0.029). Concentrations during Light Ex did not differ from either of the other two trials. The trial being undertaken did not alter the shape of the NEFA curve across time (trial x time interaction; P = 0.458).

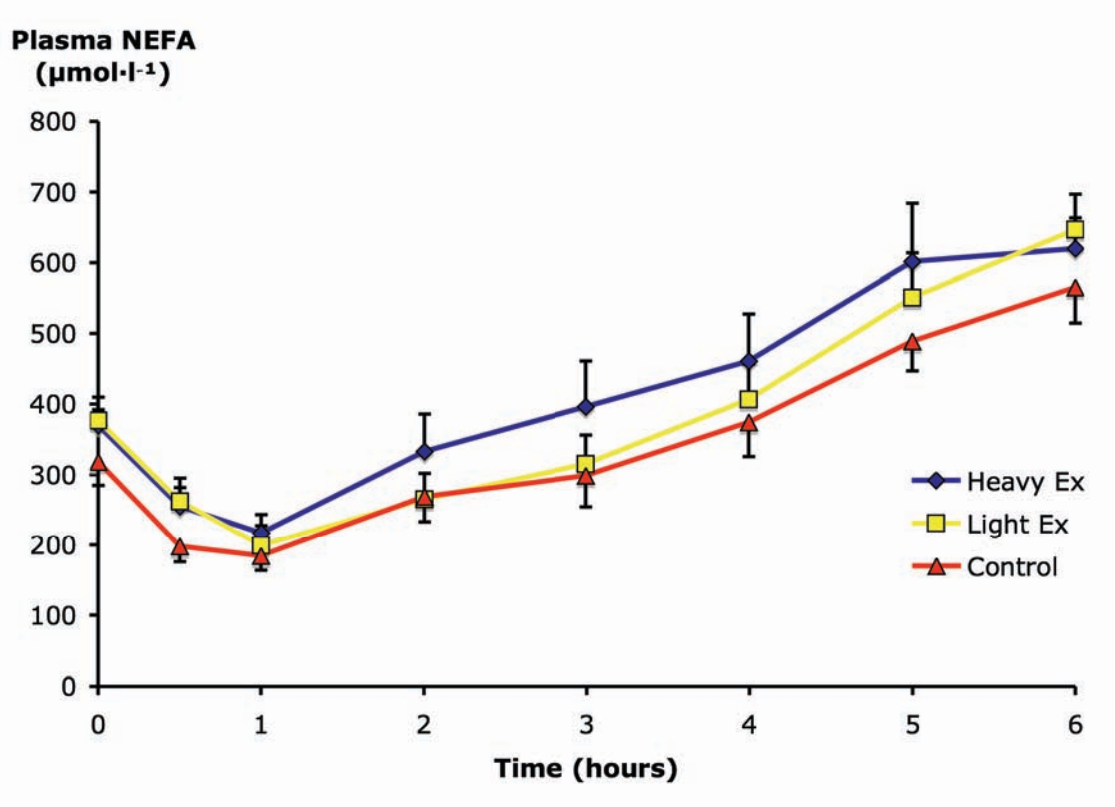


Figure 4.4. Plasma NEFA concentrations in the fasted state and for 6 h after ingestion of a high-fat mixed meal.

4.3.3.3. Glucose

Plasma glucose concentrations are displayed in **Figure 4.5**. Mean glucose concentrations peaked after 30 min in all three trials. There was a significant change in plasma glucose

across the postprandial period (P = 0.012), with the majority (51.1%) of the variation due to a linear factor (P = 0.002). The significant linear component of the glucose curves over time reflects the fact that glucose fell overall from 0.5 h to 6 h in all three trials, with this best exemplified by the Heavy Ex trial where mean values showed a relatively steady and shallow negative slope between 1 h and 6 h. Glucose concentrations were not different between the three trials (P = 0.663) and there was not a significant trial x time interaction (P = 0.145).

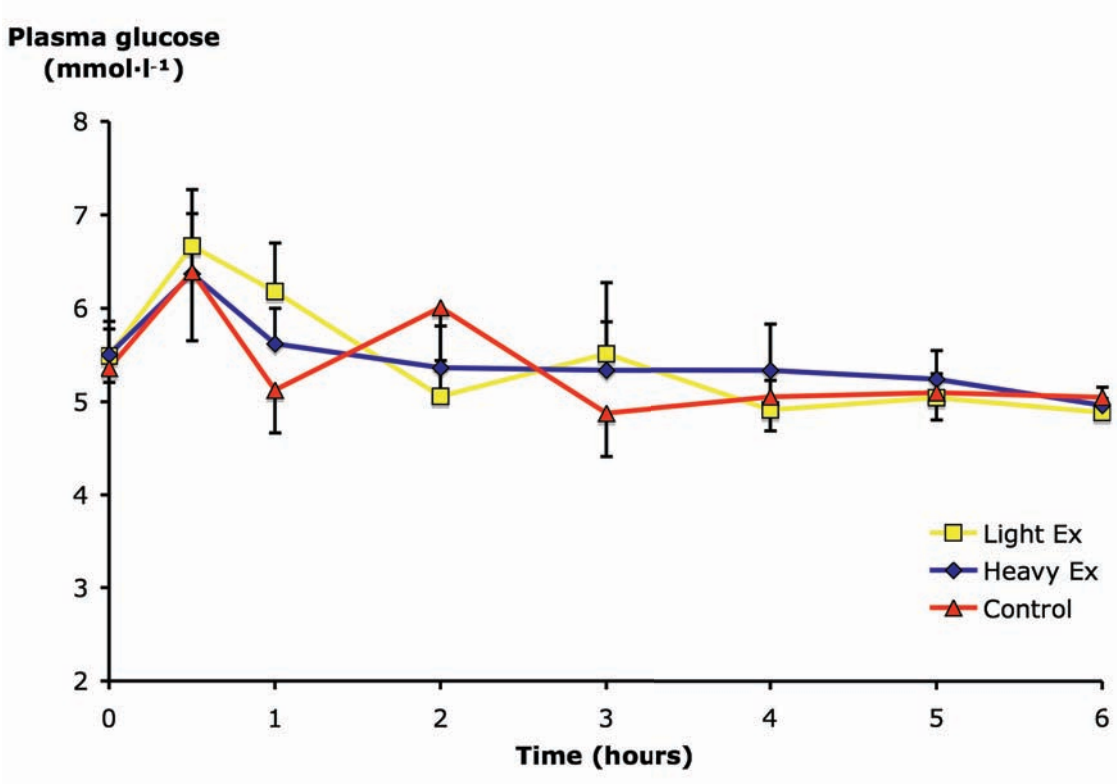


Figure 4.5. Plasma glucose concentrations in the fasted state and for 6 h after ingestion of a high-fat mixed meal.

4.3.3.5. Insulin

Serum insulin concentrations are illustrated in **Figure 4.6.** By 30 min after the test meal, mean insulin concentrations had risen 5 – 6 fold from fasting values. Concentrations peaked

after 30 min or 1 h for most participants, although peak values at 2 h, or even 3 h, were seen on a few occasions. The change in concentration across the postprandial period was significant (effect of time, $P = 0.001$), with quadratic (50.6%, $P < 0.001$), linear (20.6%, $P = 0.004$) and cubic (21.7% $P = 0.011$) components collectively explaining ~ 93% of the total variation across time. The two resistance exercise sessions did not alter serum insulin relative to a control trial, or when compared with each other (effect of trial; $P = 0.357$), and no interaction between time and trial was found ($P = 0.495$).

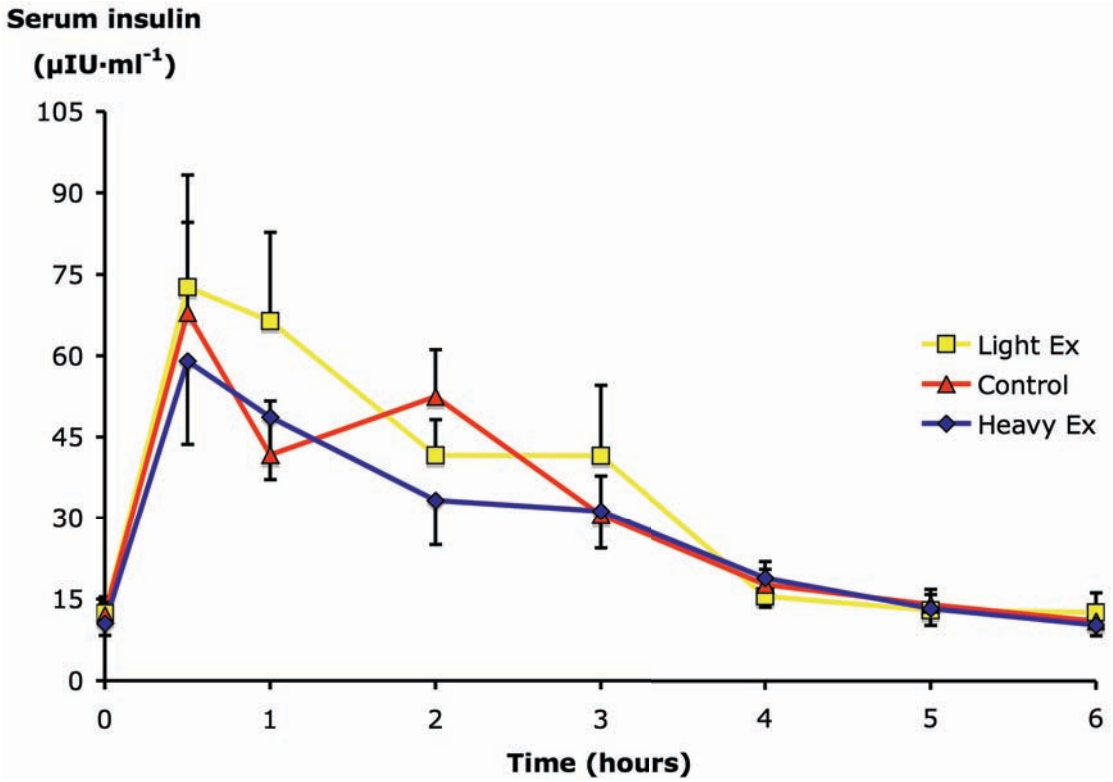


Figure 4.6. Serum insulin concentrations in the fasted state and for 6 h after ingestion of a high-fat mixed meal.

4.4. DISCUSSION

The defining outcome of this study was that performing either low-intensity or high-intensity resistance exercise, 14 – 15 h prior to ingesting a high-fat mixed meal, failed to reduce

postprandial lipaemia compared with a control trial. In support of our finding that a session of relatively high-intensity resistance exercise does not lower postprandial lipaemia, it was reported previously that a resistance exercise session where 11 different exercises were performed in 4 sets of 10 repetitions at 80% of 10-RM did not reduce the TAG response to a high-fat meal (Burns et al., 2005). Due to logistical difficulties, exercise energy expenditure was not calculated in the present study, but as the total weight lifted in our study was higher than in the study of Burns et al. (2005), it is not unreasonable to suggest that EE would also have been higher. Singhal et al. (2009) reported a higher EE during high-intensity resistance exercise than for a moderate-intensity session, despite the sessions being matched for the total weight lifted. As the relative load per repetition was higher in our study than in the study of Burns et al., this would also favour EE in our study being greater. We are therefore left to conclude that despite our session having a greater total lifting load than for Burns et al. (2005), and likely a greater EE, the high-intensity resistance exercise session used in our study still had minimal effect on postprandial lipaemia. Shannon et al. (2005) also found that postprandial TAG metabolism was not improved by 1, 3 or 5 sets of prior resistance exercise performed at 75% of 1-RM, but, as post-exercise energy balance was prevented from becoming negative in this study by addition of extra energy to the evening meal after the resistance sessions, the true exercise-derived effect may have been prevented from occurring. Other studies have found a lipaemic benefit after performing high-intensity resistance exercise (Burns et al., 2007; Pafili et al., 2009; Petitt et al., 2003; Singhal et al., 2009; Zafeiridis et al., 2007), but only one of these studies used men not accustomed to resistance exercise (Pafili et al., 2009), and the improvement in postprandial lipaemia in this study was actually attributed to a lowering of the fasting TAG concentration, not a reduction in the incremental lipaemic response, i.e. there was no true postprandial effect of the prior exercise,

the TAG response to the meal was the same, but as baseline concentrations were lower, the concentration postprandially was also lower. We hypothesised that a low-intensity resistance exercise session, with a more substantial aerobic component than previously used protocols, would be more beneficial for postprandial lipaemia than high-intensity resistance exercise; our results indicate that this is not the case. Burns et al. (2007) found postprandial lipaemia to be significantly lower on the day after low-intensity resistance exercise, but the volume of exercise used in their protocol was somewhat extreme, being far in excess of existing ACSM guidelines for use of resistance exercise to aid health. Moreover, the mean percentage reduction in total TAG response after exercise was 12%, which is somewhat lower than average reductions seen following bouts of aerobic exercise with lower durations and energy expenditures. Singhal et al. (2009) reported a borderline significant reduction in total lipaemic response after low-intensity resistance exercise, but again, the subjects used were resistance-trained, the effect size appears small and the lowering of postprandial TAG concentrations was due to a reduction in the fasting concentration rather than an improved TAG response to ingestion of the high-fat meal.

To our knowledge, only one study investigating the effect of resistance exercise on postprandial lipaemia has reported the mean heart rate during the exercise session; participants in the study of Petitt et al. (2003) worked at a mean heart rate of 131 ± 4 beats·min^{-1}. The mean heart rate during the session of Petitt et al. was approximately 25 – 30 beats·min^{-1} higher than for the two sessions within the current study, despite the Heavy Ex protocol being very similar to the protocol used by Petitt and colleagues with respect to the: relative load lifted (100% of 10-RM for both), number of sets (3 for both), recovery time (2 minutes for both), repetitions per set (8 vs. 10) and number of exercises (12 vs. 10). The

lower heart rate during the resistance exercise sessions in our study may be at least partly due to the older age of our participants; mean age in our study was 18 years above that of the participants in the study of Petitt et al (42.4 vs. 24.3 y). As adults age, maximal heart rate is reduced by approximately 1 beat·min^{-1} every year, therefore maximal heart rate will have been likely to be lower in our participant group, and as a result, the extent to which heart rate could respond to exercise may also have been diminished. However, it is also possible that the exercise sessions in our study were simply not as testing as the protocol used by Petitt et al. We were unable to calculate exercise energy expenditure for our sessions and Petitt et al. did not report the total weight lifted by participants during their protocol or the RPE, therefore it is difficult to compare our protocols directly using either objective measures of work done or subjective values of perceived effort. Mean heart rates during low-intensity aerobic exercise are generally between 100 and 115 beats·min^{-1} (Aldred et al., 1994; Tsetsonis and Hardman, 1996a,b), whereas the heart rate during aerobic exercise bouts of a moderate-intensity is nearer 150 beats·min^{-1} for young subjects (Tsetsonis and Hardman, 1996a,b) and 130 beats·min^{-1} for middle-aged men (chapters 3, 5 and 6 of this thesis). Therefore the average heart rate during resistance exercise sessions in this study was equivalent to that during low-intensity aerobic exercise, and as low-intensity aerobic exercise bouts have only been found to lower lipaemia when lasting 2 h or longer (Aldred et al., 1994; Tsetsonis and Hardman, 1996a,b), the exercise duration of the sessions in our study may have been a limiting factor. As intended, the heart rate response during the two resistance exercise sessions in our study appears different (see **Figure 4.1**), with Heavy Ex having pronounced peaks and troughs for each set lifted, whereas heart rate was contained within a tighter range for Light Ex due to the lighter load and shorter recovery. Despite these different heart rate responses, the mean heart rate was not significantly different between sessions, although there was a trend for it to be

higher during the low-intensity session. The continuous elevation of heart rate seen with aerobic exercise, and the extent to which it is elevated, may have a bearing on whether postprandial lipaemia is reduced, and, when compared with mean heart rates during the resistance exercise sessions in the present study, it may be that mean heart rate was just not high enough in our protocols to affect lipaemia the next day. Future studies may wish to investigate this further by increasing the mean heart rate during resistance exercise, either by increasing the load lifted, increasing the number of repetitions performed or shortening the recovery between sets and/or exercises. Although more intense or prolonged sessions of resistance exercise might alter the postprandial TAG response favourably, the greater intensity and duration will naturally reduce their appeal and attrition rates are likely to increase.

The only significant change in postprandial metabolism measured in this study was an increase in plasma NEFA concentration after the Heavy Ex session, relative to Control. Neither fasting NEFA, nor the incremental NEFA response were significantly different between these trials, but the small increases in both after Heavy Ex appear to have been sufficient to maintain NEFA concentrations above control values. Mechanistically, there are several potential routes through which high-intensity resistance exercise could have increased plasma NEFA concentrations, although the extent to which many of these pathways would be affected in this study is not known. Seven of the eight men had an increased fasting NEFA concentration after Heavy Ex compared with Control, therefore adipose tissue lipolysis may have been increased on the day after exercise. As VLDL secretion rate is linked to hepatic availability of NEFA, the higher plasma NEFA concentration in the Heavy Ex trial may have contributed to a greater VLDL secretion rate, which one would expect would elevate the

plasma TAG concentration. However, as TAG concentrations were not higher than control during the Heavy Ex trial, the effect of the higher NEFA concentration on VLDL-TAG is not clear. It could be that NEFA uptake was reduced after the Heavy Ex trial, although we cannot provide any evidence to support such an idea. Alternatively, if trapping of fatty acids liberated by LPL-mediated hydrolysis of TAG-rich lipoproteins within adipose tissue or skeletal muscle was lowered after Heavy Ex, this could account for the higher plasma NEFA concentration. In the fasting state, approximately 25% of plasma NEFA efflux is due to hydrolysis of fatty acids within lipoproteins by LPL (Samra et al., 1996), and during the postprandial period, the majority of plasma NEFA are derived from intravascular hydrolysis of TAG-rich lipoproteins (Frayn, 1997); therefore the theory that spillover of fatty acids from lipoproteins was increased, is a possibility, but the specific biochemical processes through which high-intensity resistance exercise would initiate such an effect are unknown. As concentrations of plasma glucose and serum insulin were not altered significantly by either exercise session in the fasted or postprandial state, neither of these metabolites can offer an explanation as to how postprandial NEFA concentrations became higher than control after Heavy Ex. The changes in NEFA AUC and iAUC after Heavy Ex from control values did not correlate with changes in TAG AUC and iAUC, therefore it appears unlikely that changes in the plasma NEFA concentration had a direct effect on the plasma TAG concentration and/or vice versa.

In conclusion, this study reports that the postprandial lipaemia seen in overweight, sedentary men after a high-fat meal is not reduced by performing resistance exercise of a low or high intensity 14 – 15 h beforehand.

CHAPTER FIVE

Is the beneficial effect of prior exercise on postprandial lipaemia lost when the meal is consumed *ad libitum*?

ABSTRACT

Background: An exaggerated increase in circulating triacylglycerol (TAG) concentration after eating a fat-containing meal has been linked with a greater risk of atherogenesis and cardiovascular disease. Undertaking aerobic exercise 12 - 16 hours prior to meal ingestion has been shown to be effective in limiting the TAG response, however the use of a standardised meal (with respect to the quantity of food given) in previous studies may limit the applicability of such findings if appetite were to be altered by exercising in a real-life situation.

Aims: To investigate whether a 90-minute evening treadmill walk alters energy intake the following morning (relative to a control trial) and whether postprandial TAG concentrations are reduced following exercise if a different quantity of fat has been consumed.

Participants: Eight sedentary men, aged 44.4 ± 13.6 years with a BMI of 33.4 ± 5.4 kg/m^2 (mean ± SD) completed the study. All were healthy and none participated in more than one hour of structured physical activity per week.

Design: All participants were provided with an *ad libitum* breakfast on two separate occasions: one breakfast was preceded 13 h earlier by 90 min of treadmill walking at 60% of individual VO_2max; the other was following 90 min of seated rest in the lab. A fasting blood sample was taken prior to the breakfast; further samples were collected at 0.5 h and 1 h after breakfast and at every hour thereafter until 6 h had elapsed since cessation of eating.

Results: On average, participants ingested 8.4% more energy on the morning following exercise than after resting ($P = 0.188$). The total area under the 6 h plasma TAG vs. time curve was 25.7% lower in response to an *ad libitum* breakfast after exercise compared with an *ad libitum* breakfast after seated rest ($P = 0.008$).

Conclusions: Exercising 13 h before an *ad libitum* breakfast does not significantly increase energy intake. Furthermore, the effect of prior exercise is sufficiently powerful to override the small additional intake of energy in an *ad libitum* situation and lower postprandial lipaemia.

5.1. INTRODUCTION

High concentrations of TAG within the plasma (as seen after fat intake) have been shown to accelerate the atherosclerotic process (Gianturco and Bradley, 1999). Many studies have shown that a single bout of aerobic exercise, performed on the evening before a fat-containing breakfast, can lower the postprandial TAG response, but the standardisation of energy intake post-intervention may limit the extent to which the results reflect a real life (non-laboratory) situation. Such investigations, where test meals provide the same energy content for exercise and control trials, have been valuable in generating proof of concept for the beneficial effect of exercise. However, it is not clear whether participants would naturally choose to eat the same quantity of food on the day after exercise as on the day after no exercise (control trial), and whether a difference in energy intake would modify the exercise-derived effect on postprandial lipaemia.

Studies investigating the effect of exercise on appetite and energy intake during the immediate post-exercise period have produced equivocal results. Hunger is attenuated during

aerobic exercise in healthy individuals (Broom et al., 2009; King et al., 1994) and hunger ratings tend to be reduced for 1 - 2 h after vigorous exercise (> 60% VO_2max), with the phenomenon referred to as “exercise-induced anorexia” (Kissileff et al., 1990; King et al., 1994). The suppression of hunger is generally short-lived, with the redistribution of blood flow from the viscera to skeletal muscle during exercise thought to play a role. The downregulation of hunger tends to be more consistent following high-intensity exercise, with bouts of low or moderate intensity activity not thought to exert the same effect (Blundell et al., 2003), however, a recent study has demonstrated that hunger ratings in the fasted state are lower immediately after 1 h of moderate exercise than before (Cheng et al., 2009). Lowered hunger ratings have not been found in all studies however. One report found hunger, both in the morning and evening, to be increased 15 minutes after 1 h of aerobic and resistance exercise when compared to values before exercise and values after 1 h of rest (Maraki et al., 2005). Additionally, another study found hunger to be significantly reduced during exercise, but not to be different from control at 1 h post-exercise (Martins et al., 2007). To further complicate matters, differences in appetite/hunger scores between trials do not always translate into differences in energy intake (Flint et al., 2000; Mattes, 1990). Energy intake in the study of Maraki et al. was not increased after exercise despite greater hunger ratings, whereas Martins et al. found energy intake to be greater after exercise despite no change in hunger. Within-subject investigations have found *ad libitum* energy intake to be reduced (Westerterp-Plantenga et al., 1997), unchanged (George and Morganstein, 2003; Hubert et al., 1998; Imbeault et al., 1997; King et al., 1994; Maraki et al., 2005; Thompson et al., 1988) and increased (Martins et al., 2007; Pomerleau et al., 2004; Shorten et al., 2009; Verger et al., 1992) after exercise, relative to control trials. It is not obvious why exercise has evoked such divergent findings in these studies, but the methodological differences between investigations

are thought to play a role (Martins et al., 2008a,b). Interestingly, in one study where total energy intake was increased after exercise, the "relative energy intake" (energy intake - energy expenditure of the exercise above resting energy expenditure), was found to be reduced with exercise compared to control (Martins et al., 2007). This observation of reduced relative energy intake has been supported by findings from studies where total energy intake was not increased after exercise (Imbeault et al., 1997; King et al., 1994; Maraki et al., 2005). Collectively, these studies suggest that while *ad libitum* energy intake may be increased in the hours immediately after physical activity, such an effect is not consistently observed. Most studies report no change in energy intake after physical exertion, and when an increase is seen, it is not sufficient to totally compensate for the energy expended during exercise.

The vast majority of the previous literature investigates the effect of exercise on appetite or food intake in the first few hours of recovery, but no studies have examined the effect of moderate-intensity evening exercise on *ad libitum* energy intake the following morning. A temporary state of negative energy balance is likely to occur after moderate-intensity exercise of 1 – 2 h duration; the extent to which a negative energy state exists may be an important factor determining how large the subsequent reduction in postprandial lipaemia will be. Adding extra energy to the evening meal consumed after exercise (to replace substrates used during exercise and prevent negative energy balance) has been shown to attenuate the degree to which prior exercise lowers postprandial lipaemia (Burton et al., 2008). However, in this study, 55% of the extra energy was given at lunch, i.e. before the walk had been performed; the effect on lipid metabolism of providing this additional energy prior to exercise is therefore a complicating variable. Furthermore, if appetite is indeed increased several hours after exercise, and not immediately after, then the appropriateness of providing additional energy in

a meal shortly after exercise is perhaps questionable. After all, *ad libitum* energy intake at this point in time would not be expected to increase, particularly given that the volitional onset of eating is significantly delayed by prior exercise (King et al., 1994). Instead, energy intake on the morning after an evening exercise bout may be greater. To test this hypothesis, and the effect which greater energy intake would exert on the exercise-induced lowering of TAG concentrations, eight male participants meeting established criteria for risk of elevated postprandial lipaemia were recruited. All men consumed an *ad libitum* breakfast on two occasions: once, 13 h after performing 90 min of brisk walking, and once, 13 h after resting for 90 min. Blood samples were collected immediately before the breakfast, and for 6 h after, to measure plasma TAG concentrations.

5.2. METHODS

5.2.1. Participants

Eight, healthy, non-smoking, sedentary, overweight men gave informed written consent to participate in the study. Physical characteristics of the participants are presented below in **Table 5.1**. Sedentariness was defined as performance of no more than one hour of structured physical activity per week; all men taking part in the study were classified as sedentary in accordance with this definition. None of the men were taking medication known to affect lipid metabolism, and all were free of cardiovascular disease and diabetes.

Table 5.1.
Characteristics of the eight study participants

	Mean ± S.D.
Age (years)	44.4 ± 13.6
Height (m)	1.83 ± 0.05
Body mass (kg)	111 ± 15
BMI ($kg \cdot m^{-2}$)	33.4 ± 5.4
Body fat (%)	29.2 ± 5.9
Estimated VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	34.1 ± 4.9

5.2.2. Study design

All participants consumed an *ad libitum* breakfast on two separate occasions. One breakfast was eaten on the morning after a brisk 90-minute treadmill walk (EX); the other was ingested following the same duration of seated rest (CON). The order in which the two different visits took place was randomised until four people had completed one order; the remaining participants followed the alternative order to ensure a counter-balanced design. The washout period between trials was at least 6 days, and no more than 10 days, for all participants. Pre-trial restrictions on diet and physical activity are outlined in **General Methods** (section 2.2.2).

5.2.3. Preliminary exercise testing

The preliminary exercise test used is described in the **General Methods** (section 2.3).

5.2.4. Main trials

Exercise and control trials were as described in the **General Methods (**section 2.4).

5.2.5. Body fat estimation

Body fat percentage was estimated as described in **General Methods** (section 2.7).

5.2.6. Evening (post-intervention) meal

An evening meal was provided as described in the **General Methods** (section 2.5). The mean energy (± S.D.) provided by the meal was 5.41 ± 1.27 MJ (1292 ± 304 kcal).

5.2.7. Ad libitum breakfasts and blood sampling

With the exception that the test breakfast was provided *ad libitum*, the remaining procedures were as described in **General Methods** (section 2.6). The breakfast consisted of a raspberry milkshake (whole milk, double cream, sugar and raspberry flavouring) and oven-cooked croissants with butter and raspberry jam. The breakfast was carefully formulated (by modification of the ratio of the different ingredients to each other) such that the macronutrient composition of the milkshake and the croissants was identical (50.7% fat, 42.2% carbohydrate and 7.1% protein, as percentages of total energy). This was done to ensure that fat would be ingested in the same proportion to carbohydrate and protein regardless of whether subjects preferred the milkshake or the croissants. The croissants provided ~ 405 kcal per 100 g, with the milkshake containing approximately 147 kcal per 100 ml. For each trial, two litres of the milkshake was made and eight croissants were toasted, and spread with butter and jam. The milkshake was constructed the same way on each occasion, with the ingredients blended together in an electric food mixer to aid dissolution of the sugar. Every croissant was cut in half and individually weighed, such that the exact quantity of butter and jam required could be calculated and provided. After being cooked until golden brown, each croissant was placed onto a large tray to receive its ration of butter and jam. After addition of the butter and jam, the croissants were cut into irregularly sized pieces to make it more difficult for participants to gauge how much they had eaten. The tray containing the segmented croissants was weighed and the value recorded. The milkshake was provided in two 1-litre sports drink

bottles, with black tape used to cover the window on the side of the bottle. A drinking glass was not provided. These steps were taken such that participants would be less able to replicate their food intake, based on visual clues (number of croissants eaten and volume of drink consumed), during their second trial. Participants were blinded to the fact that energy intake was of importance to the investigators. The men were advised to eat and drink what they felt they wanted. When participants had ingested as much of the milkshake and croissants as they wanted, the investigators were notified of this and a stopwatch was started. Blood samples were then taken for 6 h from this point. The participants were escorted back to the laboratory to rest and one of the investigators returned to the Research Kitchen to measure the remaining milkshake in a measuring cylinder (to the nearest 1 ml) and weigh the remainder of the croissants (to the nearest 0.1 g).

5.2.8. Analytical procedures

Analytical procedures were conducted in line with **General Methods** (section 2.8). Within-batch coefficients of variation were 3.7% for TAG (after correction for plasma glycerol), 0.9% for NEFA, 1.4% for glucose and 5.4% for insulin.

5.2.9. Calculations and statistics

Calculations and statistics were performed as per **General Methods** (section 2.9). For orthogonal polynomial contrast analysis, logarithmic transformation of time was found to improve symmetry for NEFA, glucose and insulin, but not for TAG; therefore, analysis for the first three variables was performed with time logged to the base *e*, whereas TAG was analysed without logarithmic transformation of time.

5.3. RESULTS

5.3.1. Responses during brisk walking

Participants walked at a speed of 5.6 ± 0.3 $km \cdot h^{-1}$ up a gradient of 4.1 ± 0.6 %. Mean oxygen uptake during the walk was 20.1 ± 1.1 $ml \cdot kg^{-1} \cdot min^{-1}$, thereby representing 59.3 ± 1.0 % VO_2max. The gross energy expenditure during the walk was 4.10 ± 0.13 MJ, with 62.4 ± 2.2 % of energy from carbohydrate and 37.6 ± 2.2 % from fat. Mean heart rate calculated from measurements taken every 5 seconds during the 90-minute walk was 130 ± 3 $beats \cdot min^{-1}$. The mean rating of perceived exertion from the Borg RPE scale was 14.0 ± 0.5, therefore suggesting that across 90 minutes of walking, the exercise was described as between "somewhat hard" and "hard".

5.3.2. Fasting measurements

Fasting plasma concentrations of triacylglycerol, NEFA and glucose, serum concentrations of insulin, and HOMA scores are presented in **Table 5.2**. Mean plasma TAG concentration was 25.5% lower after walking compared with the control trial ($P = 0.028$). NEFA concentrations tended to be higher after exercise, but the difference did not reach statistical significance ($P = 0.082$). Concentrations of plasma glucose and serum insulin were not different between trials ($P = 0.844$ and $P = 0.722$ respectively); unsurprisingly therefore, HOMA score was also not different ($P = 0.778$).

Table 5.2. Plasma and serum metabolite concentrations in the fasted state for eight participants, for both walking and control trials (mean ± S.E.M.)

	Control	Exercise
TAG ($\mu mol \cdot l^{-1}$)	936 ± 169	697 ± 126*
NEFA ($\mu mol \cdot l^{-1}$)	353 ± 37	398 ± 30
Glucose ($mmol \cdot l^{-1}$)	5.74 ± 0.25	5.69 ± 0.22
Insulin ($\mu IU \cdot ml^{-1}$)	16.9 ± 3.4	17.5 ± 4.2
HOMA	2.23 ± 0.45	2.29 ± 0.53

*Different from control ($P < 0.05$).

5.3.3. Postprandial measurements

5.3.3.1. *Ad libitum* energy intake

Figure 5.1 shows the total *ad libitum* energy intake on the morning after either exercise or control. Total energy intake was not significantly different between the trials ($P = 0.188$), although it was 8.4% higher after exercise. Statistical power was calculated as 0.24; sample size calculations based on the effect size in this study suggested that 42 participants would be needed to detect a significant difference in total energy intake between exercise and control trials. When the solid and liquid components of the breakfast were examined separately, a difference emerged. Intake of croissants was not changed by exercise (840 ± 63 kcal vs. 819 ± 88 kcal; $P = 0.732$), but a nonsignificant trend was found for participants to consume more of the milkshake after the exercise trial (963 ± 185 kcal vs. 1136 ± 233 kcal; $P = 0.070$).

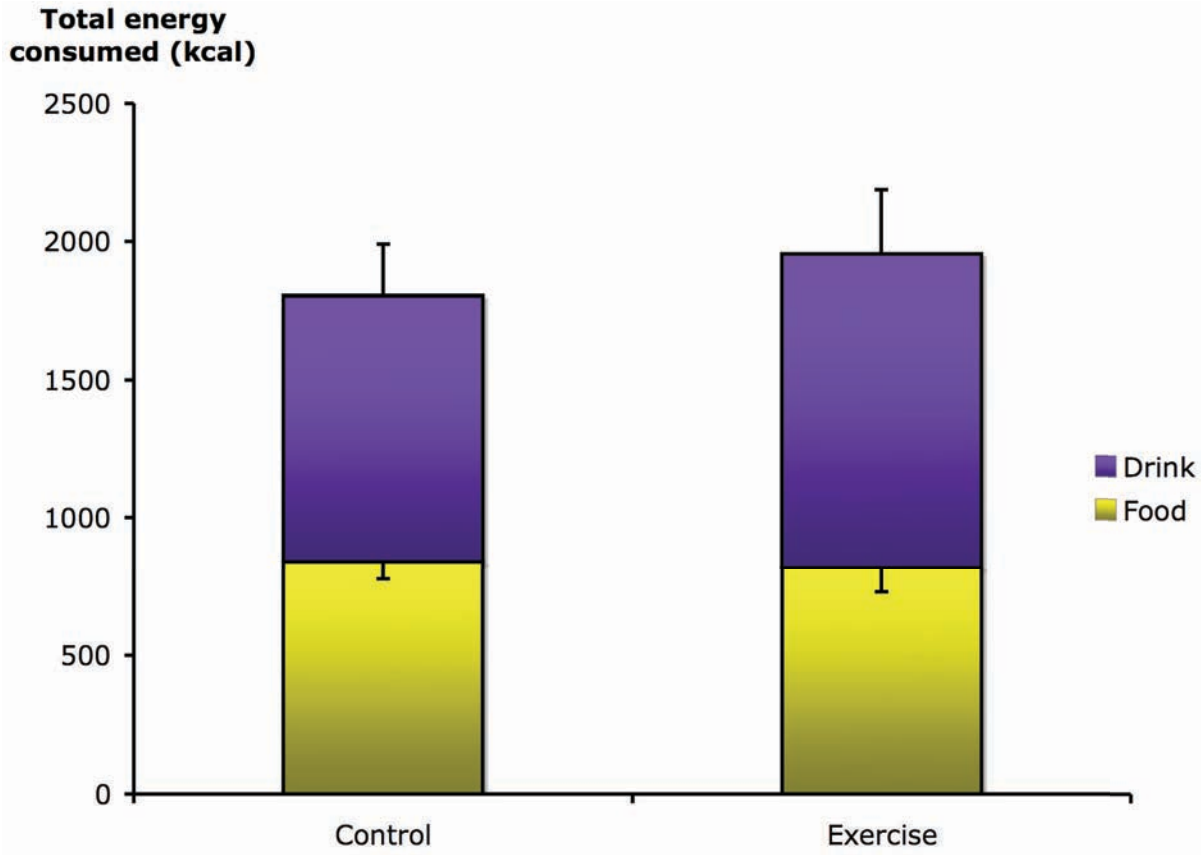


Figure 5.1. Mean (± S.E.M.) energy intake during an *ad libitum* breakfast, following prior rest or exercise.

5.3.3.2. Triacylglycerol

Figure 5.2 shows plasma triacylglycerol concentrations during the 6 h after an *ad libitum* breakfast (50.7% of energy as fat), for both exercise and control trials. The hour at which peak TAG concentration was observed following exercise was the same or earlier than control for all participants. On average, the time to peak was ~ 1 h earlier during the exercise trial (3.63 h vs. 4.75 h), a finding that was on the cusp of statistical significance ($P = 0.051$). Peak concentrations were lower for all participants after exercise ($P = 0.013$), with a mean reduction of 27.5% from the peak in the control trial. The change in TAG concentration during the postprandial period was significant (effect of time; $P < 0.001$). As the total variation over time was almost completely explained by linear (79.0% of the total variation; *P*

= 0.003) and quadratic (20.8% of the total variation; P = 0.001) factors, the shape of the change across time can confidently be described as curvilinear. TAG concentrations were lower at all time points following brisk walking (effect of exercise; P = 0.005), but the change over the postprandial period was not different between exercise and control (exercise x time interaction; P = 0.278).

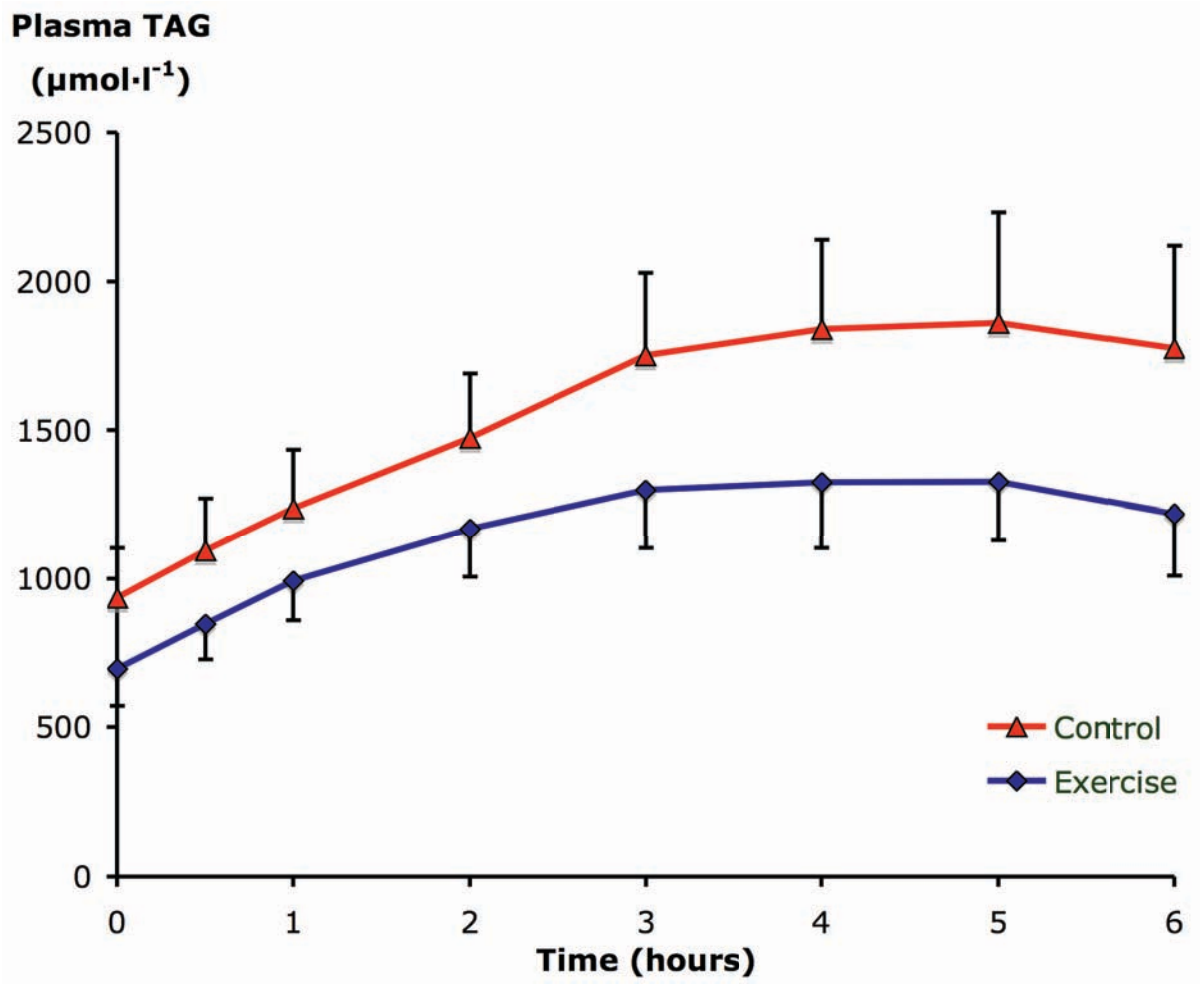


Figure 5.2. Mean (± S.E.M.) plasma triacylglycerol concentrations in the fasted state and for 6 h after an *ad libitum* breakfast, following prior rest or exercise.

Total and incremental lipaemic responses are shown in **Figure 5.3**. When the 6 h AUC scores were analysed, exercise was found to significantly reduce the total TAG response (25.7%

reduction; $P = 0.004$). The incremental TAG response (6 h change from baseline) was decreased by a similar proportion (26.1%), but due to the slightly greater heterogeneity of response for this variable, only a trend towards statistical significance was observed ($P = 0.055$). A retrospective power calculation, based on the sample size of 8 participants, found that the power within this study to reject the null hypothesis (that total TAG response was not different between trials) was 0.944.

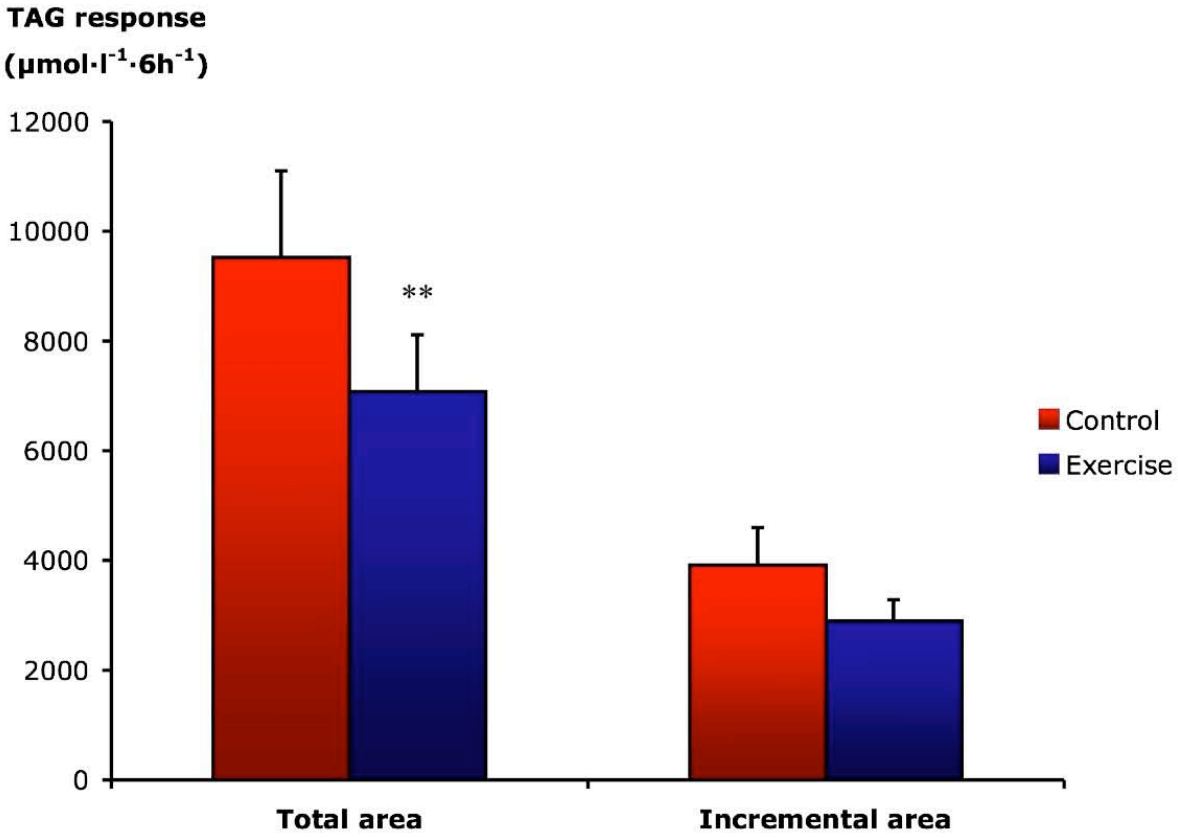


Figure 5.3. Total and incremental area under the curve scores for triacylglycerol over the 6 h postprandial period. **Significantly different from control trial ($P < 0.01$).

5.3.3.3. NEFA

Plasma NEFA concentration dropped from fasting values in the hours immediately after eating (**Figure 5.4)**. The nadir was 2 h post-feeding in most participants, after which

concentrations rose continuously. At the end of the observation period, NEFA concentrations were greater than or equal to baseline values. These changes represented a significant effect of time ($P = 0.001$); the shape of the graph was largely quadratic, with 97% of the variation explained by this function ($P < 0.001$). Mean NEFA concentration tended to be higher after exercise, but there was not a significant difference between trials ($P = 0.086$).

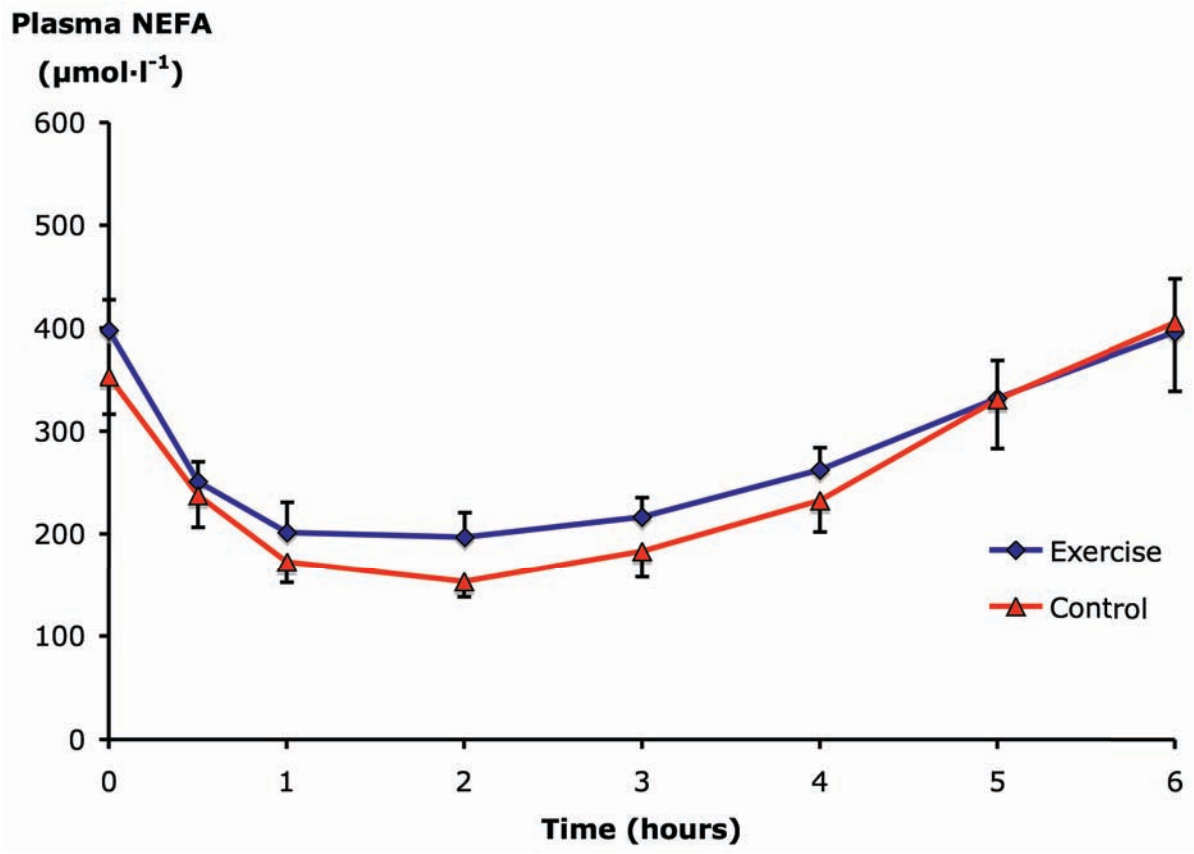


Figure 5.4. Mean (± S.E.M.) plasma NEFA concentrations in the fasted state and for 6 h after an *ad libitum* breakfast, following prior rest or exercise.

5.3.3.4. Glucose

Plasma glucose concentrations can be seen in **figure 5.5**. Plasma glucose concentration was increased above baseline by ~40% at 0.5 h after the *ad libitum* breakfast. In both trials this was the time point at which peak concentrations were observed. Glucose concentration fell

sharply from 0.5 h to 1 h in both trials, and in the control trial a more gradual fall continued until 6 h. In the exercise trial, a second, smaller peak was seen after 3 h before concentrations dropped to meet those from the control trial. The changes over time were statistically significant ($P < 0.001$), with a curvilinear relationship best reflecting the shape of the change in concentration with time. This is evident from the significant linear ($P < 0.001$) and quadratic ($P = 0.007$) components, which accounted for 36% and 25% of the total variation respectively. There was no main effect of exercise ($P = 0.214$), and despite the somewhat different changes in concentration across time with each trial, there was only a non-significant trend for exercise and time to interact ($P = 0.071$).

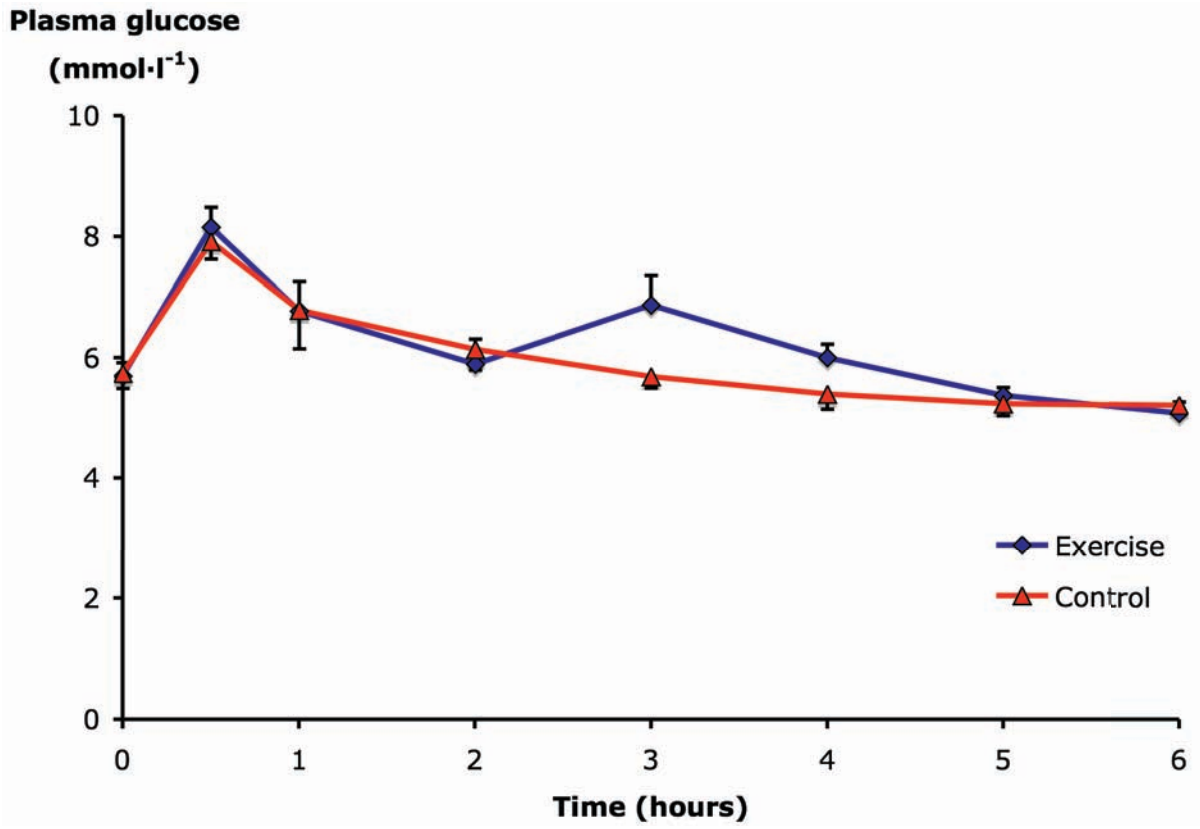


Figure 5.5. Mean (± S.E.M.) plasma glucose concentrations in the fasted state and for 6 h after an *ad libitum* breakfast, following prior rest or exercise.

5.3.3.5. Insulin

Serum insulin concentrations are displayed in **Figure 5.6**. Serum insulin concentration increased rapidly after *ad libitum* food intake, with mean values increased 7-fold above baseline at 0.5 h. Peak values were observed after 0.5 h or 1 h in both trials for almost all participants. Insulin concentrations during the control trial fell steadily between 0.5 h and 6 h. Similar to the graph seen for glucose, insulin showed a small increase from 2 h to 3 h in the exercise trial, presumably in response to the change in plasma glucose concentration. The change in concentration across the postprandial period was significant (effect of time; $P < 0.001$) and the variation was largely (68%) ascribable to a quadratic function ($P < 0.001$). Insulin concentrations were similar in the two trials ($P = 0.683$).

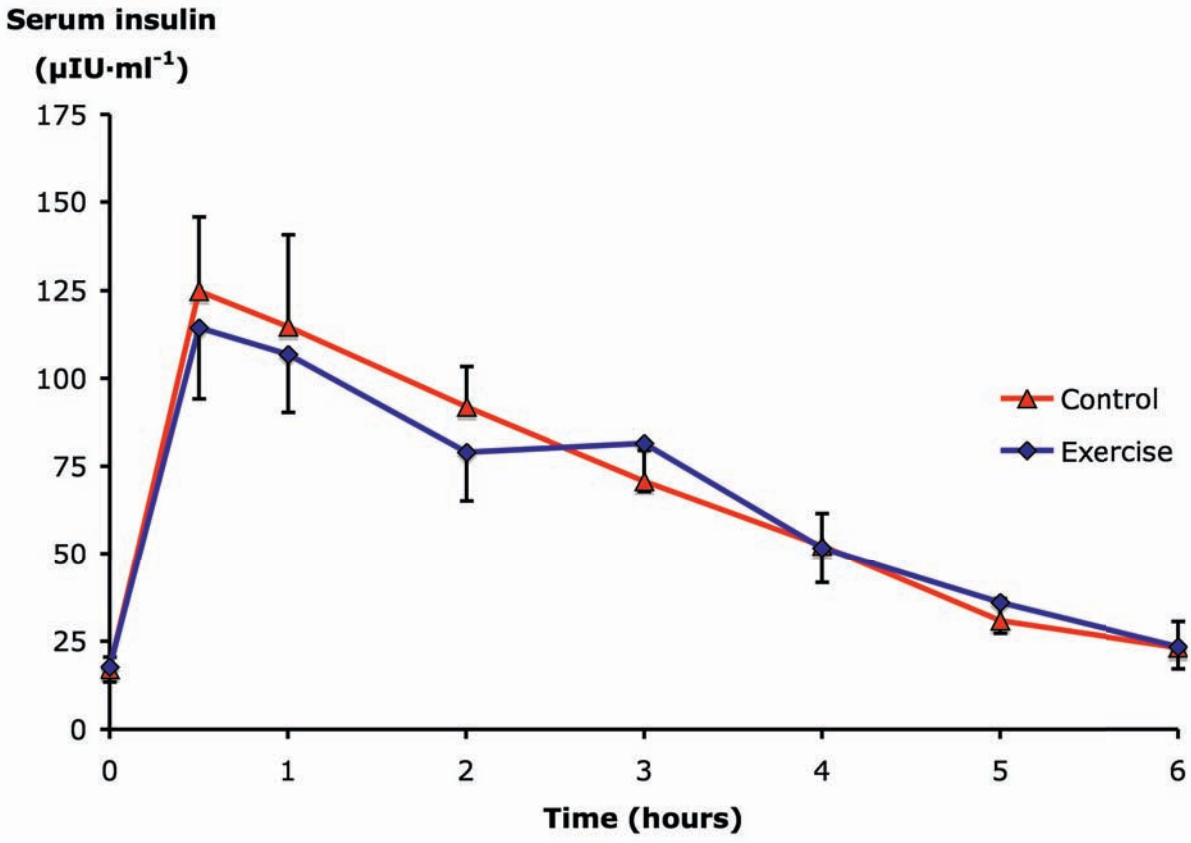


Figure 5.6. Mean (± S.E.M.) serum insulin concentrations in the fasted state and for 6 h after an *ad libitum* breakfast, following prior rest or exercise.

5.3.3.6. Correlations between variables

Fasting TAG concentration showed a positive correlation with total TAG response for both control and exercise trials ($r = 0.952$; $P < 0.001$ for both). Incremental TAG response correlated with fasting TAG in the control condition ($r = 0.713$; $P = 0.047$), but not after an exercise bout ($r = 0.595$; $P = 0.120$). The difference in fasting TAG concentration (Δ fasting TAG; exercise value minus control value) was positively correlated with the difference (Δ total TAG; exercise minus control) in total TAG response ($r = 0.740$; $P = 0.036$), but not with the difference (Δ incremental TAG; exercise minus control) in incremental TAG response ($r = 0.008$; $P = 0.985$). Δ total TAG and Δ incremental TAG were positively correlated, but not to a statistically significant extent ($r = 0.679$; $P = 0.064$).

For control and exercise trials, BMI showed a positive correlation with incremental lipaemic response and body fat percentage was positively correlated with total and incremental lipaemic responses (range: $r = 0.736 – 0.899$; $P < 0.05$ for all). The difference in total *ad libitum* energy intake between trials (Δ total energy intake; exercise intake minus control intake) correlated with the difference in milkshake intake ($r = 0.809$; $P = 0.015$), but not with the difference in croissant intake ($r = 0.625$; $P = 0.097$). Despite this finding, it was the difference in croissant intake between trials (exercise intake minus control intake), not the difference in total energy or milkshake intake, which correlated with Δ incremental TAG ($r = -0.756$; $P = 0.030$). There was also a non-significant trend for Δ total TAG to correlate negatively with Δ total energy intake ($r = -0.689$; $P = 0.059$). *Ad libitum* energy intake correlated positively with incremental insulinaemic response for both control ($r = 0.807$; $P = 0.016$) and exercise trials ($r = 0.793$; $P = 0.019$). Trends were displayed for *ad libitum* energy intake to correlate positively with peak insulin concentrations for both the control ($r = 0.672$;

$P = 0.068$) and the exercise trial ($r = 0.678$; $P = 0.064$), but these did not reach statistical significance.

5.4. DISCUSSION

This study found that moderate-intensity evening exercise did not significantly increase *ad libitum* energy intake the following morning. Furthermore, the reduction in postprandial lipaemia after exercise, seen previously in studies using meals of a fixed size, was maintained despite a slightly greater mean energy intake on the morning after the walk. We believe such findings are novel and provide evidence to suggest that aerobic exercise of a moderate intensity should be effective in lowering postprandial TAG concentrations outside of laboratory conditions, when individuals have the option to consume as much energy as they wish.

In everyday life, most people consume carbohydrate alongside fat and protein, with the proportion of energy from carbohydrate being equal to or greater than that from fat. Therefore pure fat loads and meals containing more than two-thirds of energy as fat, as seen in much of the previous literature, do not reflect normal Western diets. The breakfast in this study was intended to reflect a real meal that may be consumed habitually, with milkshakes and croissants both being common foodstuffs readily available to the general population. As the participants were instructed to eat and drink what they wanted, not a certain quantity, the option to completely reject the breakfast was available. The fact that energy intake was high after both *ad libitum* breakfasts (approximately 75% of recommended daily energy intake for a man) suggests that participants found the breakfasts palatable and easy to eat. However, in order that participants did not become suspicious of the fact that energy intake was of interest

to the investigators, no ratings of palatability or appetite were asked for. One participant stated after his first trial that he disliked the milkshake and that it could be removed for his second trial. The milkshake was retained for the second trial on the proviso that he may change his mind, but none of the milkshake was consumed. The volume consumed during the first trial was also minimal (55 ml supplying ~ 81 kcal of energy), and total energy intake was very similar, therefore this participant's data were not removed from the group data set.

There was not a consistent effect of prior exercise on total energy intake or intake of solid food (croissants) in this study, but ingestion of liquid energy (milkshake) was increased, with 6 participants drinking more milkshake on the day after exercise, one drinking the same and one drinking slightly less. The participant drinking less was the same participant mentioned previously who declared a dislike for the drink. This participant completed his exercise trial after his control trial and therefore did not drink any milkshake on the day after exercise. When this participant's data were removed from the data set and the intake of milkshake was reanalysed (with $N = 7$), exercise was found to significantly increase milkshake energy intake (20.6% increase; $P = 0.047$). The participants could theoretically have had a greater thirst on the day after exercise, with the increased intake of milkshake reflecting an attempt to rehydrate the body. This is unlikely, however, as water was freely available during the 90-minute treadmill walk and after exercise with the evening meal, and participants were free to drink as much water as they wished up until the time they came into the lab for the *ad libitum* breakfast. In further support for the greater milkshake intake after exercise in this study, increased intake of liquid-source energy, after both low and high intensity exercise, has been reported previously when *ad libitum* energy intake was measured 1 h post-exercise

(Thompson et al., 1988). In agreement with our findings, Thompson et al. found that intake of liquid-source energy was greater after exercise despite no increase in total energy intake.

Mean fasting TAG concentration was substantially and significantly lower on the day after exercise than on the day after an equivalent period of seated rest. The ability of moderate-intensity exercise to reduce fasting TAG levels is well documented (Burton et al., 2008; Gill and Hardman, 2000; Gill et al., 2001a; Gill et al., 2001b; Gill et al., 2002a; Gill et al., 2003a; Gill et al., 2004; James et al., 2007; Malkova et al., 2000; Tsetsonis and Hardman, 1996a; Tsetsonis and Hardman, 1996b; Tsetsonis et al., 1997; Zhang et al., 2007), although not all studies investigating the effect of prior exercise on postprandial lipaemia have found fasting values to be significantly decreased on the day after exercise (Gill et al., 1998; Gill et al., 2007; Kolifa et al., 2004; Malkova et al., 1999; Miyashita et al., 2006; Miyashita & Tokuyama, 2008; Zhang et al., 1998; Zhang et al., 2004; Zhang et al., 2006). While mean energy intake was slightly higher on the day after exercise, the total TAG response was significantly lower than control. Seven of the eight men had a reduced total lipaemic response with exercise compared with control. Earlier investigations have found mean reductions in total TAG response ranging from 9.3% after 1 h of walking at 50% VO_2max (Gill et al., 2002b) to 51% after 1 h of treadmill exercise at 60% VO_2max (Zhang et al., 1998), with the majority of studies reporting reductions of 20 – 30 % with prior exercise. All such studies have given participants a test meal containing a preset quantity of energy in control and exercise trials, i.e. all participants ingest a meal with the same energy content or the energy within the meal is scaled to body mass, with heavier subjects eating a larger meal. In contrast, the current study allowed participants a free choice of how much they ingested. In our study, the mean percentage reduction in the TAG area under the curve score (25.7%)

with exercise was in the middle of the range seen in the previous literature and therefore compares favourably with studies using meals of a fixed size. Fasting TAG values were correlated with total lipaemic responses for both control and exercise trials, and the change in fasting TAG with exercise correlated positively with the change in total lipaemic response. These correlations suggest that an individual's baseline TAG concentration is an important determinant of the extent to which plasma TAG will rise after eating. Furthermore, if fasting TAG is reduced after exercise, then the rise in plasma TAG concentration postprandially is also likely to be less than in a control trial. Few previous studies in the relevant literature have reported whether fasting TAG was correlated with the total lipaemic response, but of those which have, most have shown a positive correlation for both control and exercise trials (Burton et al., 2008; Kokalas et al., 2005; Kolifa et al., 2004; Tsetsonis and Hardman, 1996a). One study found a correlation between these variables in the control condition, but not after exercise (Aldred et al., 1994), while another reported a significant correlation after control without mention of the relationship following exercise (Zhang et al., 1998). As the fasting value is included in the total lipaemic response, it is logical to think that the former would correlate with the latter, particularly if the rise in TAG above baseline after eating is not substantial. There is no autocorrelation between fasting TAG concentration and incremental lipaemic response, however, and the relationship between the two is not well documented. One previous investigation found no correlation between measures of fasting TAG and the incremental lipaemic response to a moderate-fat meal (Kokalas et al., 2005). Burton et al. (2008) reported positive correlations between fasting TAG and TAG iAUC when exercise bouts were performed before intake of two moderate-fat meals, but no correlation was found when the same participants undertook a control trial. Other studies undertaken as part of this thesis also show conflicting results. In chapter 3, fasting TAG and iAUC showed a positive

correlation, whereas the work reported in chapter 6 found no such relationship. This study found a positive correlation between fasting TAG and the incremental lipaemic response in a control trial, but not after exercise. The difference in incremental lipaemic response between trials was not correlated with the difference in fasting TAG. The incremental TAG response to the *ad libitum* breakfasts was 26.1% lower on average after exercise, but the response showed greater variation between participants than the total TAG response and therefore, when compared with the control trial, the difference was not quite statistically significant. The difference in energy intake between trials did not correlate with the difference in incremental lipaemic response, but the three men whose incremental TAG response was unchanged or greater after exercise were three of the four participants who consumed more energy after the exercise trial. To draw firmer conclusions about whether *ad libitum* energy intake affects the ability of exercise to lower the incremental TAG response, studies with greater subject numbers must be undertaken. In this study, exercise lowered the fasting TAG concentration slightly more consistently than the incremental lipaemic response, but both appear to play a role in reducing the total lipaemic response when participants consume an *ad libitum* breakfast.

For most of the men (6 of 8), fasting NEFA concentrations were higher on the morning after exercise, but the mean value was not significantly different from control. While several articles have reported an increase in fasting NEFA on the day after a moderate exercise bout (Gill et al., 1998; Gill & Hardman, 2000; Gill et al., 2001b; Gill et al., 2004; Malkova et al., 1999; Tsetsonis et al., 1997), many more have not (Burton et al., 2008; Gill et al., 2002a; Gill et al., 2007; James et al., 2007; Kolifa et al., 2005; Miyashita et al., 2006; Miyashita and Tokuyama, 2008; Tsetsonis and Hardman, 1996a). As with this study, others still have found

a trend for NEFA to be higher after exercise, but not a statistically significant difference (Gill et al., 2001a; Malkova et al., 2000). Most studies report mean fasting NEFA concentration to be higher following exercise than after an equivalent period of rest, even if the difference between trials is not significant, and fasting NEFA concentration was found to drop by 50% when endurance athletes detrained for 1 week (Gill et al., 2003b). As with NEFA in the fasted state, postprandial concentrations of NEFA tended to be higher in our study after exercise than control, but the difference was not statistically significant.

Fasting plasma glucose was not different between exercise and control trials. The failure of moderate-intensity exercise undertaken on the afternoon/evening of day 1, to lower glucose concentrations on the morning of day 2, is a finding repeated throughout almost all studies investigating the effect of acute exercise on postprandial lipaemia (Burton et al., 2008; Gill et al., 1998; Gill and Hardman, 2000; Gill et al., 2001a; Gill et al., 2001b; Gill et al., 2002a; Gill et al., 2004; James et al., 2007; Kolifa et al., 2005; Malkova et al., 1999; Malkova et al., 2000; Miyashita and Tokuyama, 2008; Tsetsonis & Hardman, 1996a; Tsetsonis & Hardman, 1996b; Tsetsonis et al., 1997; Zhang et al., 2007).

Exercise did not lower fasting or postprandial insulin concentrations in this study. The vast majority of studies show no alteration to fasting insulin concentration on the day after exercise. At least four studies have shown an improvement (Gill et al., 2001b; Gill et al., 2007; Zhang et al., 2006; Zhang et al., 2007), but one of them used patients with type 2 diabetes (Gill et al., 2007), and two of the remaining three, used participants with metabolic syndrome (Zhang et al., 2006; Zhang et al., 2007). Furthermore, Zhang et al. (2006) only found fasting insulin concentration to be reduced after exercise at 70% VO_2max, not at 40%

or 60%. Several previous studies have found postprandial insulinaemia to be reduced on the day after exercise (Burton et al., 2008; Gill et al., 1998; Gill et al., 2000; Gill et al., 2001b; Gill et al., 2002a; Gill et al., 2004; Tsetsonis and Hardman, 1996b; Zhang et al., 2006; Zhang et al., 2007), but none of these studies allowed participants to consume breakfast *ad libitum*. In this study, the incremental insulinaemic response was positively correlated with *ad libitum* energy intake for both control and exercise trials, suggesting that those who consumed more energy at breakfast had larger increases in insulin afterwards. However, the difference in total *ad libitum* energy intake between exercise and control trials did not correlate with the difference in incremental insulinaemic response. Instead, it was the difference in total food (croissant) intake between trials that showed a positive association with the difference in insulin response after breakfast. Every participant eating more croissants after exercise had an increased incremental insulinaemic response compared to control and vice versa. In spite of total energy intake being the same or even greater after an exercise trial, if intake of croissants was reduced relative to the control trial, then the incremental insulinaemic response was also reduced. It must be reiterated that the macronutrient content of the croissants and the milkshake was identical in percentage terms; therefore the croissants did not provide more carbohydrate than the drink as a percentage of total energy. Moreover, the majority of energy taken in during the breakfast came from the milkshake (53.4% and 58.1% of total energy in the control and exercise trials respectively), not the croissants. These findings indicate that the effect of exercise on postprandial insulin concentrations may be moderated by intake of solid foods. If a participant's intake of croissants (solid) was lowered by exercise in this study compared with control, then postprandial insulin concentrations were also lower and vice versa. Most previous studies in the area of exercise and postprandial lipaemia have given meals which are liquid-based; therefore the beneficial effect of exercise in reducing

postprandial insulinaemia may not be so great for a solid-based breakfast. When the effect of isoenergetic meals with identical or very similar macronutrient compositions, but different physical forms (solid vs. liquid), on postprandial insulin concentrations has been studied previously, conflicting results have been reported. Two studies have found solid foods to increase insulin concentrations more than liquid foods (Habas and Macdonald, 1998; Keizer et al., 1987); another two studies found the reverse (Brynes et al., 1998; Tieken et al., 2007); and a further study found no difference due to physical form (Clemente et al., 2003). Due to the uncertain impact of physical form of meals on postprandial insulin concentrations, it must be considered that another factor, different between the croissants and milkshake, but not related to their physical form, could be responsible for the greater insulinaemic response in trials where additional croissant was eaten. While the percentage of energy from carbohydrate was the same for the croissants and milkshake, either the quantity and/or the specific type of sugars present may have differed. Raspberry jam for the croissants, and raspberry flavouring for the milkshake, were the major sources of sugar respectively. Unfortunately, the specific sugars present were not identified on the labelling of either product, nor was the total sugar content mentioned; therefore, it is difficult to arrive at a strong conclusion regarding the effect of sugar in the different foodstuffs. Regardless of whether the physical state of the croissants was intrinsically involved in the higher insulin response, it appears that if participants are allowed to eat and drink as much or as little as they wish after control and exercise sessions, the ability of prior moderate exercise to reduce postprandial insulin concentrations is minimal.

It is acknowledged that the current study design does have potential limitations. Firstly, a large volume of liquid-based energy (2 litres of milkshake) was available to the participants as

part of the *ad libitum* breakfast. As energy-yielding liquids elicit weaker suppressive appetitive responses than solids (Haber et al., 1977; Hulshof et al., 1993; Mattes and Rothacker, 2001; Tsuchiya et al., 2006), it is possible that the palatability and ease of ingestion of this form of energy was a factor in the high energy intake seen with the breakfast. Unfortunately, we do not have data regarding the participants' habitual breakfast energy intake, but, despite their overweight/obese status, it appears unlikely that they would regularly consume 1800 – 2000 kcal at one sitting for breakfast. Indeed, a recent study involving 3610 men and women reported that being obese is associated with omitting breakfast (Berg et al., 2009). Secondly, the men will have been aware that no further food would be available during the 6 h after the *ad libitum* breakfast; this may have prompted them to consume more energy than in a situation outside of the laboratory where food and drink were freely available throughout the day. If the true effect of exercise in a free-living environment is to promote only a small increase in *ad libitum* energy intake, then this subtle shift may have been overridden and masked by the high energy intake in both trials. However, the fact that *ad libitum* energy intake over a 48 h period after exercise was not significantly greater than in a control trial (King et al., 1994; King et al., 1997) suggests that acute exercise is unlikely to alter energy intake substantially. Studies measuring energy intake over a 48-hour postexercise period may dilute any exercise-induced increase in energy intake, as the effect of exercise is unlikely to last 48 hours. The measurement of *ad libitum* energy intake over the 12 h or 24 h period following an exercise bout may be a more appropriate experimental approach. A recent study has, however, reported that when *ad libitum* energy intake was measured over a 26 h period (including the morning after an exercise bout), no difference was found between a control trial and trials where a 45-minute bout of treadmill running was

performed (Guelfi et al., 2009); therefore confirming the findings of King et al. (1994) and King et al. (1997).

Eating behaviour is strongly habitual; therefore a single bout of exercise performed 13 h before breakfast, should perhaps not be expected to exert a consistent measurable effect on *ad libitum* energy intake. While the buffet breakfast provided for the participants was intended to represent a real meal, it is unlikely that all men would regularly eat such a high-fat, energy-laden breakfast. Therefore the novelty of the food may in itself have contributed to the heterogeneity in eating response between participants. Moreover, as the intra-subject variability of *ad libitum* energy intake after an overnight (12 h) fast, without prior exercise, was not tested in this study, it cannot be stated with any certainty how reproducible the eating responses of the participants would be. For this reason, it is not known how large an increase in energy intake above control would have to be recorded after exercise before the difference could confidently be considered to exceed the standard error of the measurement. If possible, future investigations should test the reproducibility of *ad libitum* energy intake for any buffet/meal they plan to provide.

As detailed in the methods section, concerted efforts were made to ensure participants could not easily replicate the energy intake from their first trial during their second visit, and the single-blind design was preserved throughout the study, with none of the participants aware that their energy intake was being recorded. Also, while participants did not have a familiarisation trial, the order in which trials were run was counter-balanced and there was no effect of trial order on either *ad libitum* energy intake or postprandial lipaemia. Therefore, despite the potential limiting factors mentioned above, we feel the study design was robust

and allowed for fair assessment of whether prior exercise exerted a true measurable effect on *ad libitum* energy intake 13 h later.

In summary, energy intake was not significantly increased, relative to a control trial, when middle-aged, overweight and obese inactive men performed a brisk 90-minute treadmill walk 13 h before an *ad libitum* breakfast. The difference in *ad libitum* energy intake between exercise and control trials showed considerable inter-individual variability and was not unidirectional; both increases and decreases in energy intake, compared with the control trial, were seen after exercise. The total area under the plasma triacylglycerol versus time curve was lower with prior exercise for almost all participants and the mean reduction in TAG response from a control trial was statistically significant. Due to the strong positive correlation observed between the change in fasting TAG and the change in total TAG response with exercise, it appears likely that the reduction in total postprandial lipaemia was driven by the reduction in baseline values. An attenuated incremental TAG response was also seen for most participants and will have contributed to the lower postprandial TAG concentrations, but the incremental lipaemic response may not always be lower following exercise if energy intake is higher than after a control trial. Based on these findings, moderate-intensity exercise is likely to reduce postprandial lipaemia in a non-laboratory setting where access to food and drink is unrestricted, and should therefore be recommended as a strategy to lower risk of cardiovascular disease through its beneficial effects on lipid metabolism.

CHAPTER SIX

Is the beneficial effect of prior exercise on postprandial lipaemia partly due to redistribution of blood flow?

ABSTRACT

Background: Performing aerobic exercise prior to intake of a high-fat meal lowers postprandial lipaemia, however, the mechanisms responsible are still not clear. This study investigated whether blood flow to skeletal muscle and/or the liver was increased in the postprandial period after exercise, relative to a control trial, and whether this resulted from increased cardiac output or redistribution of flow.

Design: Eight overweight, inactive males, aged 49.4 ± 10.5 years (mean ± SD) acted as their own controls in a counter-balanced design, either walking briskly for 90 minutes at 60% VO_2max (EX), or resting in the lab (CON), on the evening of day 1. The following morning a fasting blood sample was collected, participants consumed a high-fat breakfast and further venous blood samples were drawn hourly for 6 h. Immediately after blood sampling, Doppler ultrasound was used to measure cardiac output and blood flow through both the femoral artery of one leg and the hepatic portal vein, with the ultrasonographer blinded to trial order.

Results: The total postprandial TAG response was 22% lower after EX ($P = 0.001$). Blood flow through the femoral artery and the hepatic portal vein was increased by 19% ($P < 0.001$) and 16% ($P = 0.033$) respectively during the 6 h postprandial period following EX; however, postprandial cardiac output did not differ between trials ($P = 0.065$).

Conclusions: Redistribution of blood flow, to both exercised skeletal muscle and the liver, may play a role in reducing the plasma TAG response to a high-fat meal on the day after an exercise bout.

6.1. INTRODUCTION

As outlined in the introduction to this thesis, evidence from both case-control and mechanistic studies suggests that an elevated postprandial TAG concentration is an independent risk factor for CHD. Therefore, reducing the accumulation of triglyceride-rich lipoproteins during the postprandial period presents a viable target for lowering arteriosclerotic risk.

Researchers in the field of postprandial lipaemia and exercise have consistently shown that plasma TAG concentrations are lowered when aerobic exercise such as walking is undertaken 11-18 h before an oral fat tolerance test (OFTT). Three main mechanisms have been proposed to theoretically explain the lowering of postprandial TAG concentrations seen with prior aerobic exercise. Early studies opined that greater clearance of TAG within TRL, due to increased activity of the enzyme lipoprotein lipase (LPL) within skeletal muscle, was the major mechanism. This belief was largely based on findings from ultra-endurance events, after which substantial increases in postheparin plasma LPL activity were reported (Kantor et al., 1984; Sady et al., 1986). However, growing evidence suggests that moderate-intensity exercise does not consistently upregulate the activity of LPL, despite reductions in postprandial lipaemia in the same studies (Ferguson et al., 1998; Gill et al., 2003a; Herd et al., 2001; Katsanos et al., 2004); therefore other mechanisms are likely to contribute to the exercise-derived effect on lipaemia. The majority of the reduction in total plasma TAG concentration after an acute exercise session is due to a lowering of VLDL-TAG (Malkova et al., 2000; Gill et al., 2001a), therefore it has been postulated that hepatic VLDL secretion may be attenuated following exercise. Indirect evidence, such as an increased serum 3-hydroxybutyrate concentration after exercise (Malkova et al., 2000; Gill et al., 2001a), offers support for a post-exercise switch in the liver's partitioning of fatty acids from esterification

to oxidation pathways, however, kinetic data from studies utilising staple isotope methodologies are lacking. Thirdly, Kolifa et al. (2004) concluded that delayed release of dietary fat from the intestine was responsible for at least part of the TAG-lowering effect of exercise in their study. The rate of appearance of dietary fat into the plasma is not commonly reported in studies investigating the effect of exercise on postprandial lipaemia (presumably because it had not been measured), however, owing to the length of time between cessation of exercise and test meal intake in most studies (typically 12 - 16 hours), it is thought any impact on intestinal TAG release would not be appreciable enough to significantly influence postprandial lipaemia.

Alongside these suggested mechanisms, the possibility exists that upregulation, or redistribution, of blood flow to skeletal muscle and/or the liver may occur on the day following exercise. As the rate of substrate delivery exerts a strong influence upon the extent of substrate uptake from the plasma, it could be argued that increased blood flow to either of these tissues on the day after an exercise bout would reduce plasma TAG concentrations. It has been reported that blood flow to skeletal muscle is maintained above resting levels for at least 90 minutes after moderate-intensity exercise (Williams et al., 2005) and earlier work has shown that postprandial, but not fasting, calf blood flow is elevated above control values on the day after a 2 h treadmill run (Malkova et al., 2000). If blood flow to skeletal muscles used during a treadmill walk is increased on the day after exercise, it is possible the increased flow (and assumed greater substrate delivery) will present the opportunity for greater clearance of TAG into the muscle. As the legs contain the major muscles being worked during walking, femoral artery blood flow was monitored in the current study to assess whether blood flow to skeletal muscle exercised on the previous day was increased in the fasting state 13 h later and

during the postprandial period 13 - 19 h later. Despite the major influence of the liver on lipid metabolism and its theoretical role as the organ largely responsible for the lowering of postprandial lipaemia following moderate exercise, to our knowledge, no published work has investigated whether hepatic blood flow (in the fasted state or postprandially) is altered on the day after an exercise bout. Therefore, in addition to investigating femoral artery blood flow, this study included measurements of blood flow through the hepatic portal vein. To determine whether any increases in femoral/hepatic blood flow were due to specific redistribution or were secondary to an increase in whole-body blood flow, cardiac output measurements were also made.

6.2. METHODS

6.2.1. Participants

Eight healthy, nonsmoking, sedentary, overweight men gave their written informed consent to participate in the study. Physical characteristics of the participants are presented below in **Table 6.1**. None of the men performed more than one hour of structured physical activity per week. All men were free from cardiovascular disease and none were taking medication known to affect lipid metabolism.

Table 6.1.
Characteristics of the eight study participants

	Mean ± S.D.
Age (years)	49.4 ± 10.5
Height (m)	1.72 ± 0.06
Body mass (kg)	91.7 ± 10.0
BMI ($kg \cdot m^{-2}$)	31.0 ± 3.0
Body fat (%)	28.8 ± 4.6
Estimated VO_2max ($ml \cdot kg^{-1} \cdot min^{-1}$)	34.3 ± 5.7

6.2.2. Study design

To investigate the effect of brisk walking on postprandial lipaemia and blood flow, volunteers participated in two oral fat tolerance tests with differing preconditions: a brisk 90-minute treadmill walk (EX) and a trial where no prior exercise was performed (CON). The order in which the trials were completed was initially randomised, then counter-balanced, to ensure that an equal number of participants completed the trials in each order. A minimum wash out period of 4 days separated oral fat tolerance tests for each participant. Dietary and physical activity restrictions prior to the main trials are described in **General Methods** (section 2.2.2)

6.2.3. Preliminary exercise testing

An in-depth description of the test is presented in the **General Methods** (section 2.3).

6.2.4. Main trials

The protocol for exercise and control trials can be found in **General Methods** (section 2.4).

6.2.5. Body fat estimation

Body fat percentage was estimated as described in **General Methods** (section 2.7).

6.2.6. Evening (post-intervention) meal

Evening meals were provided as described in the **General Methods** (section 2.5). The mean energy (± S.D.) provided by the meal was 4.39 ± 0.79 MJ (1049 ± 188 kcal).

6.2.7. Oral fat tolerance tests

The OFTT protocol was as described in **General Methods** (section 2.6), with the addition that participants remained supine after every blood sample to allow ultrasound measurement

of blood flow through the aorta, hepatic portal vein and femoral artery (further details of the methodology employed can be found in section 6.2.8.). The same investigator made all measurements used to derive blood flows and was blinded throughout the study as to whether participants had exercised on the previous evening, thus eliminating any potential bias. The test meal in this study provided 0.91g fat, 1.64g carbohydrate and 0.41g protein per kg body mass (68.6 kJ per kg body mass; 50% fat, 40% carbohydrate, 10% protein as percentage of total energy). The meal contained 6.29 ± 0.24 MJ (1504 ± 58 kcal) of energy, had a mean fat load of 83.5 ± 3.2 g, and consisted of a raspberry milkshake (whole milk, double cream, sugar and raspberry flavouring), apricot cereal bars, and oven-cooked croissants with butter and raspberry jam.

6.2.8. Ultrasound measurements

Echocardiographic measurements were made using a Philips Sonos 7500 ultrasound system (Philips Medical Systems, Bothell, Washington, U.S.A.) with an S3 two-dimensional transducer (1-3 MHz). Digital images of spectral waveforms were recorded for later analysis. For each measurement point, a minimum of three spectral waveforms were recorded at end-expiration, or as close as possible to it, before being averaged. By employing this technique, measurements for cardiac output could be averaged in 60-second intervals. Heart rate and a respiratory waveform were also recorded.

An apical five-chamber view of the heart was used with Doppler mode to identify flow through the aortic valve during systole. Using pulsed-wave spectral mode at a screen sweep speed of 100 $mm \cdot s^{-1}$, the velocity profile of the aortic flow was obtained. Doppler sampling of the flow was taken immediately below the orifice of the aortic valve. The flow was

quantified automatically using the velocity time integral (VTI), which is the mean distance through which blood travels in the outflow tract during ventricular contraction. Each measurement of VTI was made from at least three velocity profiles taken towards the end of expiration. Aortic valve diameter (d) was measured from a parasternal long axis view, thus allowing the aortic valve area (A) to be calculated using the formula $A = \pi \cdot (d/2)^2$. Stroke volume (SV) was calculated from VTI x A; cardiac output was calculated from SV multiplied by HR.

Two-dimensional (2-D) and Doppler ultrasound measurements were made using the same ultrasound system (Philips Medical Systems, Bothell, WA) with a linear-array transducer transmitting a frequency of 12 MHz. Longitudinal images of the femoral artery (proximal to any branching) and the hepatic portal vein were obtained, and the average of several diameter measurements made during the R wave for each vessel was taken. At the same location, blood velocity was measured using pulsed-wave Doppler at the centre of the vessel. Using the 2-D and Doppler ultrasound measurements, blood flow through the femoral artery and the hepatic portal vein was then calculated using the equation $V\pi \cdot (d/2)^2$, where V is the mean velocity of blood flow through the vessel and d is the diameter of the vessel.

6.2.9. Analytical procedures

General Methods (section 2.8) describes the analytical procedures which were carried out. Within-batch coefficients of variation were 2.3% for TAG (after correction for plasma glycerol), 1.1% for NEFA, 2.1% for glucose and 5.7% for insulin.

6.2.10. Calculations and statistics

For orthogonal polynomial contrast analysis, logarithmic transformation of time was found to improve symmetry for NEFA, glucose, insulin, heart rate, cardiac output and portal vein blood flow, but not for TAG, stroke volume and femoral artery blood flow; therefore, analysis for the first six variables was performed with time logged to the base *e*, whereas the last three variables were analysed without logarithmic transformation of time.

6.3. RESULTS

6.3.1. Responses during brisk walking

Participants walked at a speed of 6.0 ± 0.2 $km \cdot h^{-1}$ up a gradient of 2.9 ± 0.7 %. Mean oxygen uptake during the walk was 20.4 ± 1.1 $ml \cdot kg^{-1} \cdot min^{-1}$, thereby representing 59.7 ± 0.6 % VO_2max. The gross energy expenditure during the walk was 3.51 ± 0.23 MJ, with 60.2 ± 3.3 % of the energy derived from carbohydrate and 39.8 ± 3.3 % from fat. Mean heart rate calculated from measurements taken every 5 seconds during the 90-minute walk was 128 ± 3 $beats \cdot min^{-1}$. The mean rating of perceived exertion from the Borg RPE Scale (Borg, 1982) was 13.1 ± 0.7; this equates to the descriptor “somewhat hard” on this scale.

6.3.2. Metabolite concentrations and blood flows in the fasted state

Fasting plasma concentrations of triacylglycerol, NEFA and glucose, serum concentrations of insulin, HOMA scores and tissue blood flows are presented in **Table 6.2**. Mean plasma TAG concentration was 12.1% lower after walking compared with the control trial; this difference reflects a trend for TAG to be lower with exercise, but the effect was not statistically significant (effect of trial, $P = 0.062$). Heart rate was significantly increased, relative to control, following exercise (4.0% increase; effect of trial, $P = 0.018$), whereas stroke volume

was effectively unchanged (0.7% increase with exercise; effect of trial, $P = 0.847$). Cardiac output was 4.6% greater on the morning after walking, but this was not a significant change relative to control (effect of trial, $P = 0.296$). Flows through both the hepatic portal vein and the femoral artery were increased in the fasted state following exercise (increases of 16.4% and 16.7% respectively, relative to control values), however only the increase in hepatic flow was found to be of statistical significance (effect of trial, $P = 0.044$ and $P = 0.094$ respectively). When hepatic portal vein blood flow was expressed relative to cardiac output in the fasted state in each trial, the proportion of cardiac output flowing through the hepatic portal vein was not significantly different between trials (mean increase of 10.6% with exercise; $P = 0.224$). The mean percentage of cardiac output flowing through the femoral artery in the fasted state was also unchanged by prior exercise (7.3% increase on control values; $P = 0.469$). Exercise and control trial values for plasma glucose and NEFA concentration, serum insulin concentration, and HOMA score, were similar, and none of the differences between these variables approached statistical significance ($P > 0.05$ for all).

Table 6.2. Plasma and serum metabolite concentrations, and tissue blood flows for the eight participants, in the fasted state, for both walking and control trials (mean ± S.E.M.)

Measure	Control	Exercise	*P*
TAG ($\mu mol \cdot l^{-1}$)	1211 ± 142	1064 ± 134	0.062
NEFA ($\mu mol \cdot l^{-1}$)	310 ± 46	327 ± 47	0.815
Glucose ($mmol \cdot l^{-1}$)	6.08 ± 0.40	6.27 ± 0.40	0.352
Insulin ($\mu IU \cdot ml^{-1}$)	14.1 ± 2.5	16.0 ± 4.9	0.579
HOMA	1.89 ± 0.34	2.13 ± 0.63	0.583
Heart rate ($beats \cdot min^{-1}$)	63.6 ± 2.8	66.1 ± 2.7*	0.018
Stroke volume (ml)	77.8 ± 4.2	78.4 ± 3.2	0.847
Cardiac output ($ml \cdot min^{-1}$)	4945 ± 360	5173 ± 298	0.296
Hepatic portal vein blood flow ($ml \cdot min^{-1}$)	174 ± 14	202 ± 21*	0.044
Femoral artery blood flow ($ml \cdot min^{-1}$)	372 ± 28	434 ± 51	0.094

*Different from control ($P < 0.05$).

6.3.3. Postprandial metabolite concentrations and blood flows

6.3.3.1. Triacylglycerol

Main effects and trend analysis

Plasma triacylglycerol concentrations are shown in **Figure 6.1**. Plasma TAG concentrations increased significantly during the postprandial period for both trials (effect of time, $P < 0.001$), but were lower after the walking trial than with control (effect of trial, $P = 0.002$). No significant trial x time interaction was found ($P = 0.105$), however, orthogonal polynomial contrast analysis revealed a non-significant trend for the linear component of the trial x time interaction ($P = 0.077$), which accounted for 87.5% of the total variation; as TAG concentration was higher at all time points in the control condition than following exercise, and only began to fall between 5 and 6 h for both, i.e. at the very end of the observation period, this suggests that the rate of increase was greater during the control trial than when prior exercise had been performed. The peak TAG value was substantially and significantly reduced with exercise (23.9% lower than control; $P = 0.003$), however, the time at which the peak value occurred (relative to the ingestion of the test meal) was not different between trials ($P = 0.529$).

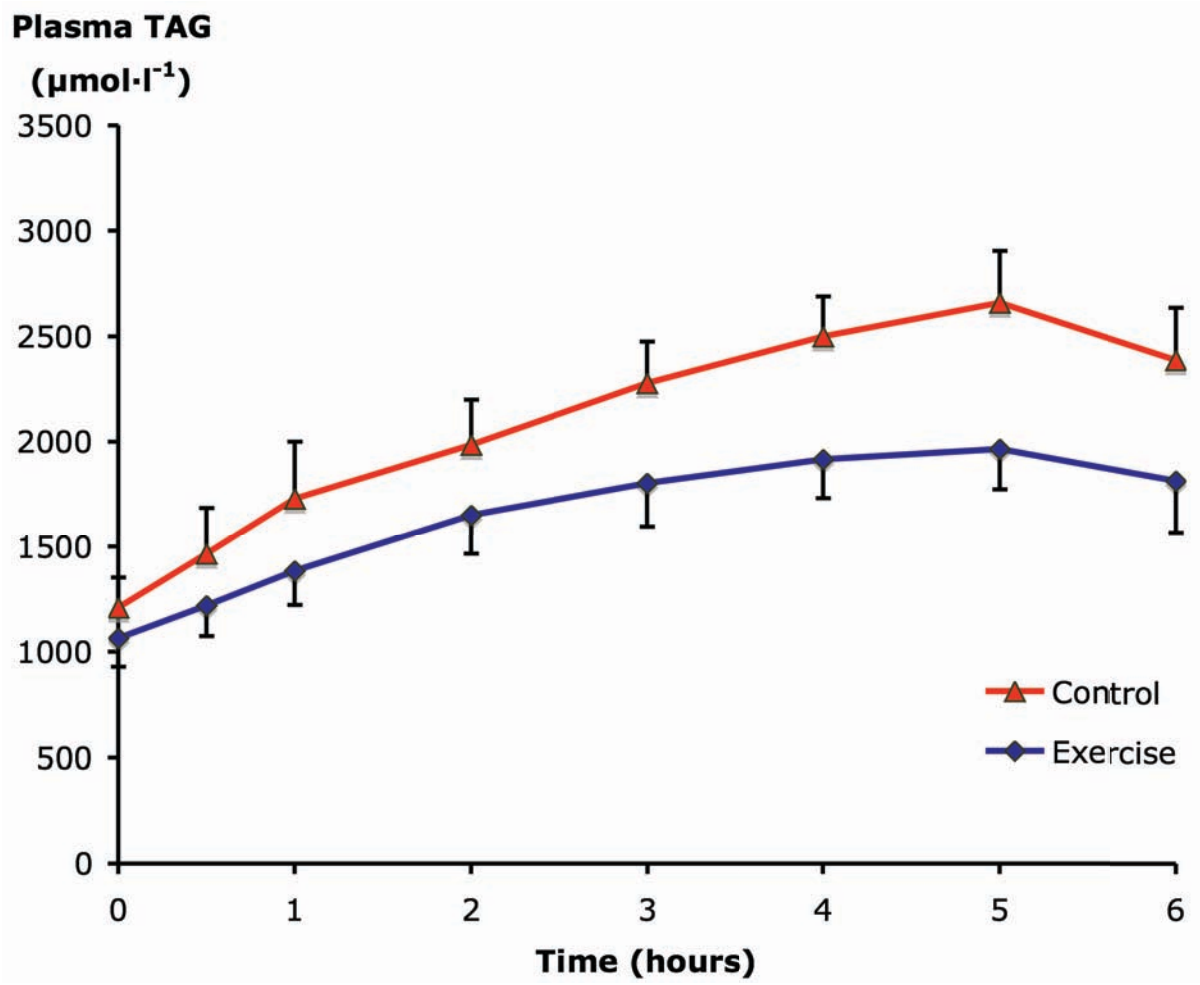


Figure 6.1. Mean (± S.E.M.) plasma triacylglycerol concentrations in the fasted state (0 h) and for 6 h after intake of a fat-rich mixed meal, following either a 90-minute walk (exercise) or seated rest (control) on the previous evening.

AUC scores

Total and incremental lipaemic responses are shown in **Figure 6.2**. Prior walking attenuated both the total and the incremental lipaemic response, relative to the control trial. The total area under the plasma TAG versus time curve was reduced by 21.6% with exercise (P = 0.001); the iAUC was 33.6% lower than control, following exercise ($P < 0.001$). After all analyses were complete, a retrospective power calculation was performed. Based on the

sample size of 8 participants, the power within this study to reject the null hypothesis (that exercise did not reduce TAG AUC) was 0.989.

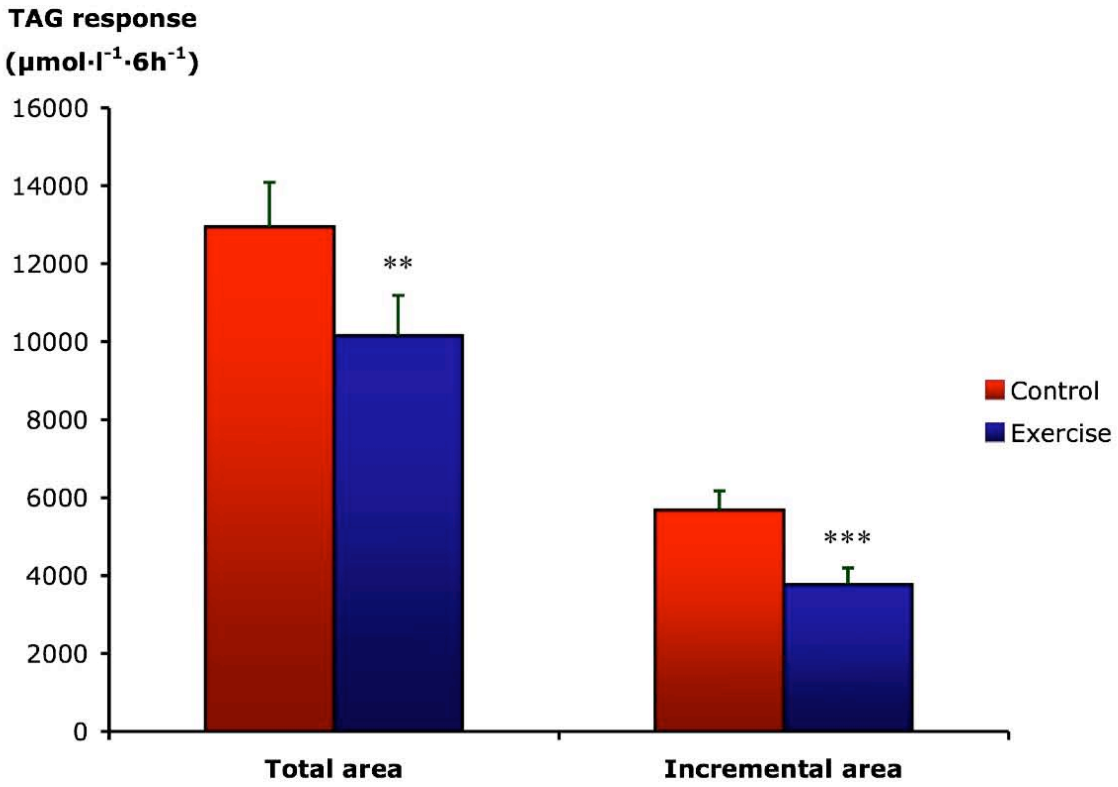


Figure 6.2. Total and incremental area under the curve scores for triacylglycerol over the 6 h postprandial period. **Different from control total area ($P < 0.01$). ***Different from control incremental area ($P < 0.001$).

6.3.3.2. Heart rate

Figure 6.3 illustrates the heart rate response. Heart rate was significantly higher in the exercise trial than the control (effect of trial; $P = 0.042$) and changed significantly across the observation period ($P = 0.004$ for effect of time). Most (68%) of this variation across time was explained by a quadratic function ($P < 0.001$). While peak heart rates occurred 1 – 2 h postprandially in both conditions, the shape of the heart rate response curves was different between trials (trial x time interaction, $P = 0.004$). A linear factor accounted for the largest

proportion of the variation in this interaction (38%), but was not quite statistically significant (P = 0.064). The difference in the shape of the curves over time appears primarily due to the time taken to return to baseline values after the meal. In the control condition the heart rate response to the meal is biphasic, with an increase and subsequent fall between 0 and 3 h, which is then repeated on a smaller scale between 3 and 6 h. In the exercise condition, heart rate increases to a peak at 2 h and then falls steadily.

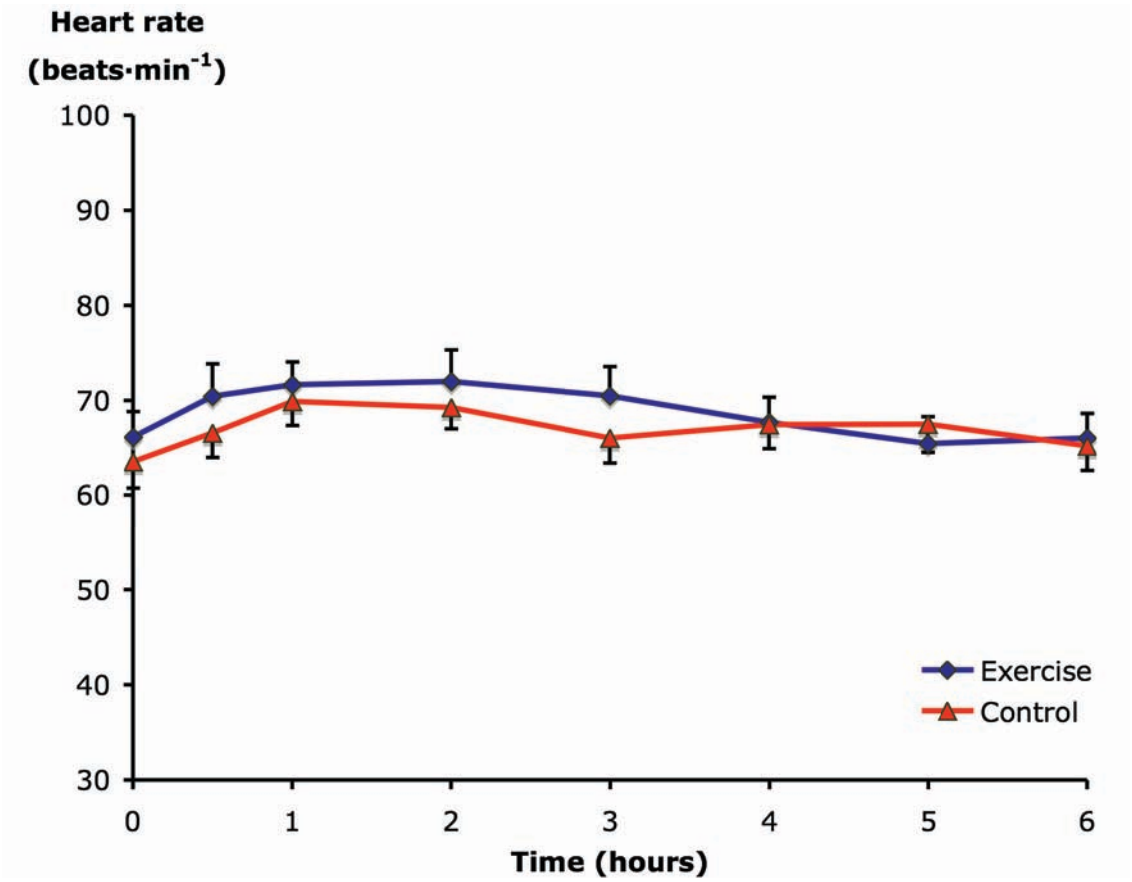


Figure 6.3. Mean (± S.E.M.) heart rate values in the fasted state (0 h) and for 6 h after intake of a fat-rich mixed meal, following either a 90-minute walk (exercise) or seated rest (control) on the previous evening.

6.3.3.3. Stroke volume

The stroke volume response is shown in **Figure 6.4**. Stroke volume increased after eating in most subjects, with peak values commonly occurring 0.5 or 1 h postprandially. A significant change over time was detected for stroke volume (P = 0.001), but the variation over time could not be assigned to one particular polynomial function in any large part, making it difficult to characterise the shape of the curve. Neither the actual values for stroke volume (effect of trial; P = 0.494), nor the shape of the respective curves (trial x time interaction, P = 0.794), differed between exercise and control trials.

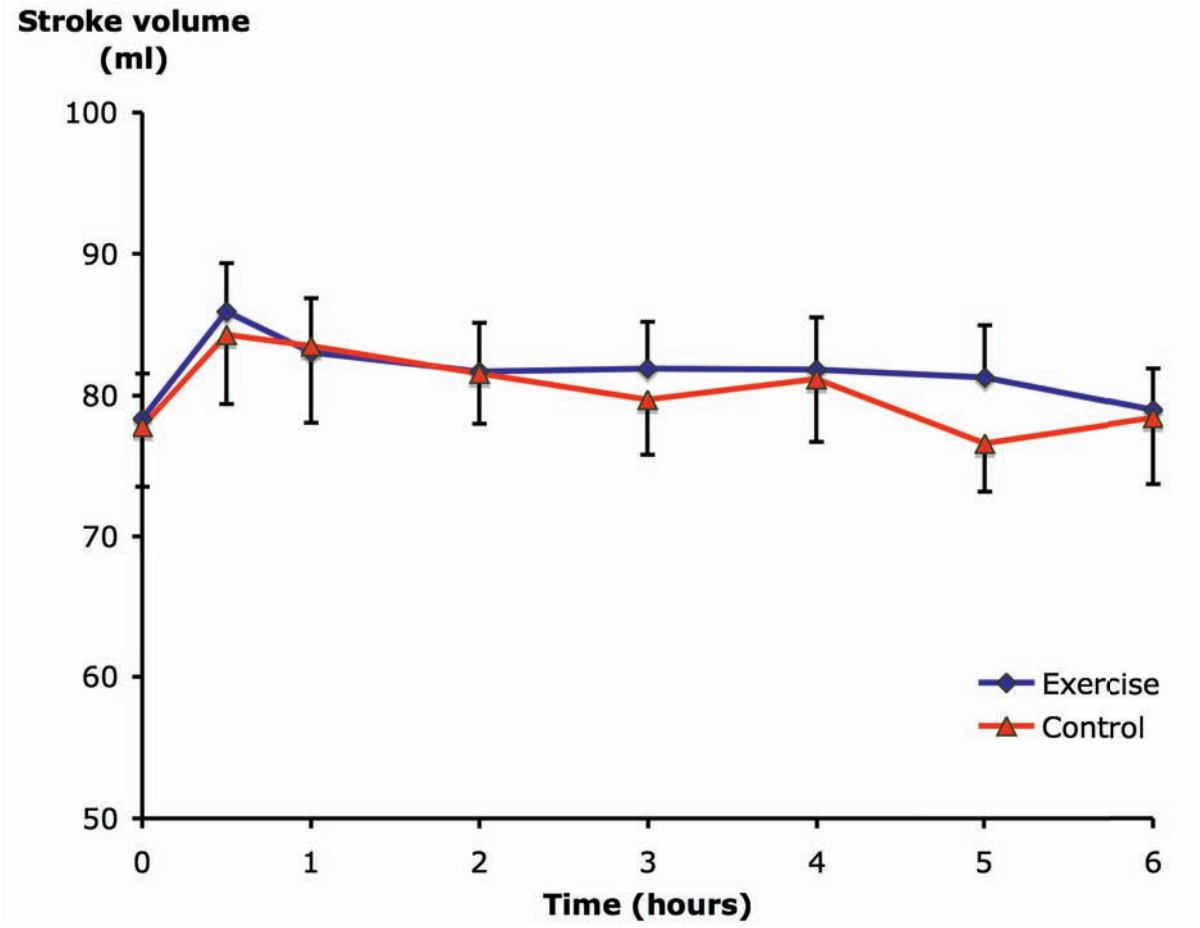


Figure 6.4. Mean (± S.E.M.) stroke volume values in the fasted state (0 h) and for 6 h after intake of a fat-rich mixed meal, following either a 90-minute walk (exercise) or seated rest (control) on the previous evening

6.3.3.4. Cardiac output

Figure 6.5 displays the mean cardiac output values. Cardiac output was significantly altered across the postprandial period ($P < 0.001$ for effect of time); a quadratic function explained 80% of this variation ($P < 0.001$). Peak cardiac output values for the majority of subjects were recorded 30 minutes or 1 h postprandially, with the greatest increase in cardiac output occurring between the fasting and 30 minute post-meal samples. While cardiac output was increased at all time points on the day after the 90-minute walk, relative to control, the difference was modest and did not quite reach statistical significance (effect of trial; $P = 0.061$).

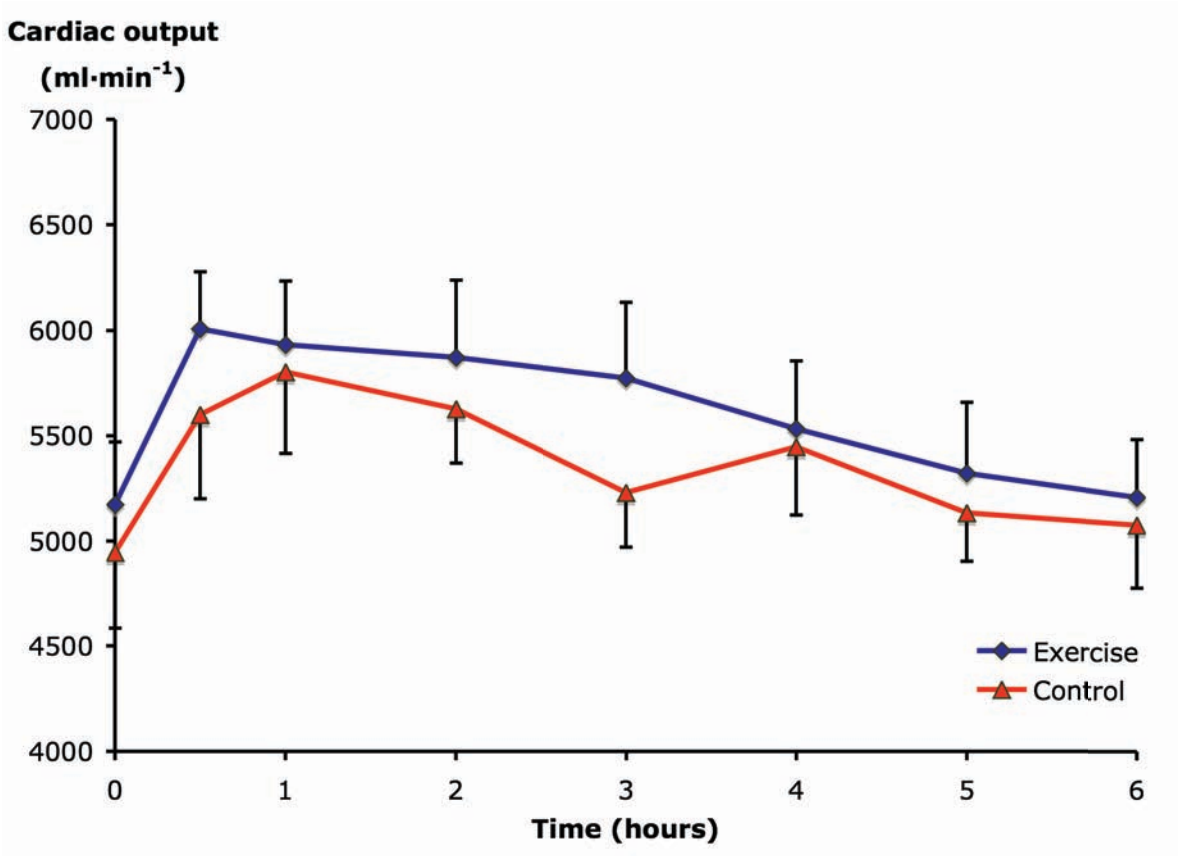


Figure 6.5. Mean (± S.E.M.) cardiac output values in the fasted state (0 h) and for 6 h after intake of a fat-rich mixed meal, following either a 90-minute walk (exercise) or seated rest (control) on the previous evening.

6.3.3.5. Hepatic portal vein blood flow

Main effects and trend analysis

Mean values for blood flow through the hepatic portal vein are shown in **Figure 6.6**. A significant change was seen in the flow through the hepatic portal vein postprandially ($P < 0.001$ for effect of time); 79% of this variation could be attributed to a quadratic function ($P = 0.002$). The temporal pattern of the change in flow was similar to that observed with cardiac output, i.e. flow increased rapidly and sharply from fasting values, with most subjects experiencing peak flow rates within 1 h of meal ingestion. Flow rates slowed over the remaining 5 h, but were still generally above baseline at 6 h. Hepatic portal vein blood flow was elevated after exercise, with flow increased at all time points relative to the control trial (effect of trial; $P = 0.027$). When hepatic portal vein blood flow was expressed as a percentage of cardiac output (to examine whether blood was being redistributed to the liver), values were higher in the exercise trial at all time points, but the effect was not statistically significant ($P = 0.105$). The percentage of cardiac output flowing through the hepatic portal vein changed with time ($P = 0.001$), and, similar to total hepatic portal vein flow, the shape of change largely reflected a quadratic function (55% of variation; $P = 0.025$), with peak percentages seen at 0.5 h in the control trial and 2 h in the exercise condition.

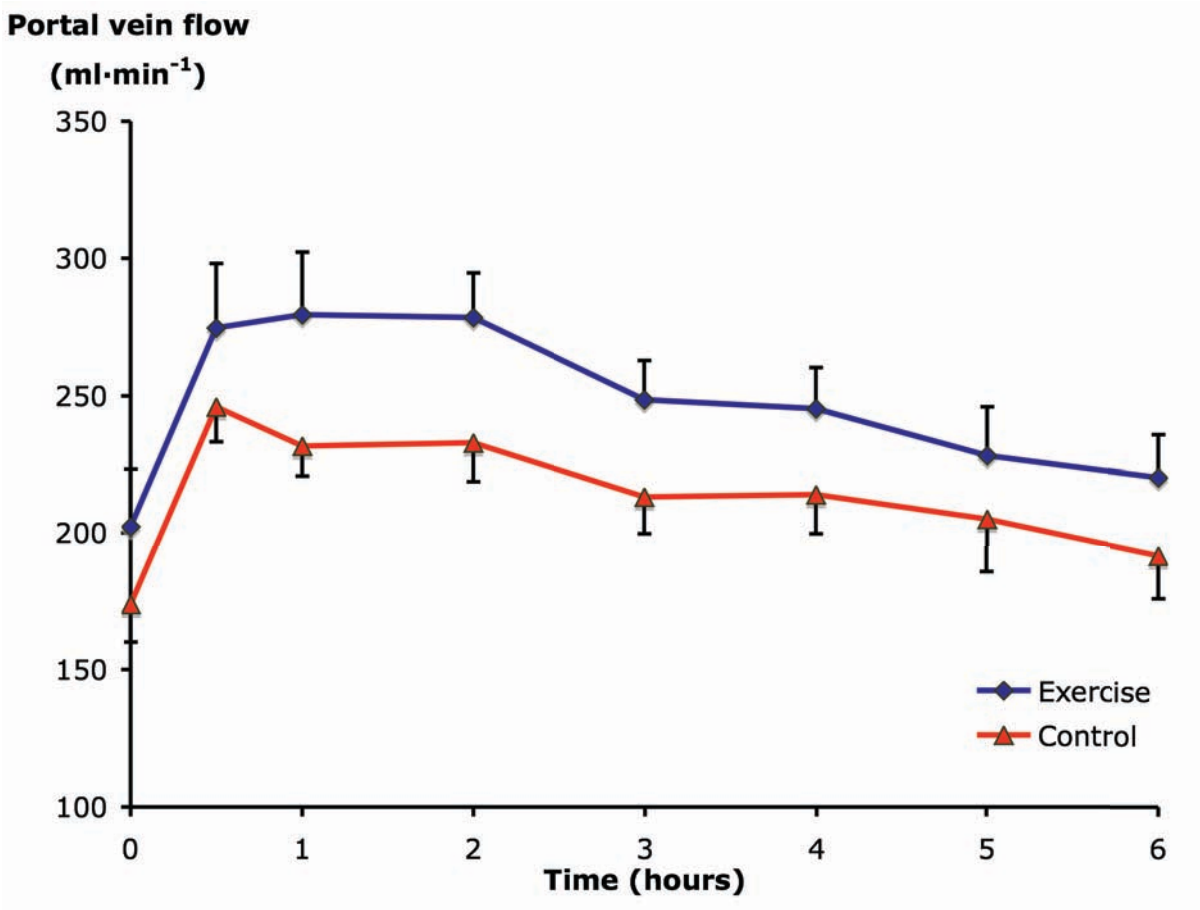


Figure 6.6. Mean (± S.E.M.) values for hepatic portal vein blood flow in the fasted state (0 h) and for 6 h after intake of a fat-rich mixed meal, following either a 90-minute walk (exercise) or seated rest (control) on the previous evening.

AUC scores

Following the exercise trial, the total area under the hepatic portal vein blood flow vs. time curve was 16.0% greater than the AUC for the control trial (effect of trial, $P = 0.033$). When the hepatic portal vein blood flow AUC was expressed relative to the AUC for cardiac output (thus allowing an estimation of flow redistribution to the liver after exercise), it represented 4.05 ± 0.22 % of cardiac output in the control condition and 4.52 ± 0.35 % after the exercise bout. This equated to an 11.6% increase in the percentage of cardiac output distributed to the liver (via the hepatic portal vein) with exercise, but was not a statistically significant change ($P = 0.113$). The area under the incremental curve was 14.2% greater with exercise than

control, however, this belies the considerable variation in the responses between subjects; no significant effect of exercise on iAUC was detected (effect of trial, $P = 0.648$).

6.3.3.6. Femoral artery blood flow

Main effects and trend analysis

Mean values for femoral artery blood flow can be seen in **Figure 6.7**. The rate of femoral artery blood flow varied across the postprandial period ($P = 0.014$ for effect of time), with a quadratic function able to account for 77% of this variation ($P = 0.006$). Depicted graphically, the temporal pattern of change in femoral artery blood flow does not appear to follow the same time course as that seen previously with cardiac output and hepatic portal vein blood flow. Indeed, the temporal pattern of blood flow through the femoral artery is not so easily characterised. It can be said, however, that blood flow through the femoral artery was greater in the exercise trial than the control trial at all time points (effect of trial; $P < 0.001$). The curves for the two trials did not differ in their overall shape (trial x time interaction, $P = 0.274$), but whereas the exercise curve has a fairly distinct increase, plateau and fall over the 6 h period, the control trial is not as parabolic, instead having several, more subtle, fluctuations over time. This graphical observation is borne out in the form of a trial x time interaction in the quadratic aspect of the curves ($P = 0.049$), which explains 48% of the variation within the main trial x time interaction term. The percentage of cardiac output received by the femoral artery was significantly increased in the exercise trial compared with control ($P = 0.010$). There was a main effect of time ($P = 0.025$), with significant linear (43%; $P = 0.014$) and cubic (36%; $P = 0.039$) components responsible for almost 80% of the total variation. As cardiac output increased immediately after the test meal, but femoral blood flow did not, the percentage of cardiac output received by the femoral artery actually dropped

from fasting to 30 min after meal ingestion, particularly during the exercise trial. The exercise trial tended to show a cubic shape across time for the percentage of cardiac output received by the femoral artery, with a sharp drop initially, a prolonged increase between 30 min and 4 h, and a small reduction at the end of the postprandial observation period. In contrast, the percentage of cardiac output received by the femoral artery did not change markedly over time in the control condition.

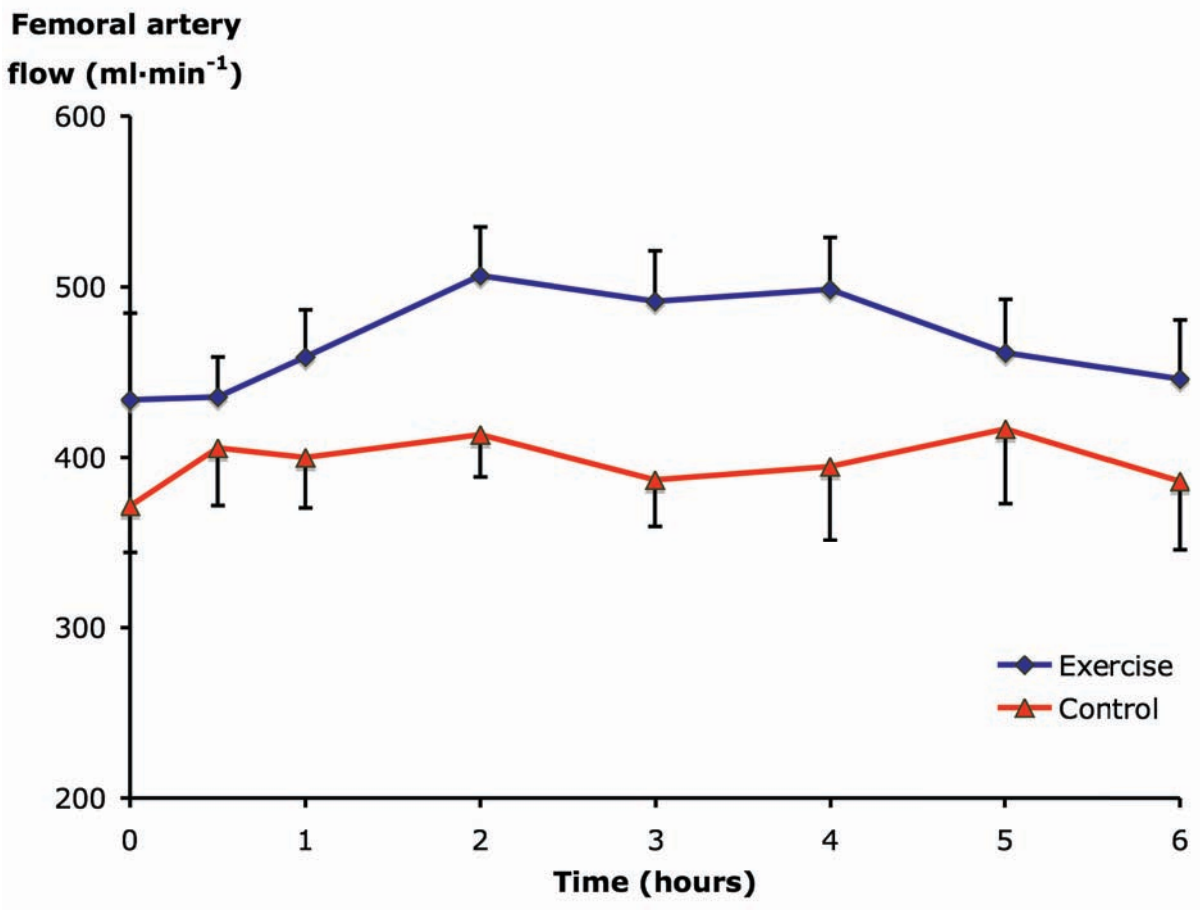


Figure 6.7. Mean (± S.E.M.) blood flow rates through the femoral artery in the fasted state (0 h) and for 6 h after intake of a fat-rich mixed meal, following either a 90-minute walk (exercise) or seated rest (control) on the previous evening.

AUC scores

An 18.8% increase in the total area under the femoral artery blood flow vs. time curve was evident when the results from the exercise trial were compared with control values (effect of trial, $P < 0.001$). The AUC for femoral artery blood flow accounted for 7.49 ± 0.58 % of cardiac output in the control trial and 8.56 ± 0.59 % in the exercise trial. A significant redistribution of cardiac output to the leg, via the femoral artery (mean increase of 14.4%), was therefore apparent on the day following exercise ($P = 0.010$). Incremental area under the curve scores demonstrated the enormous variability in this measure between individuals, and while the mean iAUC was 46.0% greater following exercise, this mean does not reflect the group well, as evinced by the nonsignificance of this relatively large mean increase ($P = 0.742$).

6.3.3.7. NEFA

Plasma NEFA concentrations are depicted in **Figure 6.8**. Plasma NEFA concentration fell from fasting values in the two hours after meal ingestion before rebounding to above baseline by 6 h (effect of time; $P < 0.001$). The shape of the NEFA curve was largely quadratic, with 86% of the variation over time accounted for by a quadratic function ($P < 0.001$). NEFA concentrations were higher after exercise than control at all time points and a significant main effect of trial was found ($P = 0.028$). The change in NEFA concentration across time was not different between the two trials (trial x time interaction; $P = 0.891$).

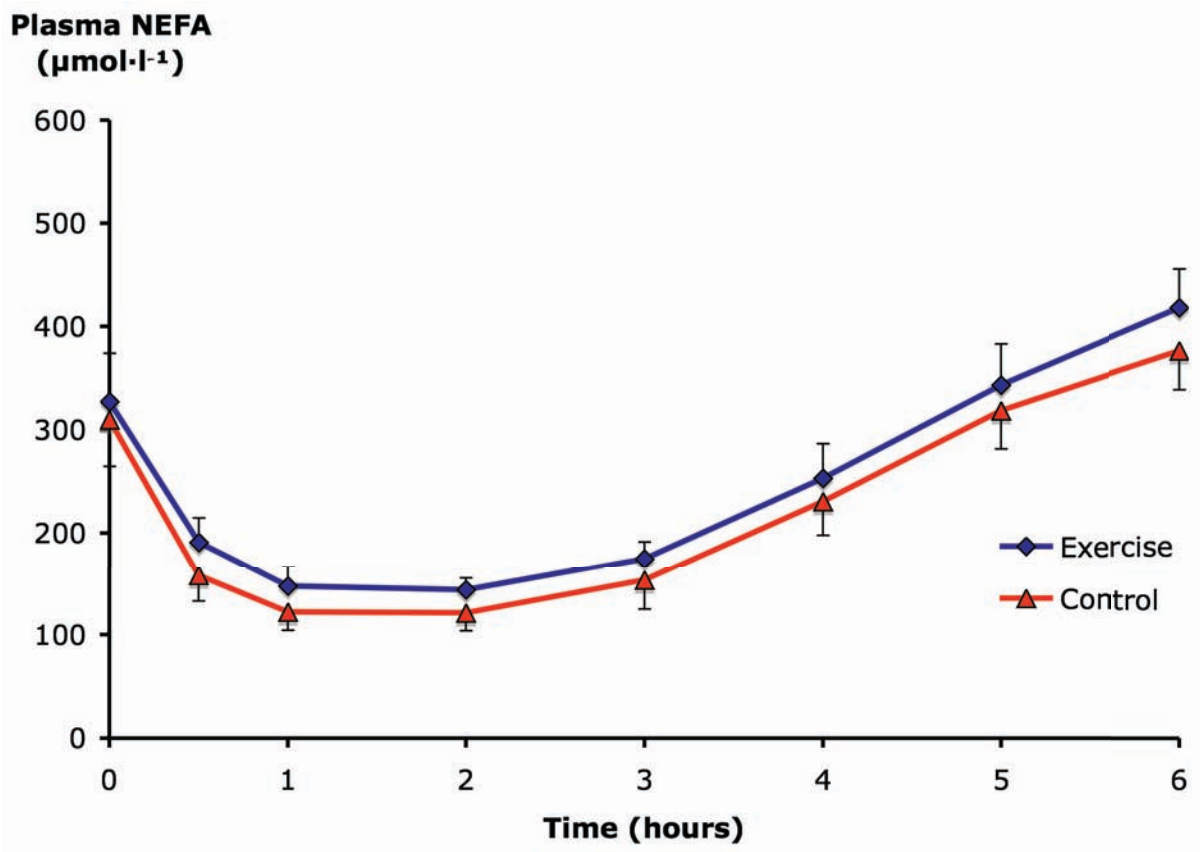


Figure 6.8. Mean (± S.E.M.) plasma NEFA concentrations in the fasted state (0 h) and for 6 h after intake of a fat-rich mixed meal, following either a 90-minute walk (exercise) or seated rest (control) on the previous evening.

6.3.3.9. **Glucose**

Plasma glucose concentrations are illustrated in **Figure 6.9**. Plasma glucose concentration changed significantly across time ($P = 0.007$), but no differences between trials were observed ($P = 0.979$). Neither the total ($P = 0.872$), nor the incremental glycaemic response ($P = 0.536$) was significantly different between trials.

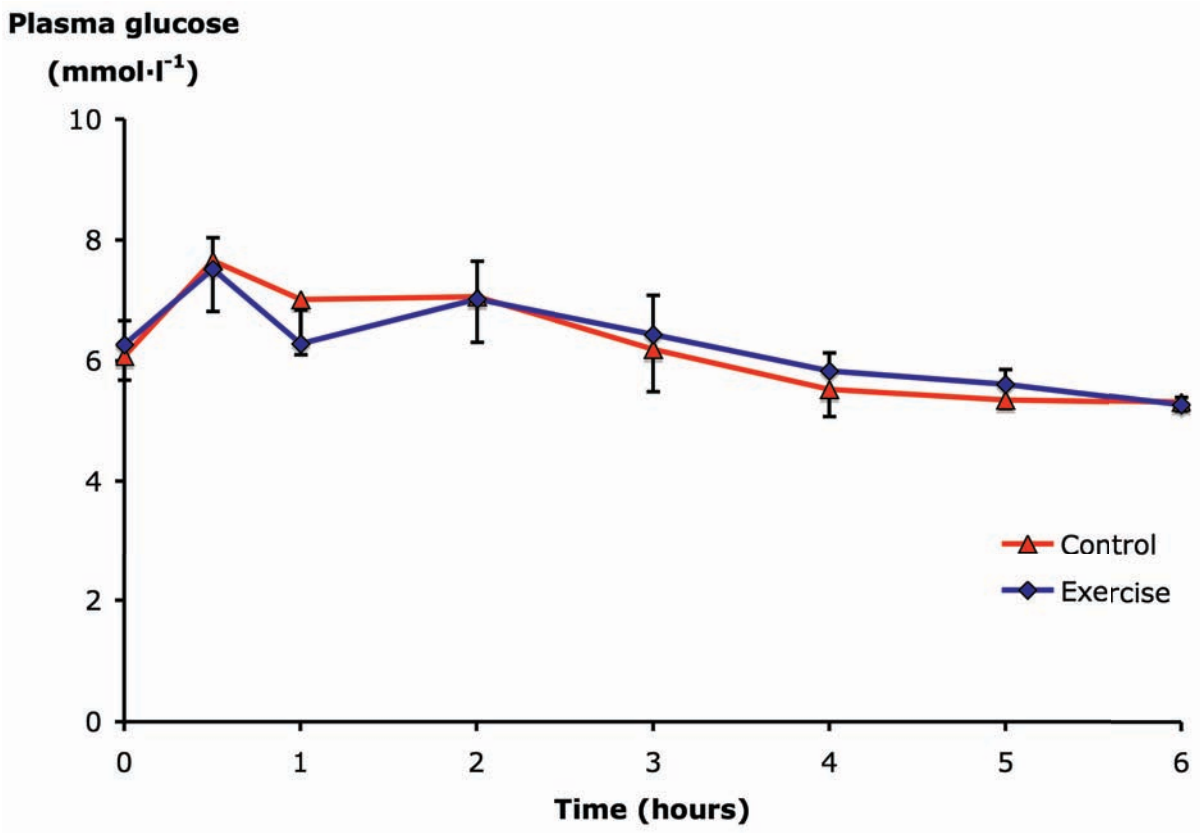


Figure 6.9. Mean (± S.E.M.) plasma glucose concentrations in the fasted state (0 h) and for 6 h after intake of a fat-rich mixed meal, following either a 90-minute walk (exercise) or seated rest (control) on the previous evening.

6.3.3.10. Insulin

Serum insulin concentrations are displayed in **Figure 6.10**. Insulin concentrations were subject to change over the postprandial period (effect of time; $P < 0.001$), with the majority of the variation (55%) explained by a quadratic function ($P < 0.001$). A sharp increase in plasma insulin concentration occurred immediately after eating, with peak values for most subjects occurring after 30 min or 1 h. Insulin concentrations following exercise were not significantly different to values from the control trial (effect of trial, $P = 0.170$). The mean value for peak insulin concentration was 17.9% lower after exercise, but this reduction did not reflect a statistically significant difference (effect of trial, $P = 0.130$).

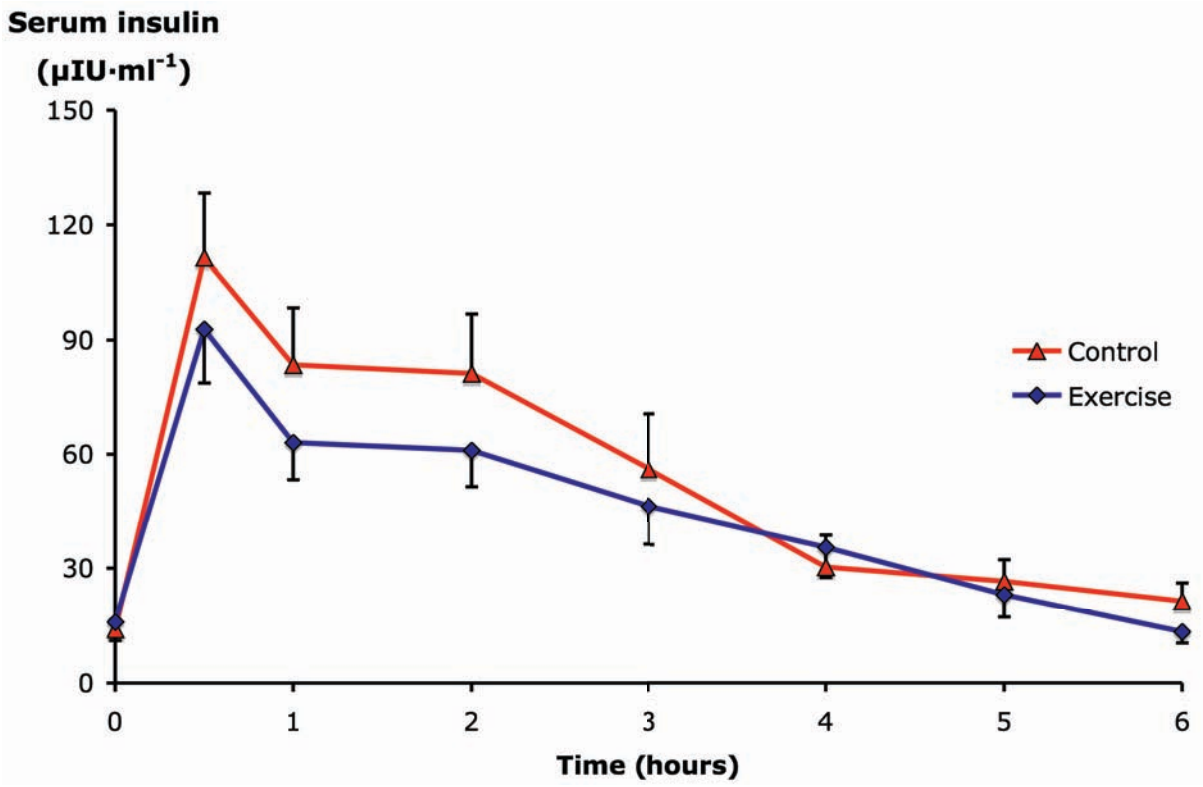


Figure 6.10. Mean (± S.E.M.) serum insulin concentrations in the fasted state (0 h) and for 6 h after intake of a fat-rich mixed meal, following either a 90-minute walk (exercise) or seated rest (control) on the previous evening.

6.3.3.11. Correlations between variables

A significant positive correlation (r = 0.801, P = 0.017) was found between the difference in total TAG response between trials (Exercise AUC – Control AUC; "Δ TAG") and the difference in femoral artery blood flow (Exercise AUC – Control AUC; "Δ Femoral") (**Figure 6.11**). The difference in TAG with exercise did not, however, correlate with differences in either hepatic portal vein blood flow ("Δ Portal"; r = -0.437, P = 0.279) or cardiac output ("Δ Q"; r = -0.186, P = 0.660). Neither "Δ Portal", nor "Δ Femoral" correlated with "Δ Q" (r values of 0.101 and 0.029, P values of 0.811 and 0.947 respectively), but they did show a borderline significant correlation with each other (r = 0.703, P = 0.052). When Femoral AUC was expressed as a percentage of cardiac output for each trial, the positive

correlation previously seen between "Δ TAG" and "Δ Femoral" became non-significant (r = 0.535, P = 0.172). When iAUC difference variables were analysed for evidence of correlation, only "Δ iPortal" (Exercise iAUC – Control iAUC) and "Δ iFemoral" (Exercise iAUC – Control iAUC) were correlated to a statistically significant degree (r = 0.817, P = 0.013).

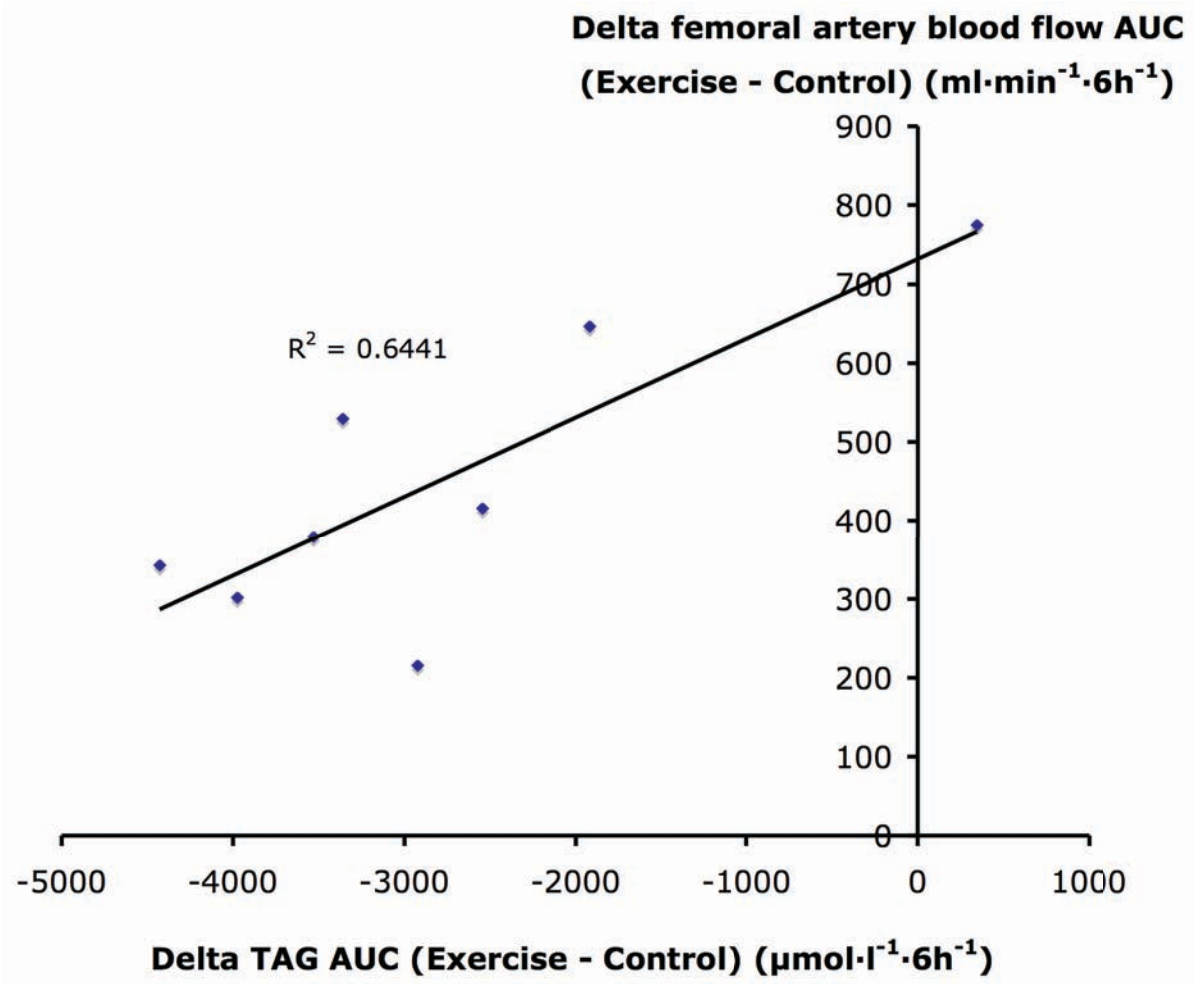


Figure 6.11. Plot showing the correlation between the difference in TAG AUC (Exercise trial minus Control trial; x-axis) and the difference in AUC for femoral artery blood flow (Exercise minus Control; y-axis)

6.4. DISCUSSION

The most novel findings within this study were the observed elevations of hepatic portal vein and femoral artery blood flow on the day after a bout of lipaemia-lowering exercise. The ability of prior exercise to attenuate postprandial TAG concentrations is well known, but to our knowledge, no published work has documented an increase in either fasting or postprandial blood flow to the liver on the day following moderate-intensity exercise. Similarly, while one previous article has reported an increase in calf blood flow, along with a reduction in postprandial lipaemia (Malkova et al., 2000), this finding was on the day after a 2 h run in normal-weight men. We believe ours is the first study to demonstrate an increase in postprandial femoral artery blood flow on the day after a 90-minute treadmill walk in a subject group at high risk of developing cardiovascular disease (middle-aged, overweight men who possess low cardiorespiratory fitness). When changes in cardiac output were accounted for, only blood flow through the femoral artery remained significantly augmented, signifying a redistribution of blood to the skeletal muscle worked 13-19 h beforehand. The upregulation of postprandial blood flow through the hepatic portal vein could not be fully explained by the small, non-significant increase in cardiac output seen on the day post-exercise (power was 0.47 and 20 participants needed to show a statistically significant difference between trials for cardiac output AUC), but equally so, this vessel did not receive a statistically significant redistribution of blood flow (power 0.34; 28 participants needed to show a statistically significant difference between trials). In other words, the significant increase in postprandial portal vein blood flow is partly due to an increase in cardiac output and partly due to receiving a greater percentage of cardiac output.

The observation that blood flow to the liver was increased on the morning after exercise, whilst the participants were still in the fasted state, was unexpected and, we believe, without precedent. The mean increase in flow was relatively small, but was consistent; all participants displayed an increase with exercise. While hepatic portal vein blood flow was significantly increased in the fasted state on the morning after exercise, when the flow was expressed relative to the cardiac output in each trial, the flow through the portal vein was not different between trials (power 0.22; 47 participants needed to show a statistically significant difference between trials). This suggests that blood was not selectively redistributed to the liver on the morning after exercise and that differences in cardiac output played a role in the increased portal vein blood flow. However, cardiac output was not significantly greater than control on the morning after exercise and three of the eight men had a lower cardiac output following the walk. Indeed, the statistical power available was 0.165 and a retrospective sample size calculation found that 68 participants would be needed to show a statistically significant difference between exercise and control trials for fasting cardiac output. Therefore, the statistically significant exercise-induced increase in hepatic portal vein blood flow in the fasted state appears to be partly due to a small increase in cardiac output and a small redistribution of cardiac output to the liver.

Previous work has shown portal vein flow to be significantly reduced during exercise (Rowell et al., 1964; Ohnishi et al., 1985; Rehrer et al., 2001), and immediately afterwards (Ersoz and Ersoz, 2003), although a return to pre-exercise values has been noted as shortly as 10 minutes post-exercise (Ohnishi et al., 1985). The reduction in portal vein flow during cycling exercise at 70% VO_2max (Rehrer et al., 2001) and after 40 minutes of treadmill walking/running (Ersoz and Ersoz, 2003) has primarily been attributed to a reduction in vessel cross-sectional

area resulting from splanchnic arterial vasoconstriction. However, reduced portal blood flow after a step test, and recovery of this flow to basal levels, resulted from reduction (and subsequent restoration) of both vessel cross-sectional area and flow velocity (Ohnishi et al., 1985). In the present study, the increased fasting portal flow on the day after exercise appears to result from vasodilation of the vessel, as the diameter of the hepatic portal vein was significantly increased following exercise, but velocity was not significantly altered (data not shown). Speculatively, therefore, the increase in fasting hepatic portal vein blood flow on the day after exercise may result from hyperaemia within the splanchnic arterial vasculature as a reaction to the reduction in flow to the viscera during exercise. Alternatively, a local increase in insulin sensitivity may play a role, as discussed in more detail later.

Hepatic portal vein blood flow was increased after eating in both trials within this study. This effect was unsurprising, having been reported previously on more than one occasion (Gaiani et al., 1989; Iwao et al., 1996a; Ludwig et al., 1998; Sabbá et al., 1991; Stanley et al., 1998), however, the significantly greater total flow response in the exercise trial is apparently a new finding. The magnitude of the increase in flow from baseline to 30 min postprandially was not significantly greater with exercise (in fact it was almost identical to control), and given that the incremental flow response was not significantly increased with exercise, it appears a large part of the enhanced total portal blood flow after exercise stems from the increase in the fasting flow. That said, the increase in flow above the basal rate in the control trial can be seen to fall after just 30 min, whereas the increased flow in the exercise trial is increased even more at 1 h and maintained at 2 h. The temporal pattern of change in hepatic portal vein blood flow followed a very different pattern to that of plasma triacylglycerol concentration, i.e. hepatic portal vein blood flow peaked very early in the postprandial period, whereas TAG

did not peak until 4 or 5 h after the test meal. This alone does not suggest that changes in hepatic blood flow play no role in altering TAG metabolism, as the regulation of plasma TAG is a complex process with many other factors known to affect the TAG concentration during the postprandial period. However, the observation that neither the total, nor the incremental AUC change with exercise was correlated for TAG and hepatic portal vein blood flow, suggests that any effect on postprandial TAG metabolism of the hepatic blood flow changes seen in this study is small.

Mechanistically, postprandial portal hyperaemia is thought to occur primarily as a result of vasodilation within the superior mesenteric artery (Iwao et al., 1996a; Sabbá et al., 1991). Differences in the extent of postprandial portal hyperaemia have been observed between groups despite similar superior mesenteric artery blood flows (Iwao et al., 1996a); in this instance the lower hyperaemia in a group of cirrhotic patients versus normal controls was attributed to an increase in collateral blood flow after eating. Superior mesenteric artery blood flow was not measured in the present study, which in turn prevented collateral blood flow from being calculated, however it is interesting to consider the possibility that exercise may have altered these parameters. As increased postprandial hyperaemia after exercise in the current study appears to stem from a higher fasting portal vein blood flow, superior mesenteric artery blood flow may also have been increased in the fasted state. Alternatively, portocollateral runoff may have been reduced on the day after exercise. We acknowledge that these mechanisms are entirely speculative at this point in time; unfortunately, no work has been published where such measurements were made on the day after exercise.

Inter-observer variability when measuring portal vein flow is large (Sabbá et al., 1990; Iwao et al., 1996b; Fisher et al., 1998), therefore to limit variability as much as possible, the same ultrasonographer (GMB) made all measurements in the current study. Intra-observer coefficients of variation in published work suggest that Doppler ultrasound is a reliable method by which to measure portal vein flow, if the same observer is retained (Sabbá et al., 1990; Iwao et al., 1996b; Lycklama à Nijeholt et al., 1997). Measurement of hepatic blood flow by Doppler ultrasound has also been shown to exhibit a strong positive correlation with flow as assessed by the invasive indocyanine green constant infusion technique, thereby lending weight to the argument that ultrasound is a suitably accurate, noninvasive alternative (Bolognesi et al., 1995).

Femoral artery blood flow was not significantly increased in the fasted state on the day after exercise, neither was the incremental flow response significantly elevated, yet the total flow response to the test meal was greater (power 0.9999). This apparent contradiction may be explained anecdotally by the observation that most people with increased femoral flow in the fasted state following exercise did not also show a greater response to the meal, and vice versa. As a result, all eight subjects demonstrated a larger total femoral artery blood flow response (range: 8.8% - 35.7% increase in total AUC) on the day after exercise than on the day after control. While neither measure was significantly different between exercise and control trials, the increase in fasting femoral flow appears a more robust exercise-induced change than the increase in incremental femoral blood blow. This observation is based on retrospective power and sample size calculations for the two measures which showed that the statistical power to detect a difference in the fasted state was 0.56 (17 participants required for a significant difference to be found), whereas in the postprandial state (incremental response)

the power was 0.09 (statistically significant increase would require 189 participants). As neither fasting, nor 6 h incremental femoral flow was significantly increased, it is difficult to suggest a main mechanism which could explain the finding of enhanced total femoral flow in all subjects; proposition of two possible mechanisms may be more appropriate. Firstly, participants who had elevated fasting femoral blood flow may simply have been seeing the tail end of the massively increased skeletal muscle blood flow from during exercise the previous day. Blood flow to exercising skeletal muscle increases (due to vasodilatation) within seconds of the first muscle contraction (Clifford, 2007), and during maximal exercise may increase 20 to 30 fold above resting values (Snell et al., 1987), therefore it is feasible that a modestly elevated flow, of the magnitude seen in this study (~ 1.2 fold increase), may persist in the fasted state some 13 h after cessation of exercise. While possible, this mechanism does not have strong support, and leg blood flow has been reported to return to pre-exercise values within 2.5 h of completing a 1 h cycle at 60% VO_2max (Williams et al., 2005). In our study, femoral artery blood flow was not measured in the period between cessation of exercise and the initial fasting measurements on the morning of day 2, therefore the rate at which leg blood flow returned towards pre-exercise values is not known. If femoral artery blood flow did return to pre-exercise values within 2.5 h of exercise being completed, as in the study of Williams et al., then increases in fasting femoral artery blood flow may instead be explained by a different mechanism: improved insulin sensitivity. A single bout of moderate-to-high intensity exercise can increase whole-body insulin sensitivity, as measured by a euglycaemic-hyperinsulinaemic clamp (Mikines et al., 1988) or a hyperglycaemic-hyperinsulinaemic clamp (Perseghin et al., 1996), such that the increase is still detectable 48 h later. Infusion of insulin and maintenance at a high, but physiological concentration has been shown to increase leg blood flow (Laakso et al., 1990). Therefore, if

insulin sensitivity was increased within the skeletal muscles exercised in our study (primarily the legs), then the small concentration of serum insulin present in the fasted state could have increased femoral artery blood flow relative to the control trial. Our own data showed no correlation between the difference in fasting insulin or HOMA from control to exercise trials, and the difference in femoral artery blood flow, but this does not necessarily discredit the idea that insulin sensitivity may still have been increased at the level of the muscle. In the case of those subjects who did not present with increased femoral blood flow at baseline, but did experience a large increase in flow postprandially in the exercise trial, these individuals may have been more insulin-sensitive during the postprandial period on the day after exercise. In studies showing increases in leg blood flow after insulin infusion (Laakso et al., 1990; Laine et al., 1998), serum insulin is elevated and maintained at a concentration equivalent to or above the peak insulin concentration seen after a mixed meal. However, the elevations in insulin after an oral glucose load (Baron et al., 1990) and a small mixed meal (Vincent et al., 2006) have also been sufficient to increase skeletal muscle blood flow. Therefore, it is perhaps most likely that any exercise-induced improvement in insulin sensitivity would increase femoral artery blood flow most substantially during the postprandial period, when insulin concentrations are high. Exercising aerobically for 60 minutes at ~ 63% VO_2max was shown to improve postprandial, but not fasting, endothelial function, 17 h later, with the improvement attributed to greater insulin sensitivity after exercise (Weiss et al., 2008). Prior aerobic exercise may therefore increase skeletal muscle blood flow through improving local insulin sensitivity and meliorating endothelial dysfunction. Insulin is known to effect a distinct phosphorylation-dependent mechanism at the level of the vascular endothelium, ultimately leading to vasodilatation via increased production of nitric oxide (Zeng and Quon, 1996). Increased sensitivity to insulin action at the site of the vascular endothelium within

skeletal muscle could therefore represent a viable mechanism through which femoral artery blood flow is increased on the day after exercise. HOMA scores in this study were not lower after exercise and therefore do not provide support for this proposed mechanism, however HOMA only provides an estimation of whole-body insulin sensitivity in the fasted state, it does not offer information regarding the insulin sensitivity of specific tissues, particularly after a meal. Furthermore, HOMA is primarily intended for use as an estimation of insulin sensitivity within a medium to large population; the likelihood of HOMA reflecting insulin sensitivity as measured by clamp, in a very small group of subjects (N = 8 in the current study), is not high. It may be asked, "Why would the insulin-induced increase in blood flow be greater the day after exercise?" Interestingly, postprandial endothelial function (measured by flow-mediated dilation) was improved by exercise performed 16 – 18 h earlier (Tyldum et al., 2009; Weiss et al., 2008). Moreover, insulin-sensitive Akt phophorylation has been shown to be elevated 24 hours after a 60 min exercise bout (Wadley et al. 2007). As there is evidence that free fatty acids appear to impair eNOS phosphorylation (Symons et al. 2009, Wang et al. 2006), the prior exercise might lower the free fatty acid concentration in the local milieu, thus releasing this inhibition of eNOS.

As both femoral artery and hepatic portal vein flow were increased with exercise, and postprandial lipaemia was reduced, correlations were run to determine if the exercise-induced changes were associated. The exercise-induced change in TAG AUC did not correlate with the change in AUC for hepatic portal vein blood flow, but did correlate, positively, with the change in femoral artery blood flow. It was expected that those people with the largest reductions in lipaemia would have the largest increases in femoral blood flow; this correlation suggests the opposite is true. While this correlation could be interpreted as implying that

TAG was being cleared into adipose tissue (the other main site of TAG uptake along with the liver and skeletal muscle), from viewing of the correlation plot (**Figure 6.11**), it appears that one individual lies far out from the others and essentially forms the basis for the positive correlation. Removal of this individual from the data set resulted in the correlation becoming nonsignificant (r = 0.548; P = 0.203). Furthermore, the physiological relevance of the correlation is questionable, as the men with the largest reductions in lipaemia still had a positive change with respect to the AUC for femoral artery blood flow.

In conclusion, this study found that a bout of moderate-intensity exercise, which significantly reduced postprandial lipaemia, also increased postprandial blood flow to the liver and recently exercised skeletal muscle. In the fasted state, blood flow through the hepatic portal vein was significantly greater 13 h after exercise than after rest, but the mean increase in femoral artery blood flow with exercise was not statistically significant. The increase in portal vein and femoral artery flow above baseline was not significantly different between exercise and control trials, suggesting that elevated flow rates in the fasted state were largely responsible for the increased postprandial blood flows. The change in postprandial TAG between exercise and control trials did not show a physiologically relevant correlation with the change in liver or leg blood flow, therefore the extent to which increases in blood flow to these tissues will directly or indirectly affect postprandial TAG concentrations is still not clear.

CHAPTER SEVEN

GENERAL DISCUSSION

7.1. Introduction

While the fasting concentration of plasma triacylglycerol (TAG) is included within criteria for clinical identification of the metabolic syndrome (National Cholesterol Education Program Adult Treatment Panel third report, 2002), the lack of a consistent correlation between TAG concentration in the fasted state and coronary heart disease (CHD) in multivariate analyses has prevented widespread acceptance of fasted TAG as an independent CHD risk factor. The postprandial concentration of TAG, particularly the concentration later in the postprandial period (6 h or 7 h after meal ingestion), has been shown to have stronger prognostic value for CHD or atherosclerosis than fasted measures (Groot et al., 1991; Karpe et al., 1998; Patsch et al., 1992). Despite this evidence that postprandial lipids provide better feedback on cardiovascular risk than measures in the fasted state, the serial measurement of TAG concentrations after a fat-containing meal is not commonly performed in general practice due to the logistics involved. Specifically, the monitoring of plasma TAG over the 6 – 9 h period after a fat-containing meal, as seen in experimental research, is not an approach which lends itself particularly well to the time-constrained world of the General Practitioner. Therefore, although the oral glucose tolerance test (OGTT) is widely used as a clinical tool to detect diabetes, a standard oral fat tolerance test (OFTT) has not yet been adopted to aid in identifying individuals at increased risk of CHD from exaggerated postprandial lipaemia. Lab-based scientific investigations are not constrained by the time pressures of the GP surgery; therefore, in laboratory settings, the OFTT provides an excellent research model in which to study the impact of interventions upon the cardiovascular risk factor of postprandial lipaemia. The investigations in this thesis all employed the 2-day experimental design first

popularised by Aldred et al. (1994) and used this robust model to advance understanding of the circumstances in which exercise interventions will attenuate postprandial lipaemia.

7.2. Research findings from the current thesis

The first study in this thesis addressed a question of much practical relevance: does an aerobic exercise bout reduce the postprandial lipaemia associated with a moderate-fat meal to the same extent as it reduces the lipaemia seen after a high-fat meal? An extensive body of research has shown that performing aerobic exercise of low- to moderate-intensity on the evening before a high-fat meal will lower postprandial lipaemia (Aldred et al., 1994; Gill et al., 1998; Gill and Hardman, 2000; Gill et al., 2001a; Gill et al., 2001b; Gill et al., 2002a; Gill et al., 2003a; Gill et al., 2004; Herd et al., 2001; Malkova et al., 1999; Malkova et al., 2000; Tsetsonis and Hardman, 1996a; Tsetsonis and Hardman, 1996b; Tsetsonis et al., 1997; Zhang et al., 1998; Zhang et al., 2004; Zhang et al., 2006; Zhang et al., 2007, among others). The meals given in such studies typically contain more than 60% of energy as fat (Zhang and colleagues use a meal containing more than 90% fat) and cannot be said to represent the average macronutrient composition of the British diet, where fat accounts for only 35 - 36% of daily energy intake (Henderson et al., 2003). A small number of studies have also recently demonstrated that exercising before a moderate-fat (35 - 37% fat) meal will reduce the subsequent total lipaemic response (Burton et al., 2008; Kokalas et al., 2005; Kolifa et al., 2004; Miyashita, 2008), but as none of these studies reported a reduction in incremental AUC, this suggests that the attenuation in total lipaemia was largely due to a lowering of the fasting TAG concentration rather than an improved response to the meal. Of the studies which have found the total lipaemic response to a high-fat meal to be reduced following aerobic exercise, the vast majority report a significant reduction in the incremental lipaemic response as well

(Gill and Hardman, 2000; Gill et al., 2001a; Gill et al., 2001b; Gill et al., 2003a; Gill et al., 2004; Herd et al., 2001; Malkova et al., 1999; Malkova et al., 2000; Tsetsonis and Hardman, 1996a; Tsetsonis and Hardman, 1996b; Tsetsonis et al., 1997), a proportion do not report the incremental lipaemic response (Aldred et al., 1994; Gill et al., 1998; Zhang et al., 1998; Zhang et al., 2004; Zhang et al., 2006; Zhang et al., 2007) and one study found a trend for incremental lipaemic response to be lower, but the difference was not statistically significant (Gill et al., 2002a). Therefore, it appears that exercising before very high-fat meals has a true postprandial effect which favours reduced lipaemia, but the same finding has not yet been confirmed for a meal where the fat content is typical of that habitually consumed by the British public. Our study therefore fills a gap in the literature by testing the lipaemic response to meals of both high and moderate fat content, with and without prior exercise, using the same participants in all four trials. If exercise affects the lipaemic response to the two meals differently, the design of this study ensures the differences will not be not the result of having used different participants to consume each meal: a charge which could be levelled against previous studies when comparisons where drawn between them. Our findings suggest aerobic exercise such as brisk walking is just as effective in attenuating the increase in TAG concentrations seen after a moderate-fat meal as that seen with a much higher lipid load. The total and incremental lipaemic responses were reduced in exercise trials compared with control trials and the percentage reduction from control trials was very similar for both meals. In this study, the increase in plasma TAG after the high-fat meals was slightly higher than with the moderate-fat meals, but not to the extent of becoming statistically significant. When a moderate-fat meal was consumed, TAG values tended to peak later in the postprandial period than when ingesting a high-fat meal. Our participants tended to be older, more overweight and less fit than those in previous studies; the fact that these men displayed

multiple risk factors for exaggerated postprandial lipaemia and coronary heart disease may have contributed to the substantial lipaemic response seen after they consumed a moderate-fat meal, i.e. the combination of middle-age, overweight/obesity and low cardiorespiratory fitness may reflect a cardiometabolic state wherein the ability to deal with even a moderate dietary fat load has become compromised.

The study described in the second experimental chapter of this thesis was conceived after consideration of the reason why postprandial lipaemia is consistently reduced after brisk walking, whereas findings using resistance exercise have been far more equivocal. Eight studies have been published which investigate the effect of single resistance exercise sessions on postprandial lipaemia (Burns et al., 2005; Burns et al., 2006; Burns et al., 2007; Pafili et al., 2009; Petitt et al., 2003; Shannon et al., 2005; Singhal et al., 2009; Zafeiridis et al., 2007), although it must be stated that only three (Burns et al., 2005; Petitt et al., 2003; Shannon et al., 2005) were in existence when the study described in **chapter four** commenced. At the time of designing the study, only one investigation had found a benefit of resistance exercise and this study used weightlifters with an average of six years training experience (Petitt et al., 2003). An almost identical protocol failed to show any effect on postprandial lipaemia when using recreationally active men who did not engage in regular resistance exercise (Burns et al., 2005). This disparity hinted that training status might influence the ability to attenuate lipaemia when using resistance exercise as the exercise intervention. More recent studies have done little to dispel this notion, if only because the majority have used resistance-trained volunteers (Burns et al., 2007; Singhal et al., 2009; Zafeiridis et al., 2007), but one study has reported a reduction in postprandial TAG concentrations after resistance exercise using a group of participants who had not performed such exercise for at least 12 months (Pafili et al.,

2009). However, as brisk walking has been shown to reduce postprandial lipaemia in untrained individuals on more than one occasion (Burton et al., 2008 and Gill et al., 2004 are examples), it does not appear that differences in training status can completely explain the failure of resistance exercise to lower lipaemia as consistently as brisk walking. Instead, it is perhaps more likely that a factor intrinsic to the activity of brisk walking is responsible. Brisk walking is by its very nature an exercise performed at a moderate, rather than a high intensity (as the gait employed is a walk rather than a jog or a run), and as the intensity stays constant for the entire session, metabolism remains predominantly aerobic throughout. Resistance exercise is however an activity which is largely intermittent, involving bursts of high-intensity effort, followed by periods of no work, during which the exerciser recovers. The anaerobic basis of metabolism during resistance exercise may therefore be a factor which limits lipaemic moderation. By increasing the time spent exercising aerobically during resistance sessions, the benefit to lipaemia may be greater and more consistently observed than with a high-intensity resistance exercise session. This theory was tested by having participants undertake a resistance exercise session where the load for each exercise was fairly light, but many repetitions were performed, with a relatively short recovery after each set. The lipaemic response to a high-fat meal was examined on the day after this session and was compared with the responses to a high-intensity resistance exercise session and a control trial. Unfortunately, we cannot provide firm evidence that the lighter session with more repetitions had a more substantial aerobic component than the other resistance exercise session, but mean heart rate tended to be higher during the former and the heart rate responses to the sessions were very different over time (a smaller heart rate range was seen for the lighter session). In opposition to our hypothesis, this study found that neither of the resistance exercise sessions lowered lipaemia when compared with a control trial. The relatively low mean heart rates

recorded may be an indication that the average intensity across the sessions (lifts plus recoveries) was not high enough to lower lipaemia; however, the fact that the reduction in total lipaemic response from control values was greater for four participants than the previously published within-subject OFTT variability for men of 10.1% (Gill et al., 2005), suggests that this was not the case for all men. Overall, our study does not offer support for the idea that resistance exercise lowers postprandial lipaemia; however, this finding may reflect a situation unique to individuals who are not weight trained. When studies are excluded which obviously deviate from the standard experimental design, i.e. Shannon et al., (2005) where the exercise-induced energy deficit was abolished and Burns et al. (2006) where the test meals were given 1 h after exercise rather than 13 – 17 h post (all other studies), studies using resistance-trained participants have all found a lipaemic benefit of prior resistance exercise (Burns et al., 2007; Petitt et al., 2003; Singhal et al., 2009; Zafeiridis et al., 2007). When compared with chapter three, where we showed that aerobic exercise significantly reduced the lipaemia associated with high and moderate fat meals, the percentage drop in total TAG response is much larger with aerobic than resistance exercise in our studies using high fat loads. It must be stressed, however, that these studies were performed with different participants and therefore a direct comparison is not strictly valid; further research would be necessary to confirm that aerobic exercise is a better strategy than resistance exercise for reducing postprandial lipaemia in middle-aged, overweight men who currently perform no exercise.

Anecdotally, many people describe feeling hungrier after exercise, and a general belief has formed that exercise increases appetite and energy intake. If true, then exercise alone would not be a successful weight loss strategy, as energy expended during time spent being

physically active, would be replaced soon after. Studies investigating the effect of exercise on postprandial lipaemia are generally, well-controlled affairs conducted in a laboratory environment - a somewhat necessary process if the effect of exercise is to prevail against the background variation in diet and other aspects of lifestyle. However, real life is not conducted in a laboratory, and findings from studies investigating the effect of exercise on postprandial lipaemia in a laboratory setting are not guaranteed to transfer out into the real world, particularly if individuals are likely to take in more energy after exercising. The relevant literature does not present a clear message regarding the effect of single exercise bouts on appetite/*ad libitum* energy intake during the immediate post-exercise period: some studies show an increase, at least one has reported a reduction and many more show no significant change in energy intake. The inconsistent nature of these reports makes it difficult to predict whether exercise outside of a controlled lab situation would actually lower lipaemia, as additional energy intake may counteract the exercise effect by preventing negative energy balance. The study in **chapter five** attempted to move investigation of exercise and postprandial lipaemia closer to the real world by having the participants eat their breakfast in an *ad libitum* fashion. A single blind design (with respect to measurement of *ad libitum* energy intake) ensured that participants were unaware of the wider aims of the research, therefore minimising the impact of changes in eating behaviour which occur when individuals are conscious of the fact that energy intake is of interest to the investigators. This study found that mean *ad libitum* energy intake was not increased by a brisk walk performed ~ 13 h previously, and, as a result, postprandial lipaemia was lowered significantly, in line with percentage reductions seen in previous studies where test meals of a fixed size were provided. Individual participants did consume up to 35% more energy after exercise, however, as half of the men ate slightly less on the day after exercise compared with a control

trial, it is not clear how much these changes in energy intake simply reflect day-to-day variation and how much they are attributable to a true effect of exercise. It is obviously possible that exercise could affect different people in different ways, with energy intake increased for some and reduced for others; but exactly which factors would be responsible for these divergent responses to essentially the same exercise stimulus, i.e. a brisk walk at 60% VO_2max, is not obvious. Recent research has focussed on so-called "hunger hormones", with the anorexigenic protein "Peptide YY", and the appetite-stimulating hormone "ghrelin", receiving much attention (see Hameed et al. (2009) for an up-to-date review). Plasma concentrations of these factors were not measured in this study, but it is tempting to speculate that differential effects on these hormones may have contributed to the heterogeneity of response in *ad libitum* energy intake after exercise.

The final study in this thesis (**chapter six**) sought to explore an aspect of physiology which is well accepted, but which has not received a great deal of attention in studies of postprandial lipaemia: exercise-induced changes in blood flow. The need for cardiac output to increase, often several-fold, during exercise is well known and the metabolic needs of the exercising muscles are such that the local hyperaemia can be many times greater than that experienced on a whole body level. As well as blood being pumped more quickly during exercise, redistribution of blood occurs, with splanchnic perfusion reduced to accommodate the requirement for extra blood in skeletal muscle. The increased perfusion of muscle capillaries during exercise may leave a legacy (in metabolic terms) which lasts long after the exercise bout has finished, with increases in whole-body insulin sensitivity observed up to 48 hours post-exercise. It could be that the opening of these muscle capillaries presents a scenario where access to the hydrolysing enzyme lipoprotein lipase is increased and clearance of

triacylglycerol from the plasma is upregulated. Therefore, measurement of postprandial blood flow to recently exercised skeletal muscle is of interest, as the ability of prior moderate exercise to lower the lipaemia brought on by ingestion of a fat-containing meal, may be partly mediated by increases in blood flow to sites where TAG can be cleared from the circulation. The liver plays a major role in regulating plasma TAG concentrations through uptake of non-esterified fatty acids and secretion of VLDL-TAG. For this reason, measurement of hepatic blood flow during the postprandial period may also provide answers to questions asked regarding the mechanistic rationale behind the lipaemic benefit of moderate exercise. We calculated cardiac output, and blood flow through both the hepatic portal vein and the femoral artery over the 6 h period after a fat-containing mixed meal, using measurements of vessel diameter and blood velocity obtained via ultrasonography. These measurements were made immediately after collection of blood samples in which TAG concentration was to be determined, therefore allowing the temporal changes in TAG concentration and blood flow to be plotted alongside one another. We found increases in postprandial blood flow through both the hepatic portal vein and the femoral artery after a bout of brisk walking which significantly reduced postprandial lipaemia. Cardiac output was not significantly higher than in a control trial, but the statistically non-significant increase in cardiac output appears to have been partially responsible for the increases in blood flow to the liver and the legs, as redistribution of flow cannot account for all the increase seen in blood flow to these two sites. Although lipaemia was reduced and hepatic blood flow was increased, no correlation between the two was observed. The increase in femoral blood flow after exercise correlated with the reduction in lipaemia, but the correlation was positive, rather than negative as expected. This suggested that individuals experiencing larger increases in femoral blood flow with exercise were likely to have smaller reductions in lipaemia, but closer scrutiny of the data showed that

the positive relationship was heavily influenced by one individual datum (an outlier) and the association disappeared completely when this point was removed from the scatter plot. It would be easy to conclude that while blood flow changes were observed in the postprandial period after exercise, these changes are not related to indices of lipaemia; however the small numbers in this study mean that firm conclusions cannot be drawn in either direction. Instead, this study has demonstrated that a bout of exercise commonly performed in previous studies, with a beneficial effect on postprandial lipaemia, also increases hepatic and lower limb postprandial blood flow. Future investigations, with more volunteers, appear merited – particularly where the option to measure adipose tissue blood flow alongside hepatic and femoral flow is available.

7.3. Limitations

While every attempt was made to ensure the experimental design of all the studies was robust before investigations began, as with all experiments, there are potential limitations which should be discussed. Individual studies have specific limitations which are addressed in their respective chapters, but there are also overarching limitations which affected all experiments. All four studies had an experimental design which centred around the analysis of blood samples (for TAG) taken after a fat-containing meal, i.e. they all involved an OFTT or a slight modification thereof (the *ad libitum* study, for example). The reproducibility of an oral fat tolerance test was reported to be high for men when preceding lifestyle was well controlled, but the lipaemic response was significantly affected by phase of menstrual cycle for women (Gill et al., 2005). Furthermore, male deaths from CHD in the U.K. outnumber female deaths, and the percentage of all-cause mortality attributable to CHD is higher for men than women (Allender et al., 2008b). Due to the low reproducibility of OFTTs for women when

performed in successive weeks and the greater burden of CHD for men, the decision was made to recruit only male participants for the studies in this thesis. The extent to which findings from these studies can be extended to women, when only male participants were tested, is questionable and is an obvious limitation of the research. A further limitation of the research concerns the degree to which the participants recruited were "at risk" of elevated postprandial lipaemia and CHD development. In all studies, we observed fairly large heterogeneity of the lipaemic response despite recruitment of (outwardly) fairly homogeneous participant groups. The decision to actively recruit individuals with previously described risk factors for exaggerated postprandial lipaemia (maleness, middle-age, overweight/obesity, sedentariness) was taken such that the relevance of the findings would be enhanced. Individuals displayed greatly exaggerated postprandial lipaemia, but this was not a consistent finding among all participants. It may be that the participant groups were not homogeneous enough, and the criteria needed to be even stricter, but it is also possible that other (as yet unrecorded) risk factors influence the postprandial TAG response. In **chapter three**, two participants of almost identical age, BMI and cardiorespiratory fitness showed noticeably different lipaemic responses (in one trial, one of the participants' responses was more than double that of the other). This observation implies that other factors outside of sex, age, weight status and cardiorespiratory fitness influence the extent to which TAG concentrations are elevated postprandially. Additionally, the lipaemic response of one individual was consistently found to be three to four times higher than another study participant, despite the two being of similar age, BMI and body fat percentage. The individual who displayed four-fold higher postprandial lipaemia did however have lower cardiorespiratory fitness. It may therefore be the case that different risk factors are far from equal when predicting who will display exaggerated postprandial lipaemia, with fitness potentially more important than age or

weight status – a statement supported by the fact that total lipaemic response correlated negatively with VO_2max, but did not correlate with age or BMI. Another partial limitation concerns the number of participants who completed each study. Although work investigating the effect of preprandial exercise on postprandial lipaemia has previously been published for studies including eight participants, with significant findings (Gill et al., 2001a; Gill et al., 2003b; Harrison et al., 2009; Herd et al., 2001; Kokalas et al., 2005; Maraki et al., 2009; Miyashita, 2008; Tolfrey et al., 2008), and sample size calculations suggest that an effect of exercise on postprandial lipaemia should be detectable with only five people, ideally, each study within this thesis would have greater participant numbers. The strength of correlations between variables may be questioned due to the low *N*; therefore these relationships require confirmation from studies with more participants. The greatest limitation of all the studies in this thesis, and indeed all the studies conducted which have investigated the effect of single exercise bouts on postprandial lipaemia, concerns the clinical relevance of any reduction observed. While studies have reported a statistically significant difference in plasma TAG concentrations between exercise and control trials, it is not clear what magnitude of reduction in lipaemia would need to be frequently induced before cardiovascular risk would be reduced to any measurable extent. Until a "normal" range for postprandial TAG concentrations is defined, as has been done for fasting concentrations, the concentration thought to confer cardiovascular risk, and the extent to which interventions must reduce lipaemia, will remain unknown. The fact that a standard fat test meal has not been agreed upon, such as the 75 g of glucose used in an oral glucose tolerance test, makes it difficult to set TAG concentration guidelines, as different test meals are likely to induce increases in lipaemia to different degrees. Also the issue of how to diagnose postprandial hypertriacylglycerolaemia would need to be addressed, i.e. would an area under the curve (AUC) score be used or would TAG

concentration at a specific postprandial time-point be selected for diagnosis? A recent study (Rector et al., 2009) added to the literature in this area, reporting that the TAG concentration at 4 h after a high-fat meal accounted for more than 90% of the variation in the 8 h area under the TAG versus time curve. Drawing a single postprandial blood sample and analysing it for TAG concentration presents no more hassle than a fasted measure, but with no concensus across the literature regariding the fat load and type to be given within test meals, it is perhaps unlikely that such a procedure will become commonplace in clinical settings. Evidently there are currently a number of impediments to be overcome before the clinical relevance of exercise-induced reductions in postprandial lipaemia can even be investigated.

7.4. Future research directions

This thesis has generated new data regarding the effect of prior exercise on postprandial lipaemia and should add to the literature base in this respect. However, through acknowledgement of the limitations of the studies in this thesis, it has also been possible to generate some ideas for areas considered worthy of future investigation. Firstly, as the clinical relevance of exercise-induced reductions in postprandial lipaemia is not established, it should be a priority to conduct long-term prospective studies which examine this factor. It cannot be denied that such studies would be difficult to carry out, and it would not perhaps be straightforward to conclude that any reduction in the number of cardiovascular events was directly attributable to changes in postprandial lipaemia, as many other factors are likely to be affected by regular moderate exercise, but the complications involved should not prevent what is necessary work from being conducted. We feel this thesis made an important advance with respect to the population recruited for the studies contained herein. The vast majority of previous work has recruited participants who are easily available, rather than those who are

particularly at risk of exaggerated postprandial lipaemia or CHD. Therefore the external validity of much of the literature is not as impressive as could be the case. Despite our adherence to strict criteria when recruiting participants, in order to obtain a homogeneous "at risk" group, the inter-subject variability in lipaemic response was still quite large. This suggests that factors beyond age, sex, weight status and cardiorespiratory fitness were responsible for differences observed between individuals. A genetic predisposition to exaggerated postprandial lipaemia appears an obvious underlying risk factor, indeed, ethnicity has already been suggested to influence plasma TAG concentration. Black males have been reported to have lower TAG concentrations than white males (Zoratti, 1998), South Asians living in the U.K. tend to have higher TAG concentrations than Africans (Cappuccio, 1997), and postprandial lipaemia has been shown to be lower in African Amercian women than White women (Bloomer et al., 2009a; Shannon et al., 2008). The vast majority of individuals who participated in the studies presented in the experimental chapters of this thesis were White and British; therefore, other, non-genetic factors are also likely to contribute to the variability in lipaemic response in a seemingly homogeneous population. As the total lipaemic response to a meal is almost always found to correlate with the fasting TAG concentration, future studies should perhaps focus on recruiting participants with fasting hypertriacylglycerolaemia. Factors previously reported to increase the likelihood of postprandial TAG concentrations being high, e.g. older age, maleness, low habitual physical activity/fitness and increased body weight/fatness are also in need of further investigation, as it is not currently known which of these factors correlates most strongly with lipaemia. Future research could also focus on identifying specific metabolites or biomarkers which correlate strongly with TAG concentrations in the late postprandial period. TAG concentrations 6 h or 7 h after a fat load have been shown to correlate with CHD incidence

(Patsch et al., 1992) and a marker of early atherosclerosis (Karpe et al., 1998), therefore identification of (fasting) biomarkers which predict the TAG concentration many hours after a meal, could remove the need for studies to be conducted in the postprandial state. Studies which clarify the best time to exercise in relation to ingesting a fat-containing meal are also justified, as knowledge of the time delay between exercise and meal which produces the optimal reduction in lipaemia would allow at-risk individuals to plan exercise sessions around meal times or vice versa. Finally, an area of research which has not been given substantial attention concerns the mechanisms through which prior exercise attenuates postprandial lipaemia. The effects of increased LPL-mediated TAG clearance and reduced VLDL-TAG secretion have both been proposed to account for the reduction in plasma TAG concentration during the postprandial period following aerobic exercise, but the extent to which these two mechanisms contribute has not been scrutinised experimentally. Methodologies are now available which could enable present day researchers to investigate both of these mechanisms within the one study. The use of stable isotope tracers, in combination with either skeletal muscle and adipose tissue biopsies, or arteriovenous difference measurements, provides a framework in which mechanistic questions could be answered. The identification of the mechanism which is affected most beneficially by prior exercise is important if the general public are to be convinced to begin including aerobic exercise in their daily lives as a step towards improved health. Therefore, future research should now prioritise studies which employ stable isotope tracers and methodologies capable of measuring tracer enrichment in multiple tissues to investigate the mechanistic basis of the exercise-induced effect on TAG metabolism.

7.5. Conclusions from research studies within this thesis

When considered in light of the research previously published in the field of exercise and postprandial lipaemia, the overall conclusions drawn from the studies conducted within this thesis are as follows: 1. Aerobic exercise is effective in attenuating meal-induced postprandial lipaemia, regardless of the fat-derived energy of the meal consumed. 2. Sessions of low- and high-intensity resistance exercise do not appear to offer the same lipaemia-lowering benefit as aerobic exercise for overweight, sedentary men. 3. Evening aerobic exercise does not induce a significant increase in energy intake the following morning, suggesting that exercise performed in a free-living situation will continue to lower postprandial TAG concentrations. 4. Moderate-intensity aerobic exercise which reduces postprandial lipaemia, also increases postprandial blood flow to the liver and the legs, but the idea that exercise-mediated reductions in postprandial lipaemia are achieved through these haemodynamic alterations can neither be confirmed nor denied. The general message from this thesis would be that moderate-intensity aerobic exercise should be advocated as a strategy to lower risk of cardiovascular disease, as experimental evidence suggests that it will be effective in reducing postprandial lipaemia in the general population.

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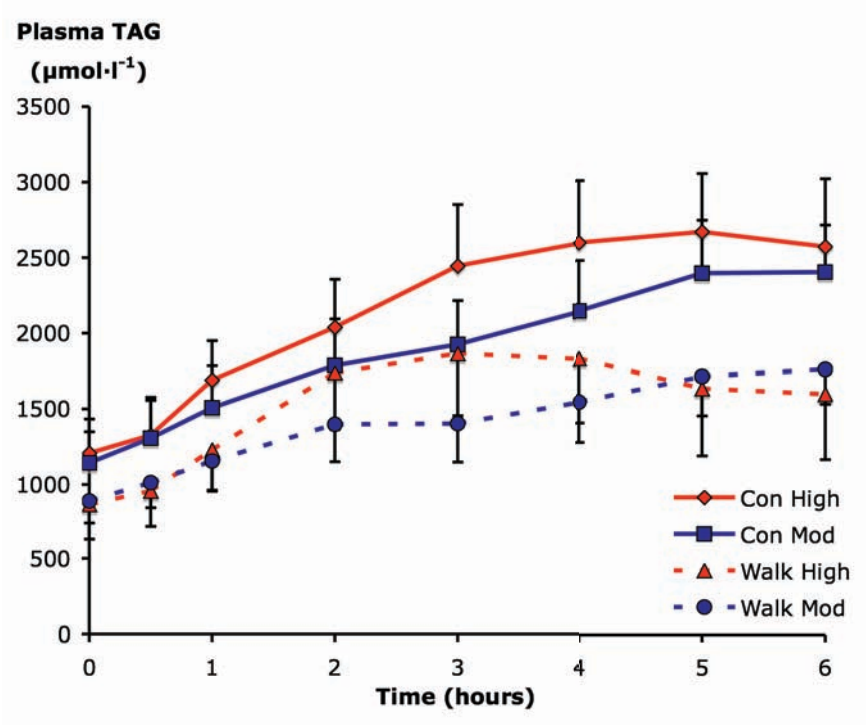
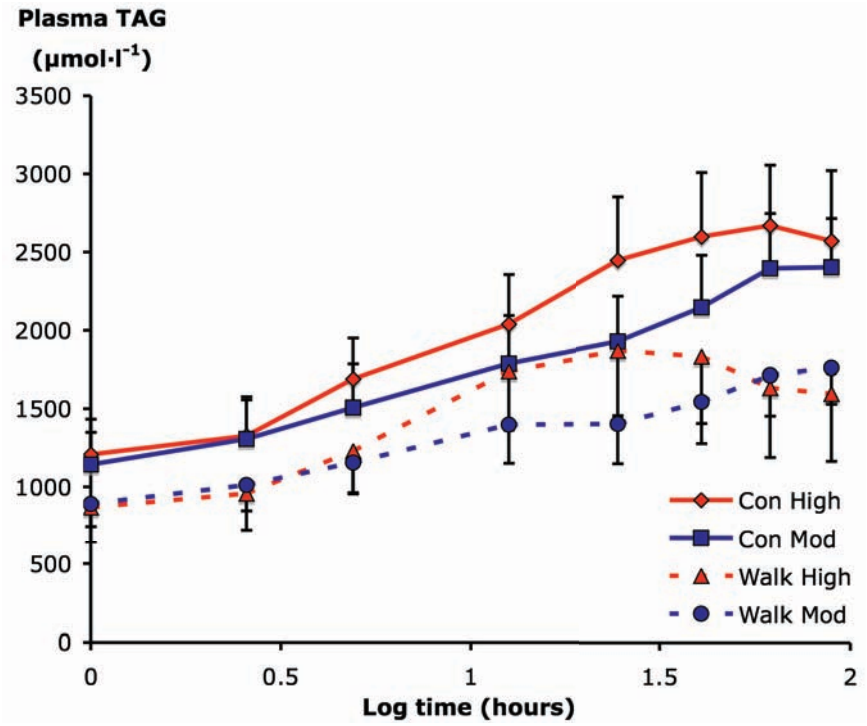
APPENDICES

Analysis of metabolite data for time effects included orthogonal polynomial contrast analysis. This was undertaken to penetrate beyond main effects of time and trial x time interactions, i.e. to determine which single degree of freedom contrasts within a main effect or interaction were statistically significant and how much of the total variation across time was explained by each polynomial contrast. As explained previously in experimental chapters, orthogonal polynomial contrast analysis is best applied when the data analysed shows a symmetrical pattern across time. Therefore, in an effort to ensure that data curves were as symmetrical as possible, prior to analysis, real time and log transformed time (base *e*) curves were generated. The decision regarding which metric to use when analysing the data was based upon visual inspection of the curves. This process is evidently subjective and different observers may arrive at different conclusions after scrutinisation of the real time and log transformed time curves. For these reasons, all metabolite data curves for which orthogonal polynomial contrast analysis was performed are presented herein, to permit scrutiny of whether the real time or log transformed time curve was more symmetrical. Appendices are provided for all four experimental chapters, in order of appearance within this thesis. For each appendix, the two curves for each metabolite are presented together, with the real time curve shown first and the log transformed time curve immediately below. The metric that was used for orthogonal polynomial contrast analysis for each metabolite is also reiterated.

Appendix A: Real time and log transformed time metabolite curves related to Chapter 3

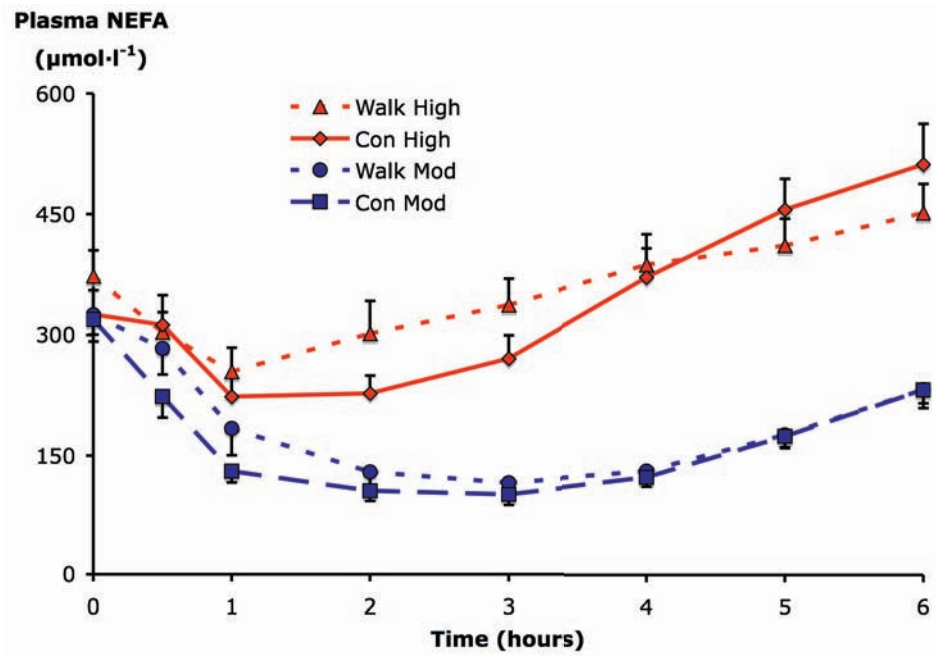
Triacylglycerol

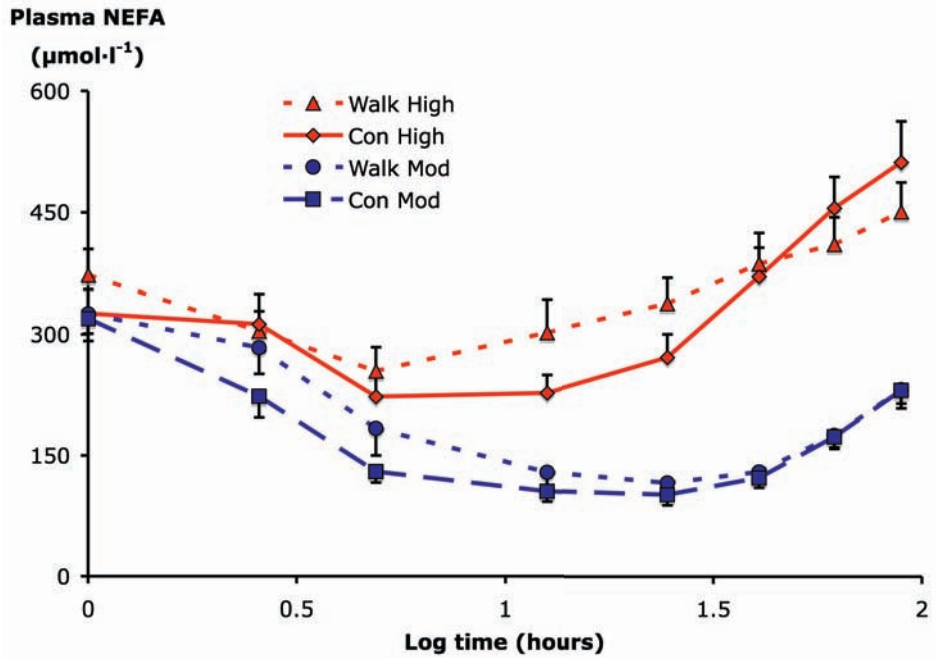




Metric used for analysis: Real time.

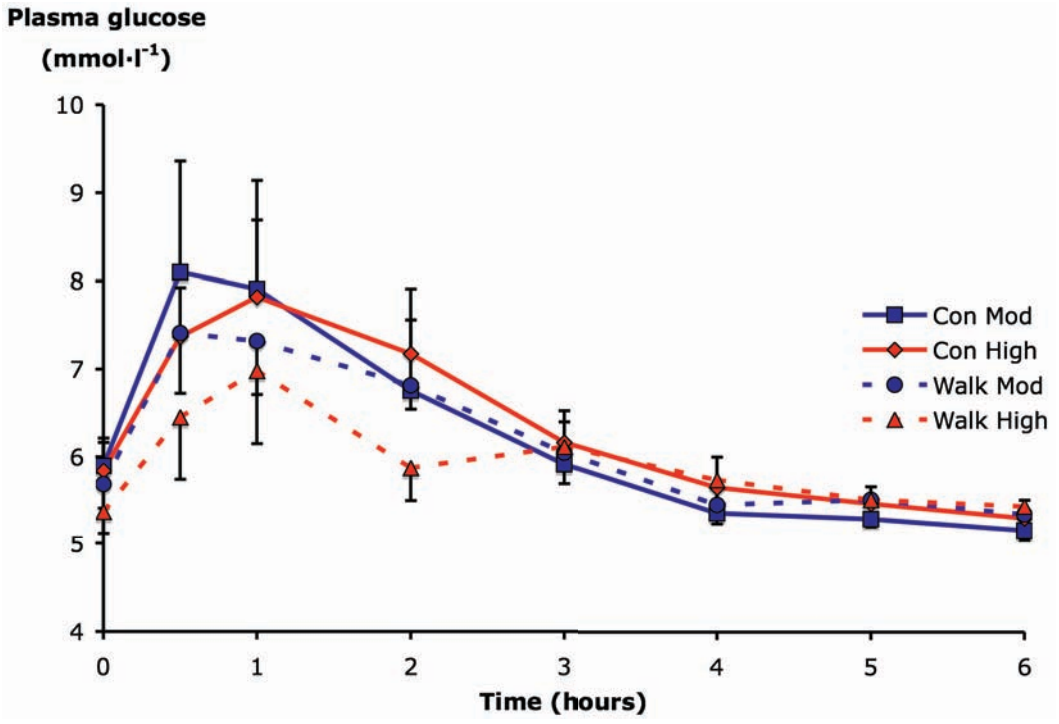
Chapter 3: Non-esterified fatty acids (NEFA)

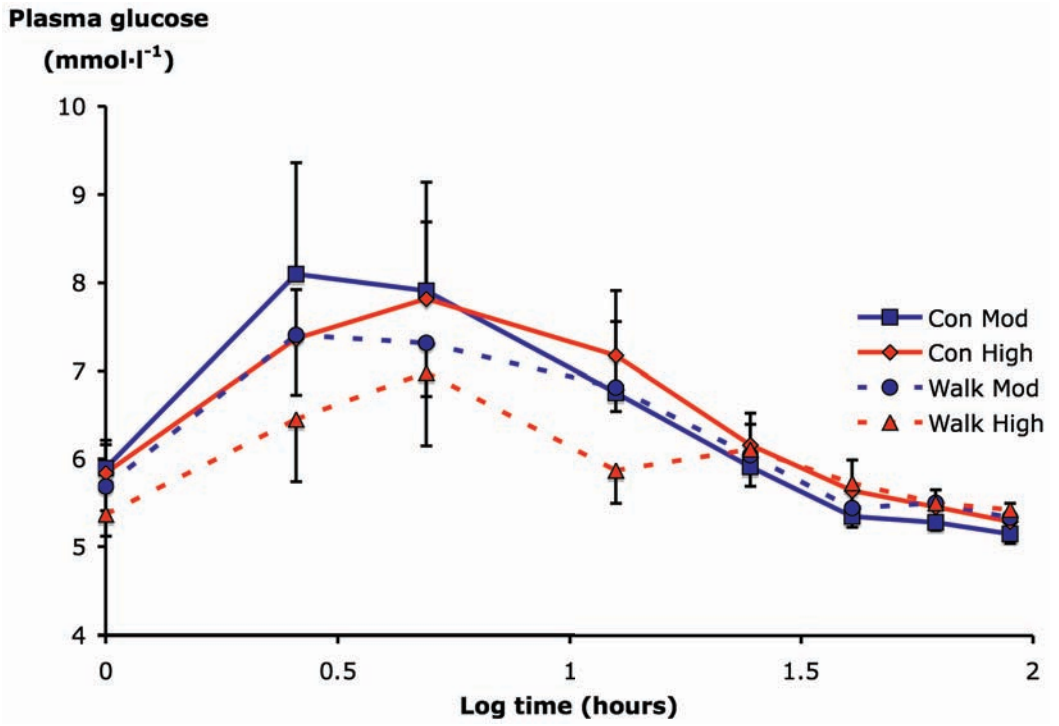




Metric used for analysis: Log transformed time.

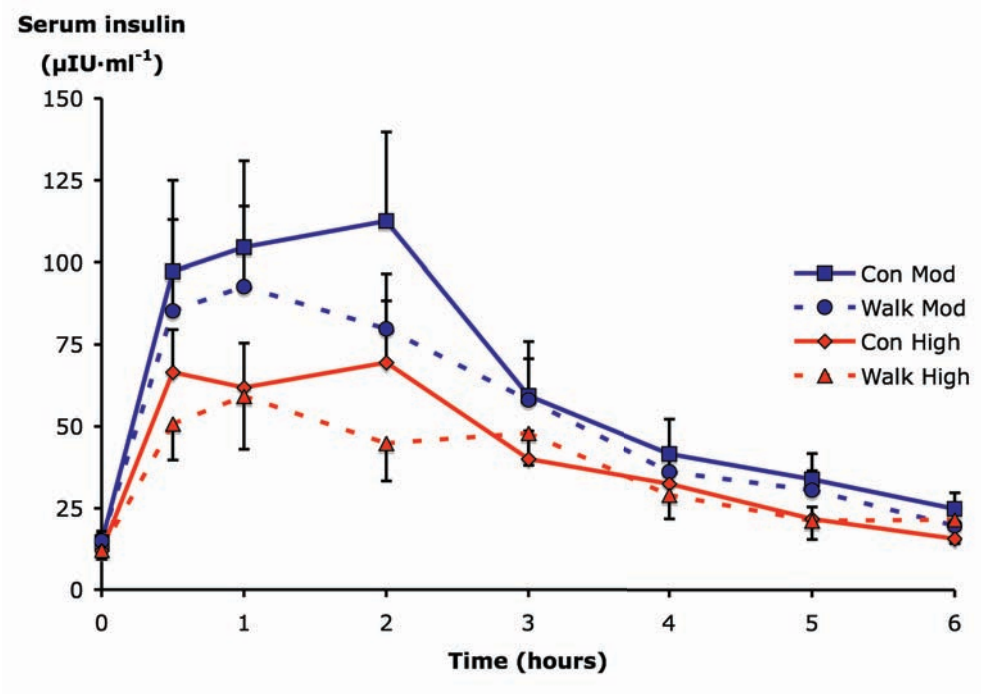
Chapter 3: Glucose

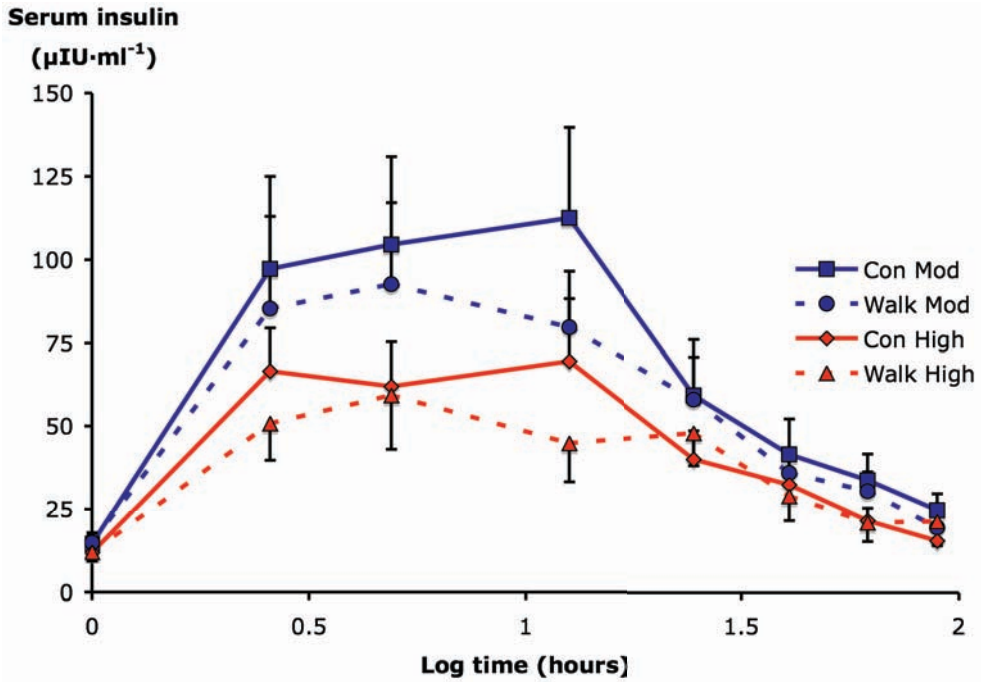




Metric used for analysis: Log transformed time.

Chapter 3: Insulin

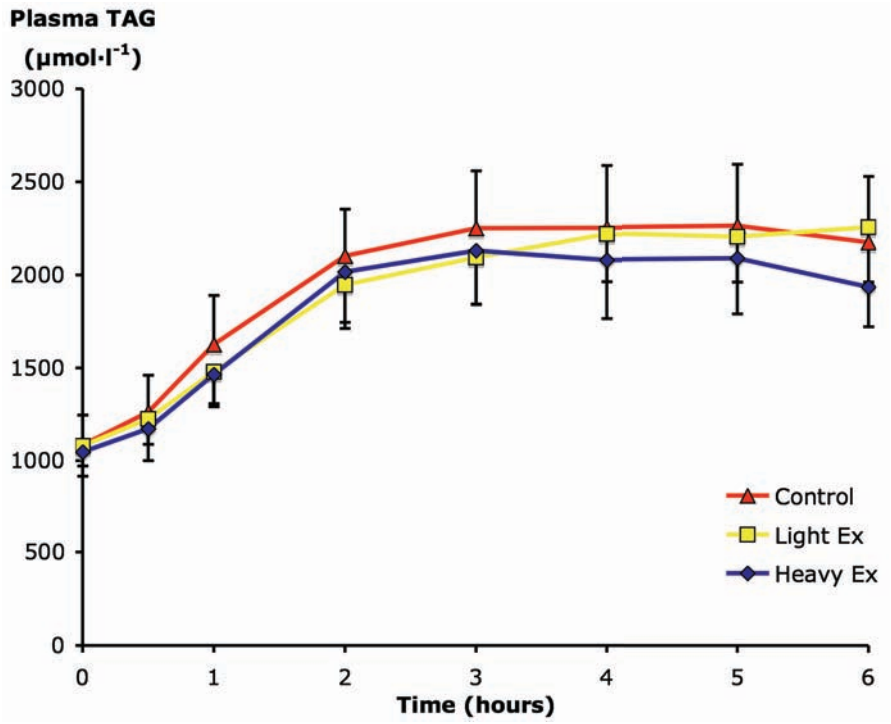


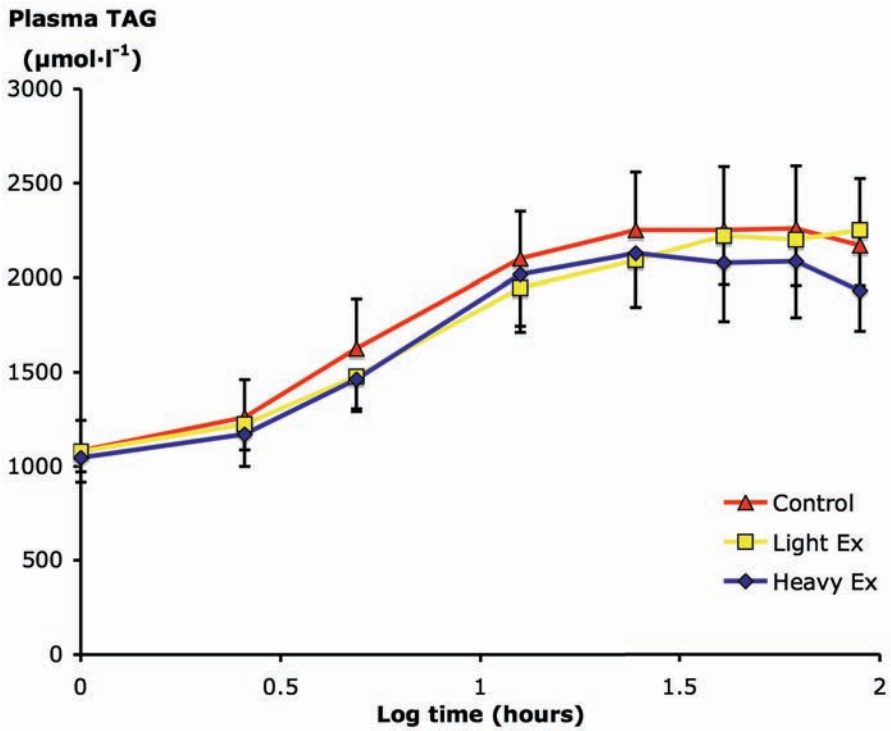


Metric used for analysis: Log transformed time.

Appendix B: Real time and log transformed time metabolite curves related to Chapter 4

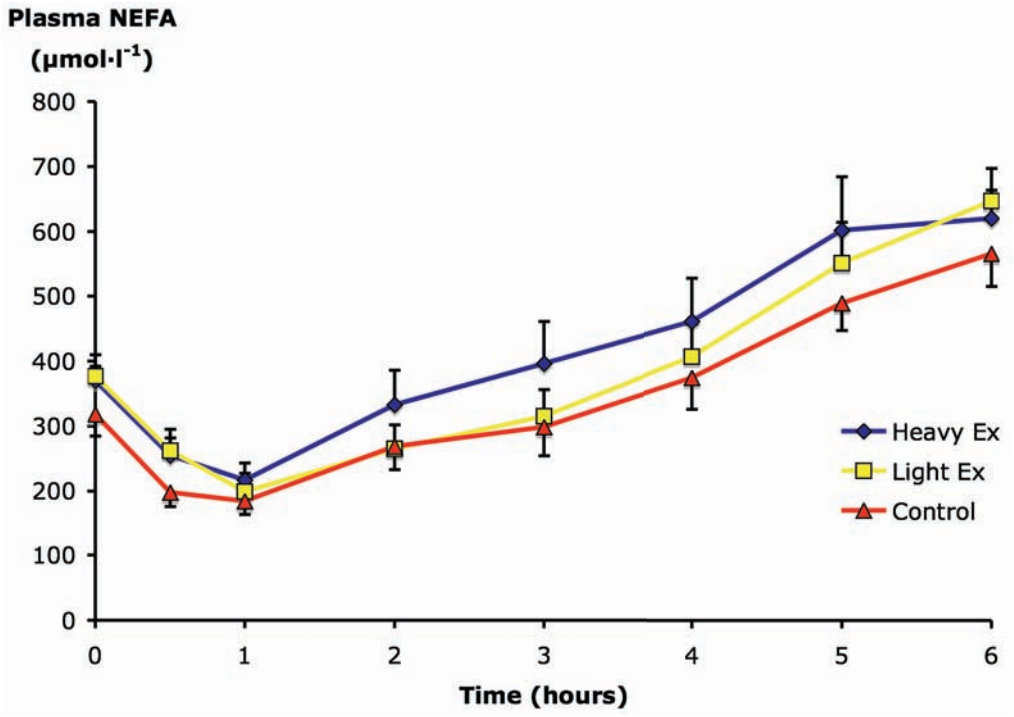
Triacylglycerol

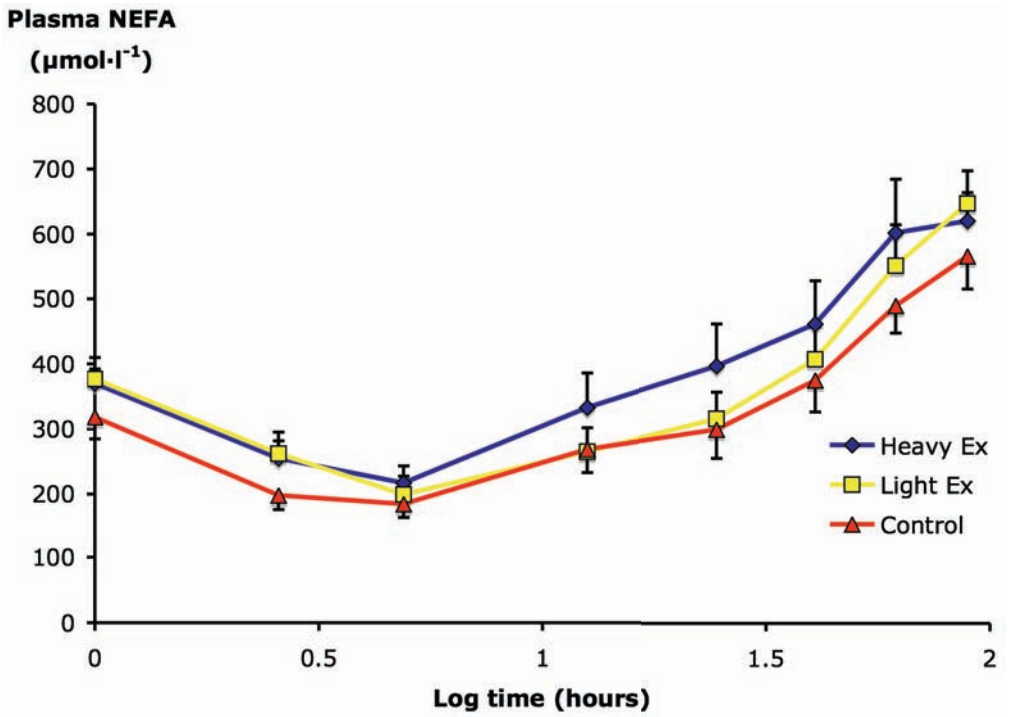




Metric used for analysis: Real time.

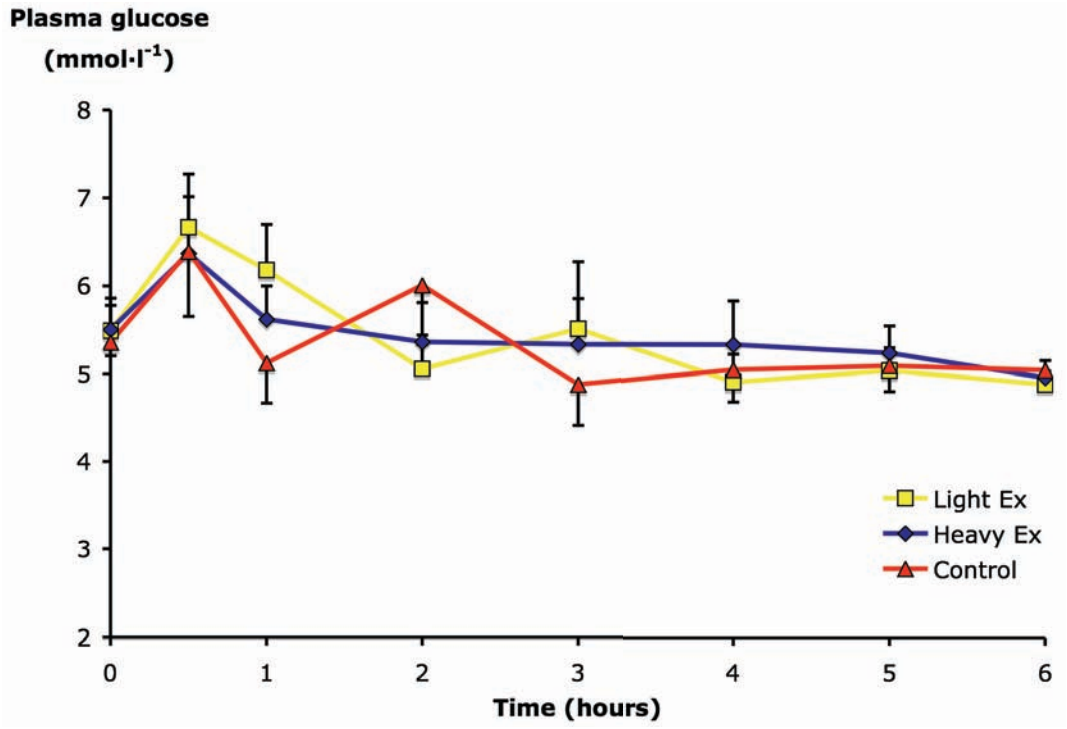
Chapter 4: Non-esterified fatty acids (NEFA)

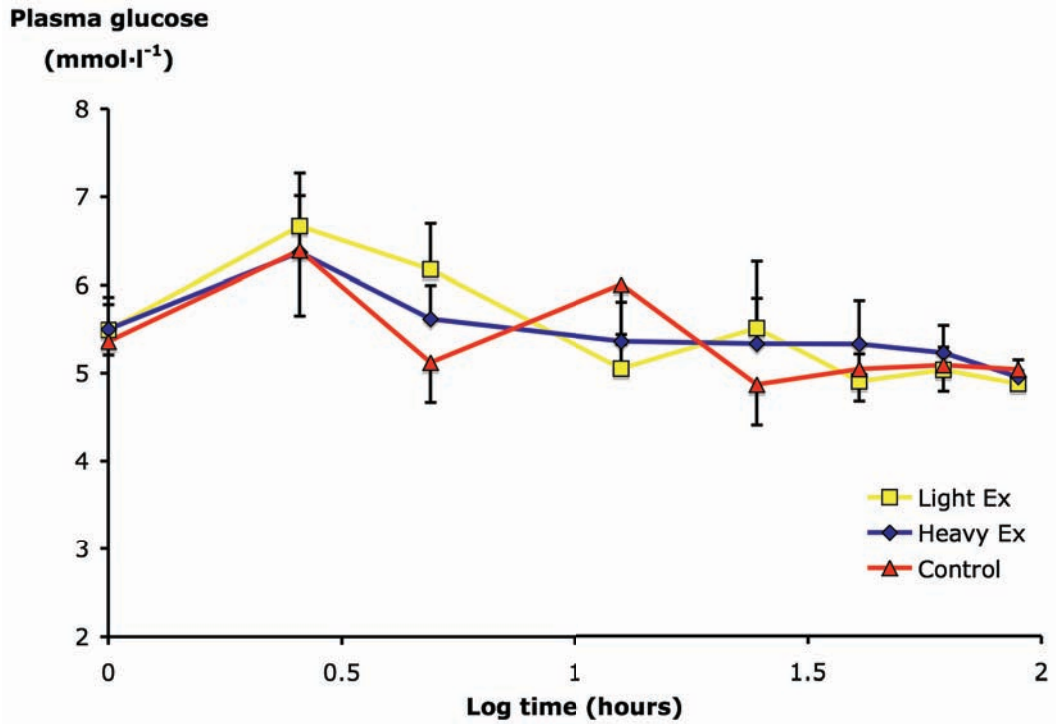




Metric used for analysis: Log transformed time.

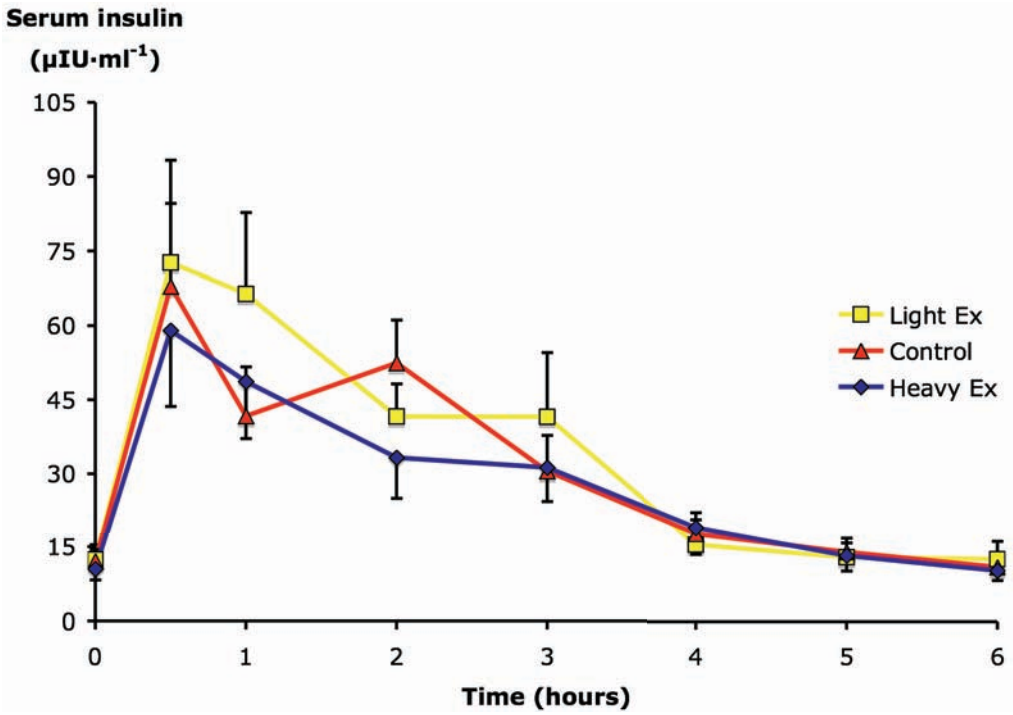
Chapter 4: Glucose

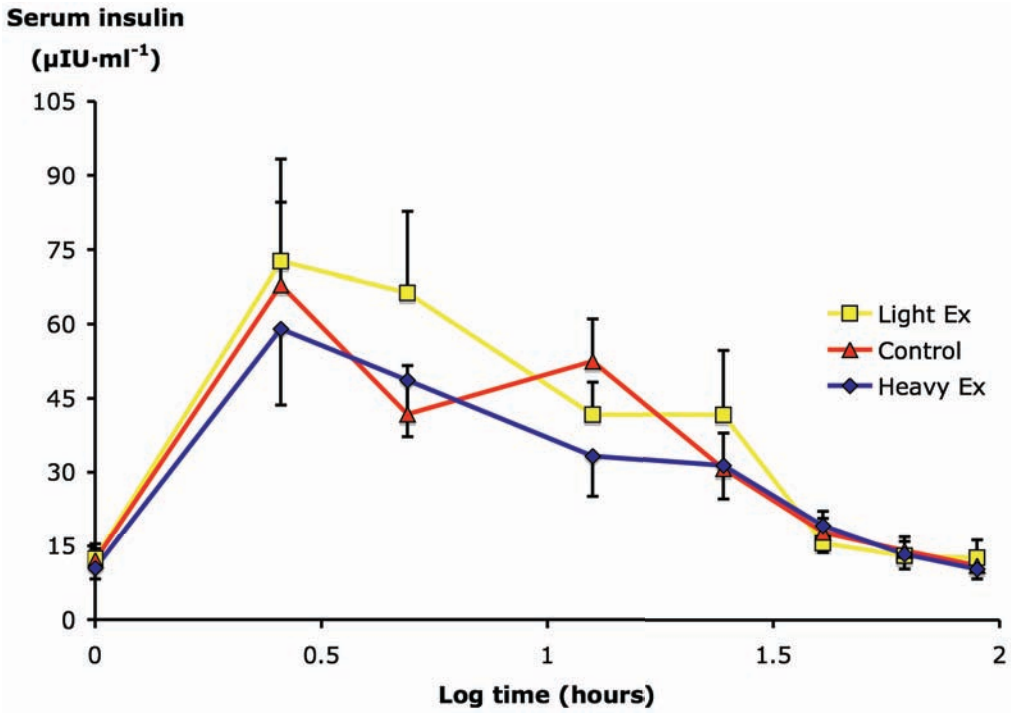




Metric used for analysis: Log transformed time.

Chapter 4: Insulin

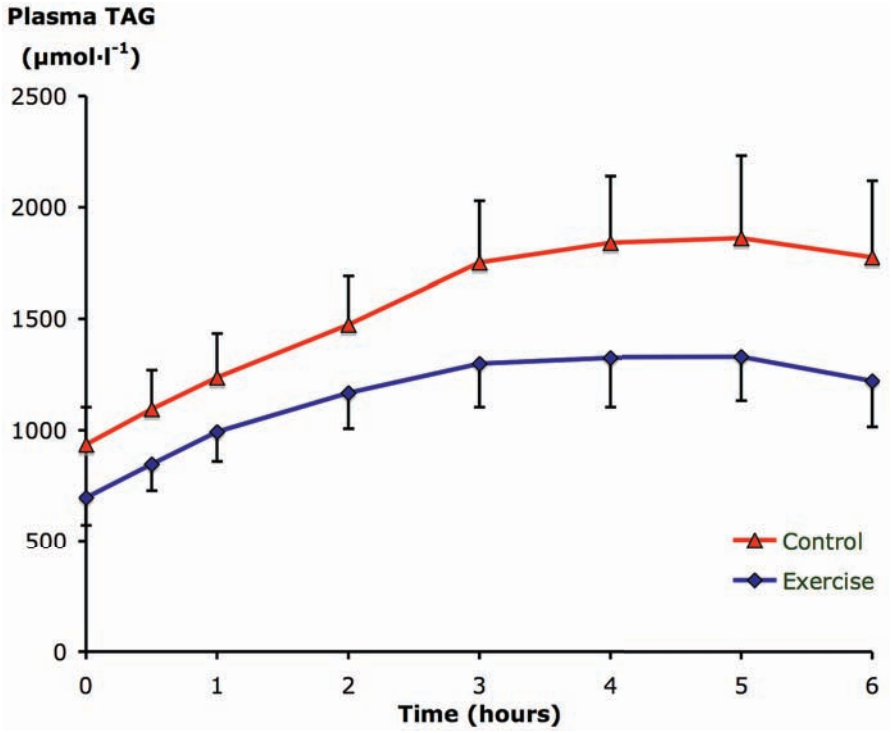


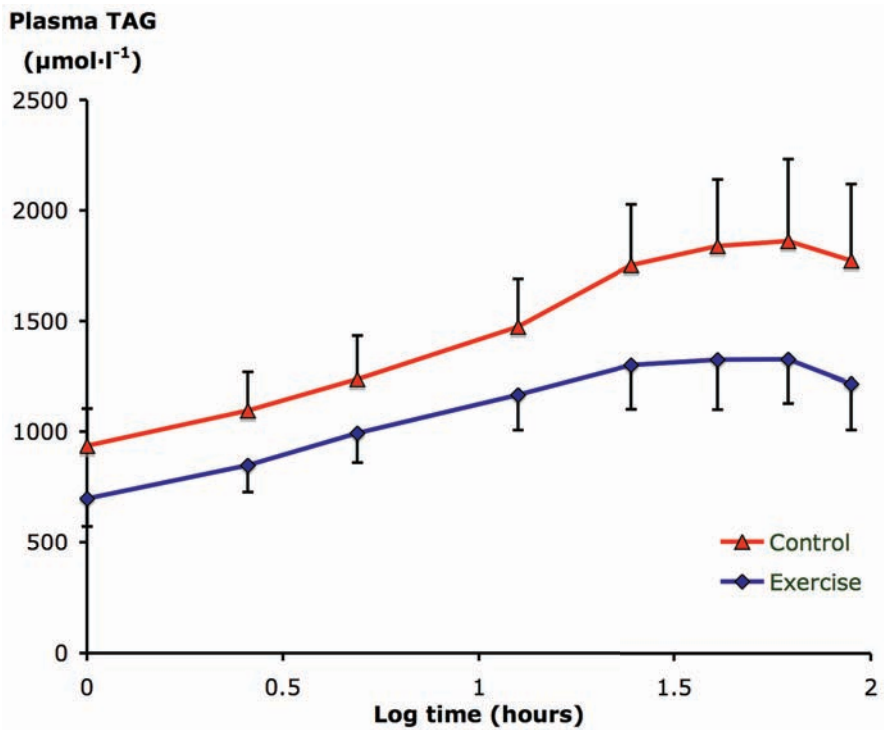


Metric used for analysis: Log transformed time.

Appendix C: Real time and log transformed time metabolite curves related to Chapter 5

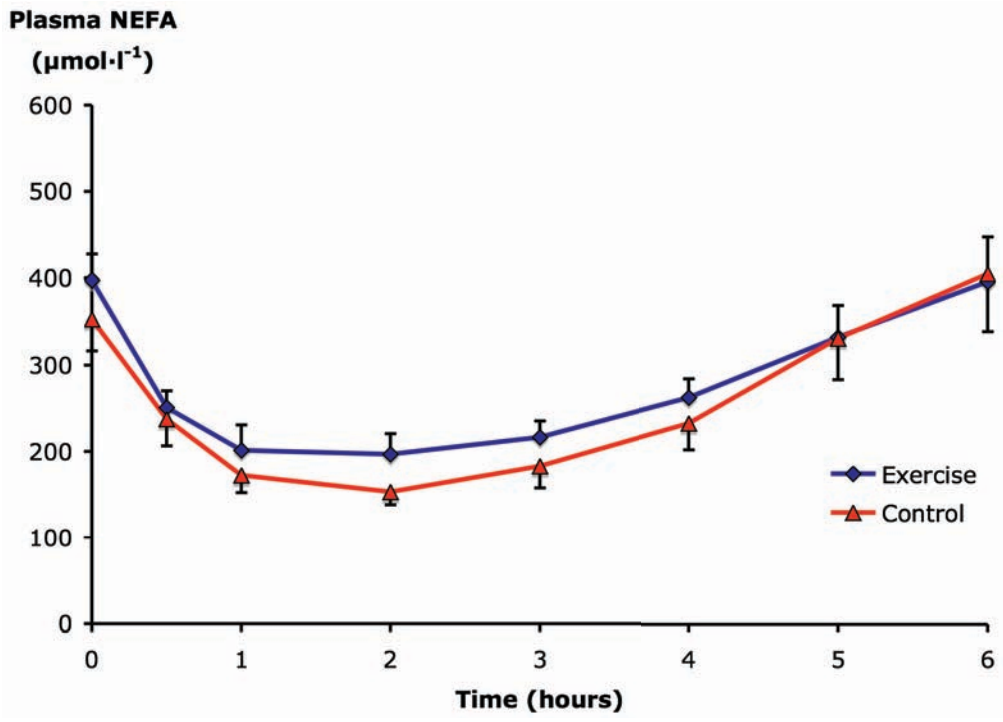
Triacylglycerol

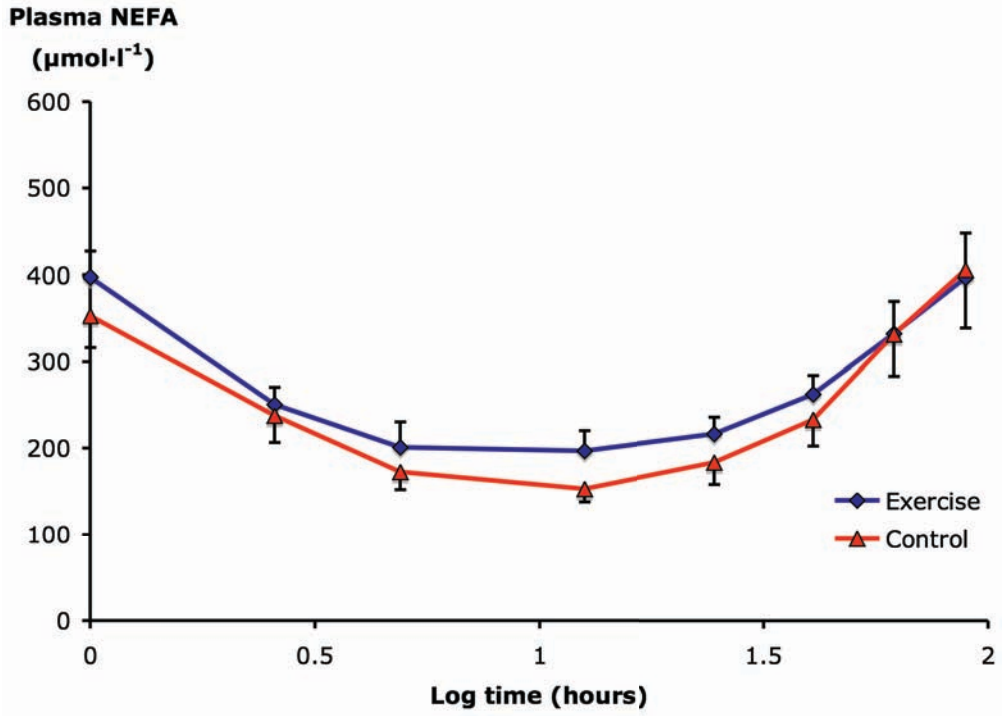




Metric used for analysis: Real time.

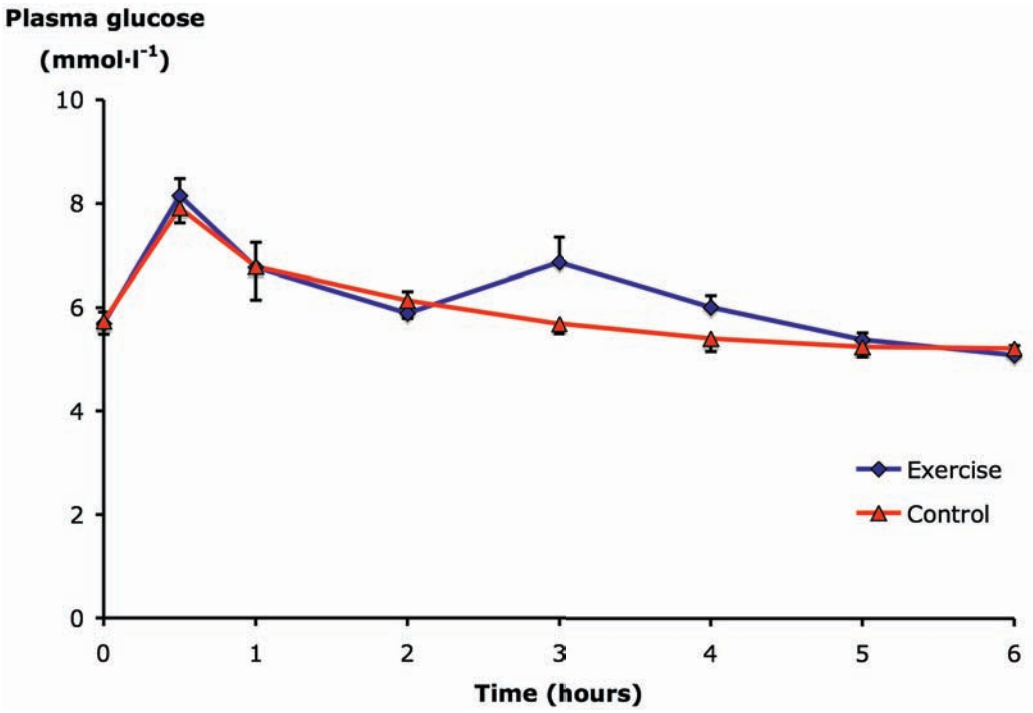
Chapter 5: Non-esterified fatty acids (NEFA)

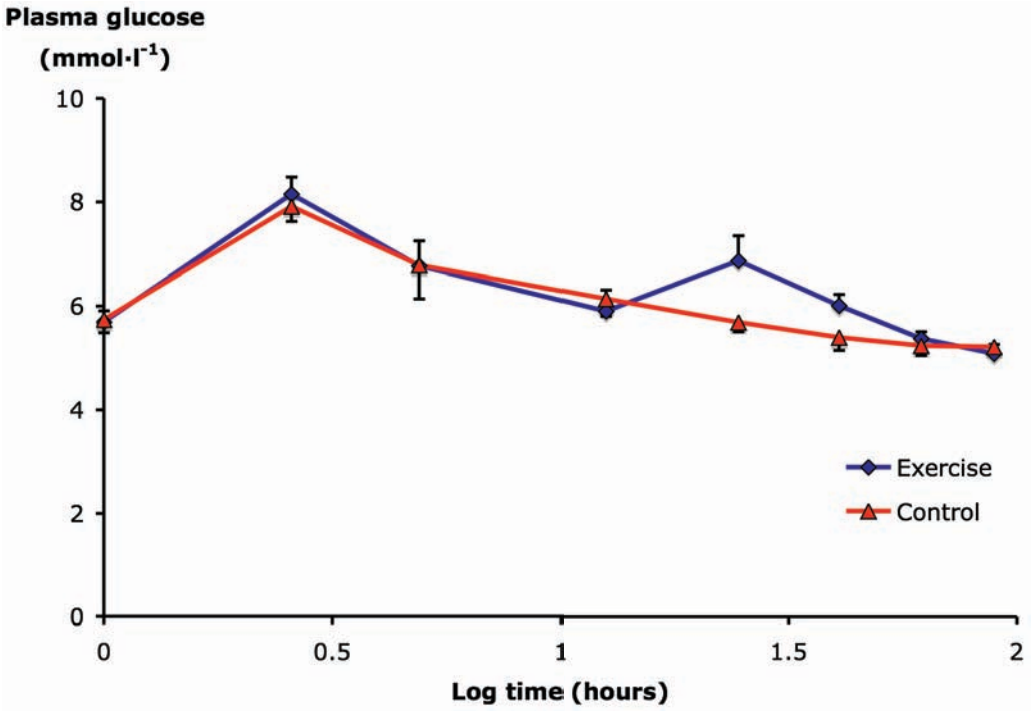




Metric used for analysis: Log transformed time.

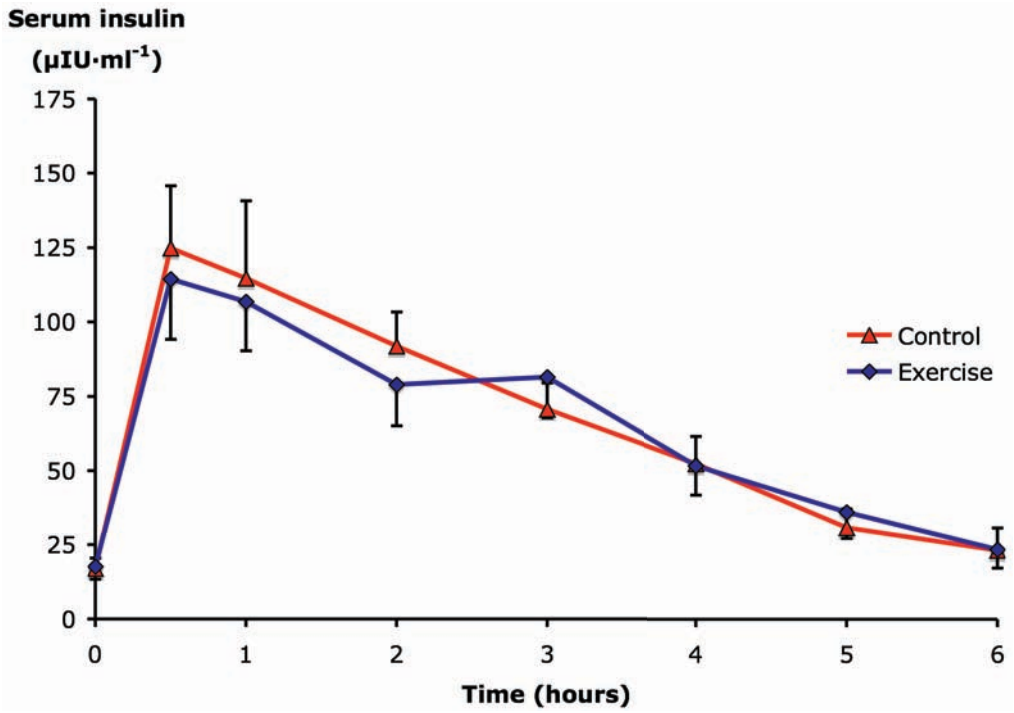
Chapter 5: Glucose

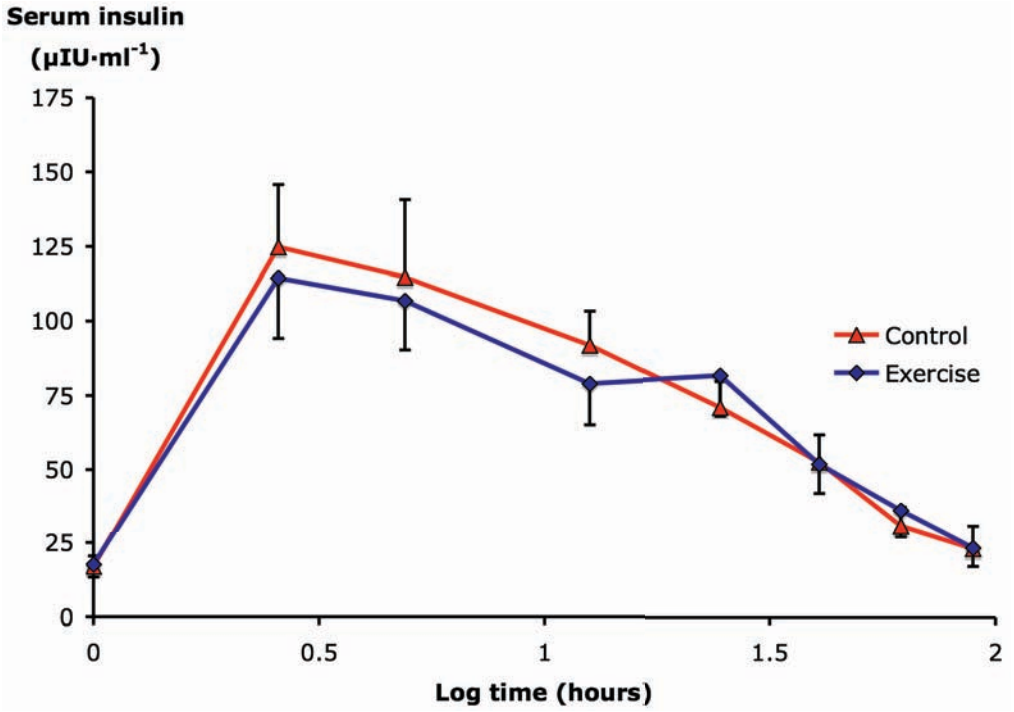




Metric used for analysis: Log transformed time.

Chapter 5: Insulin

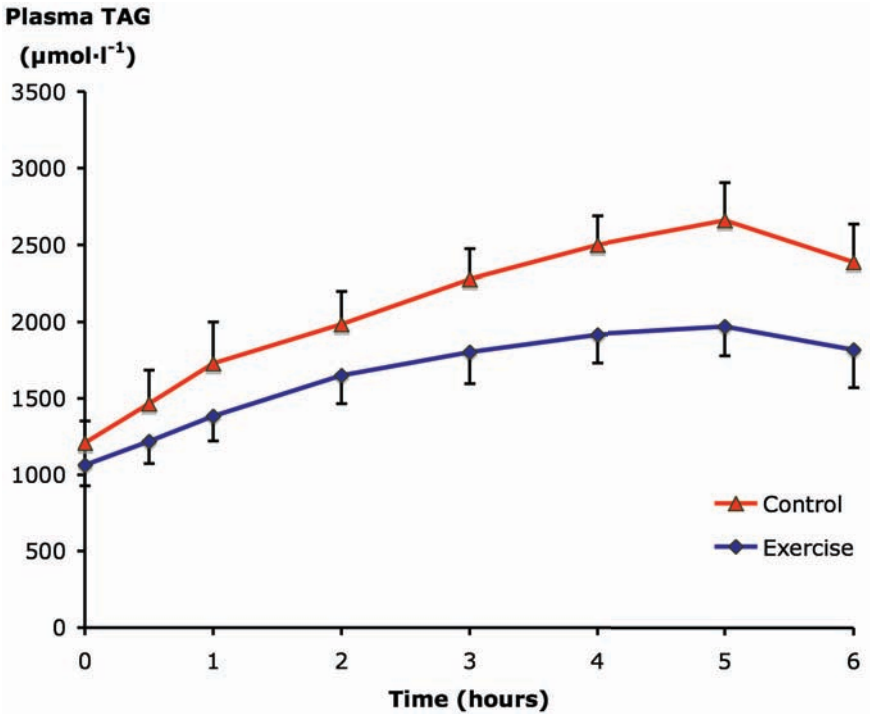


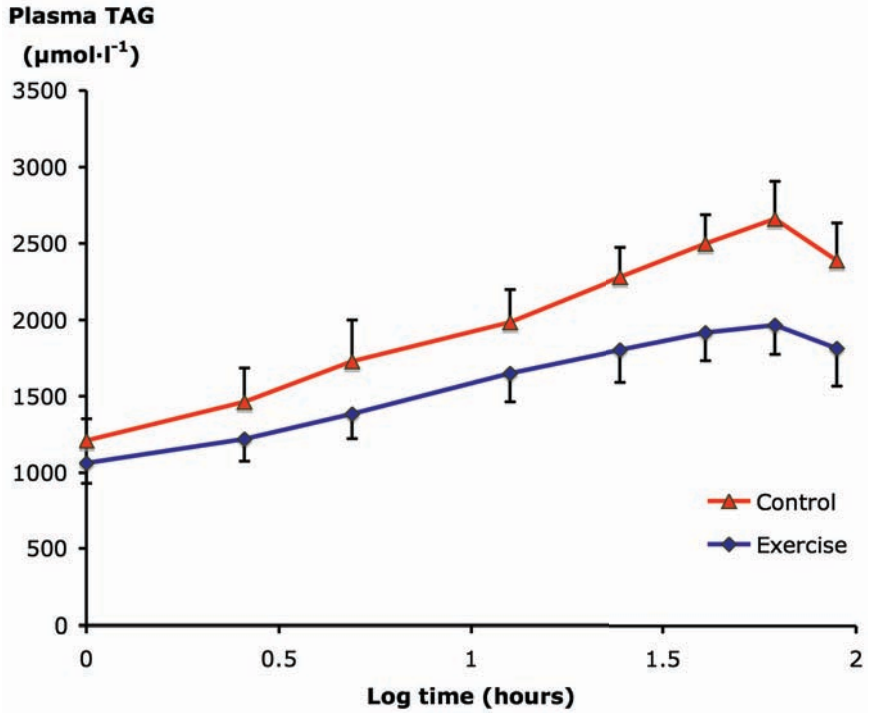


Metric used for analysis: Log transformed time.

Appendix D: Real time and log transformed time metabolite curves related to Chapter 6

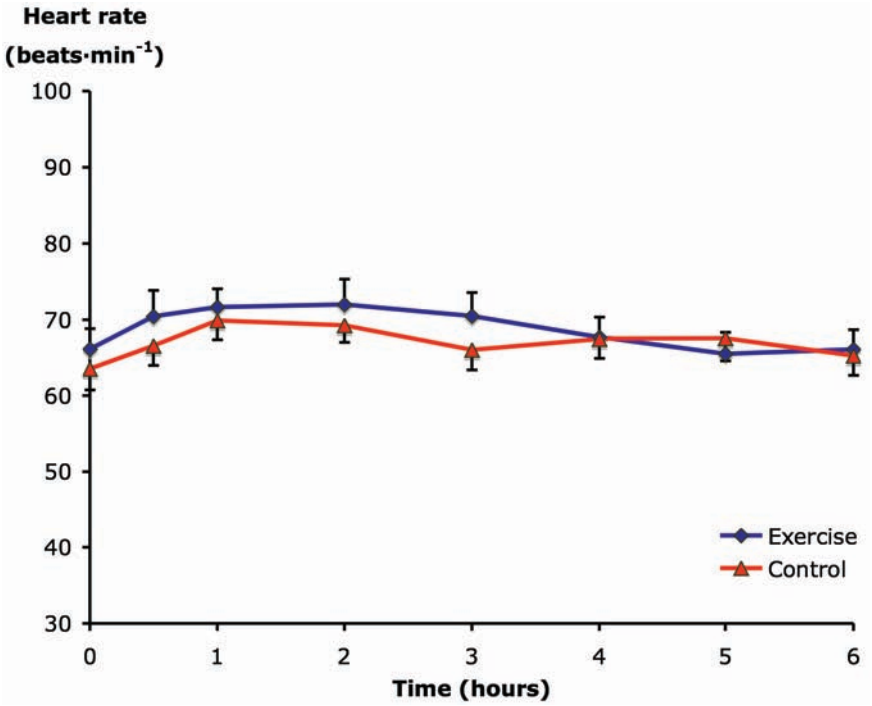
Triacylglycerol

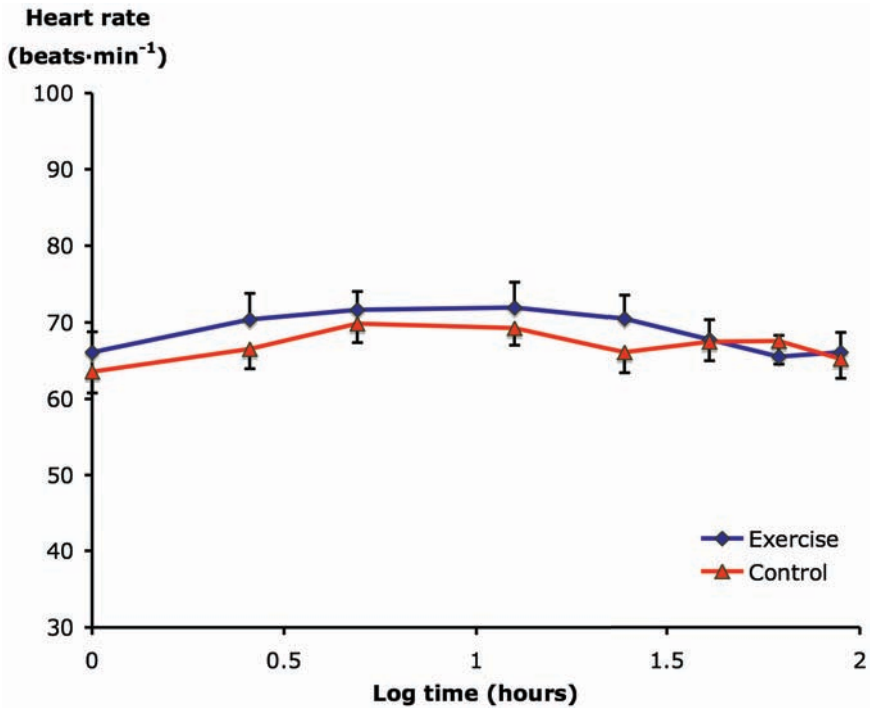




Metric used for analysis: Real time.

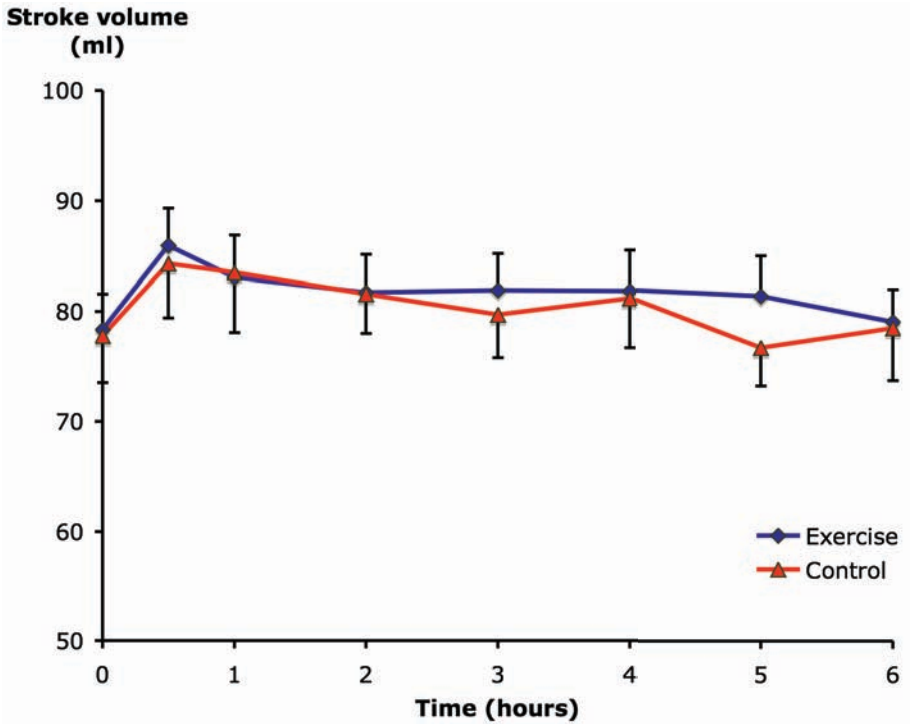
Chapter 6: Heart rate

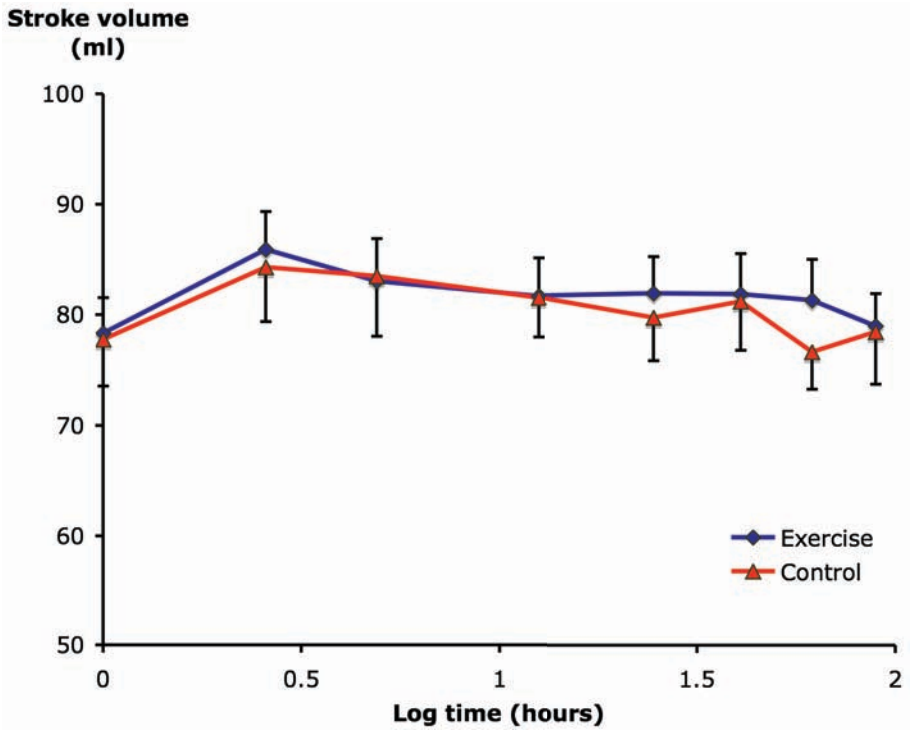




Metric used for analysis: Log transformed time.

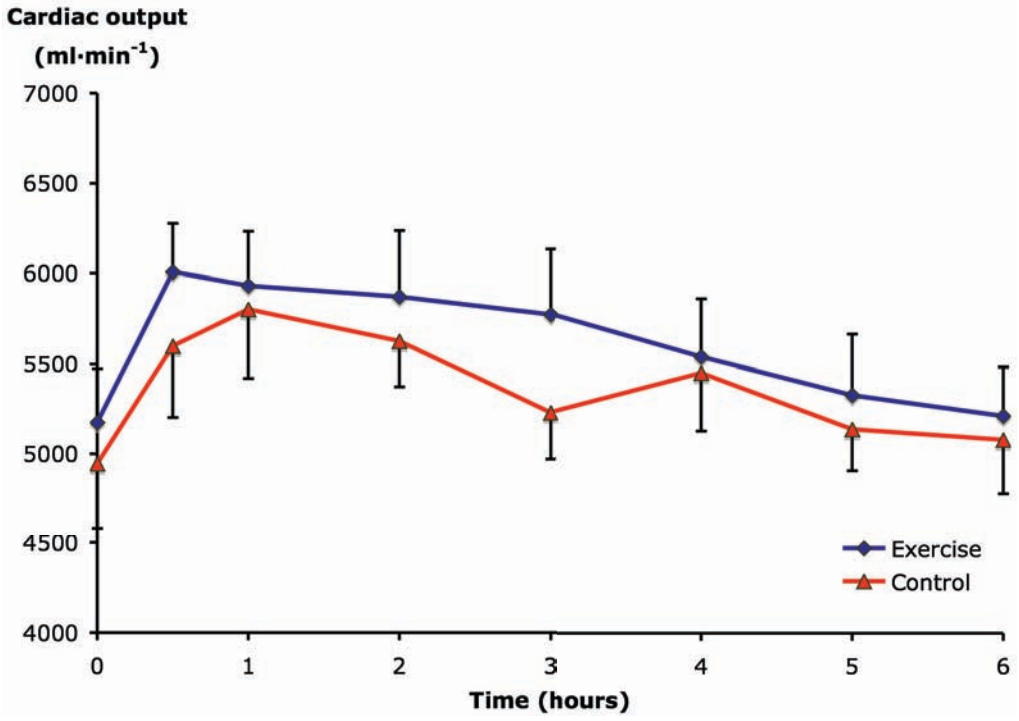
Chapter 6: Stroke volume

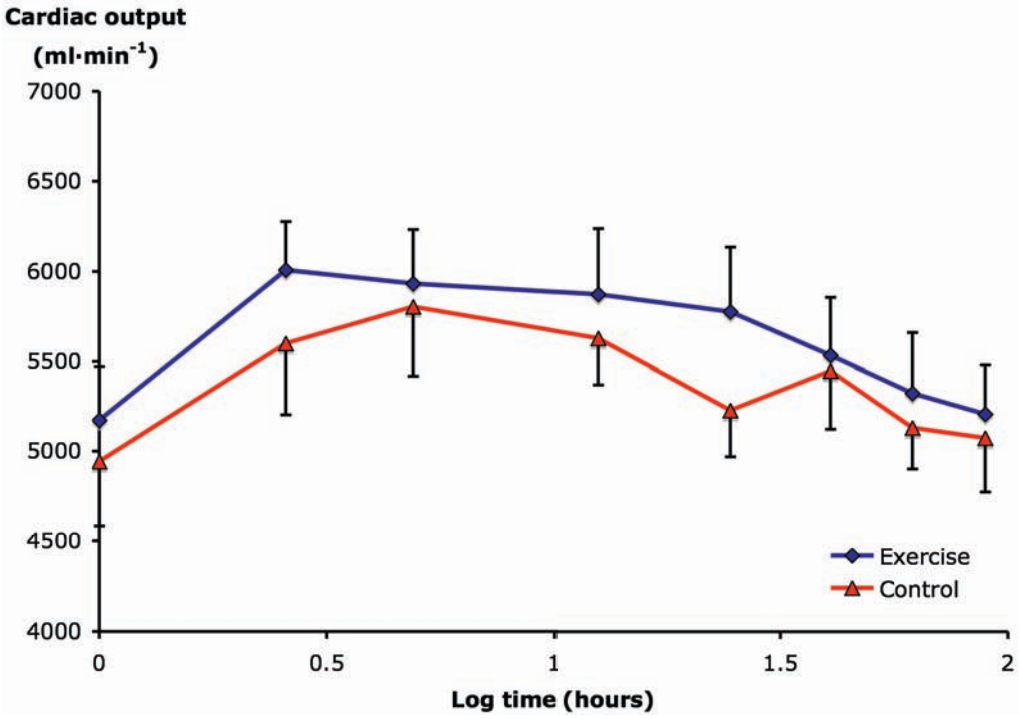




Metric used for analysis: Real time.

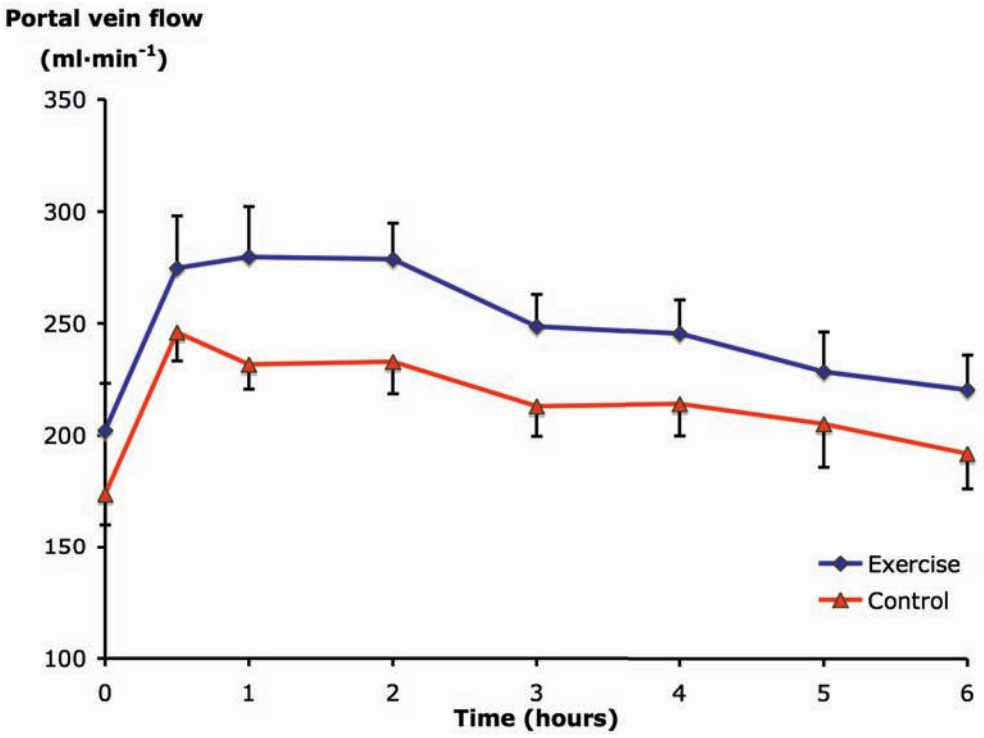
Chapter 6: Cardiac output

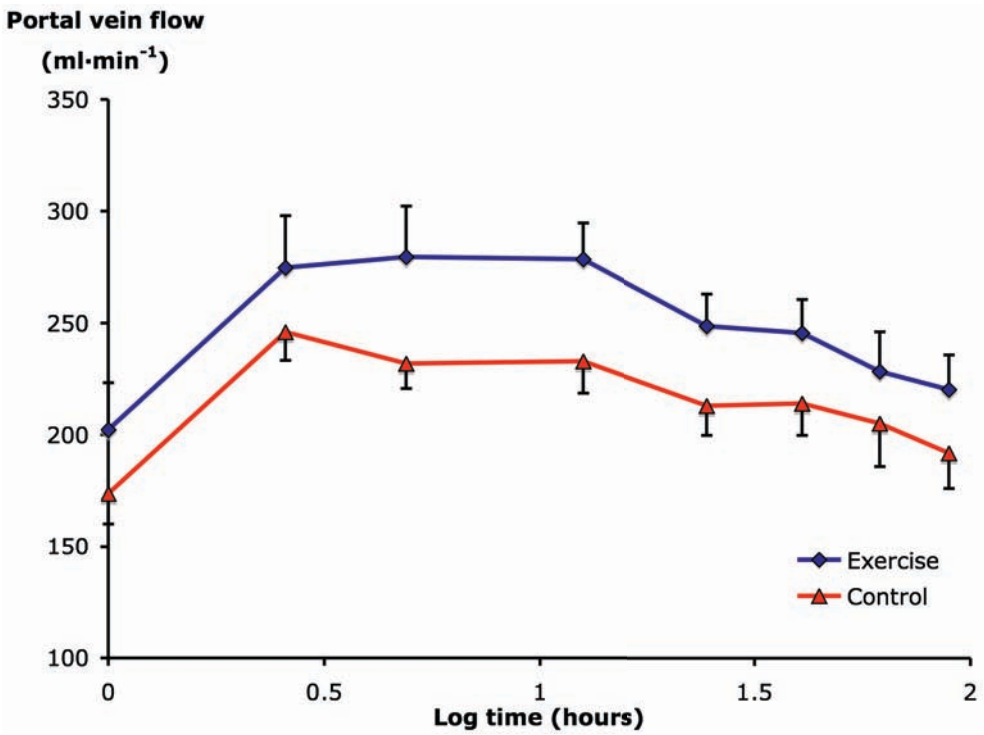




Metric used for analysis: Log transformed time.

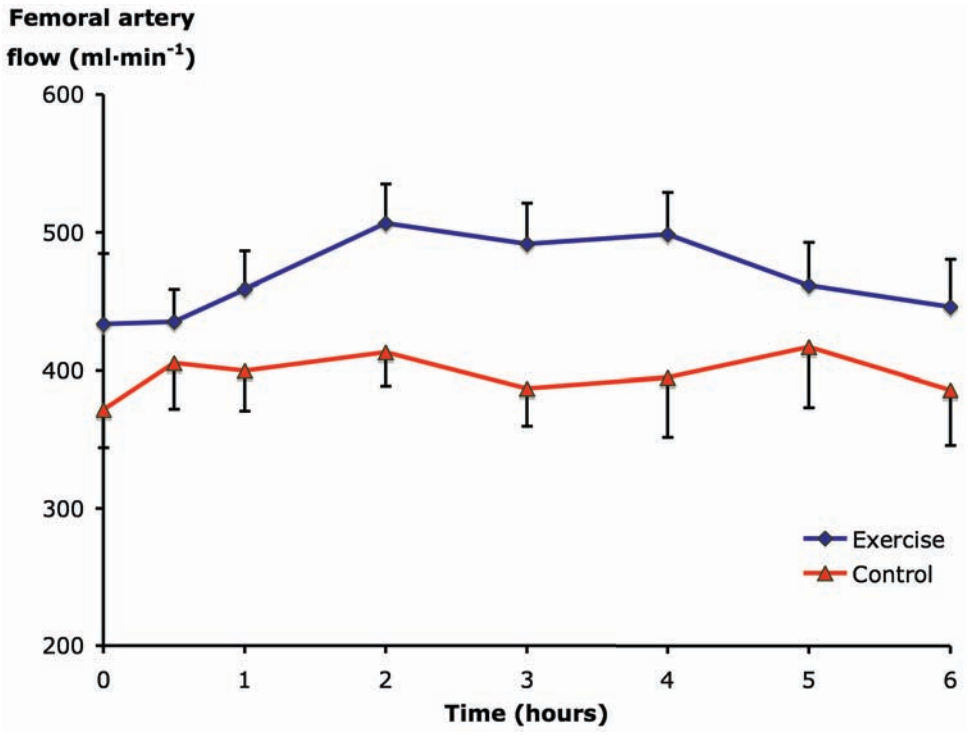
Chapter 6: Hepatic portal vein blood flow

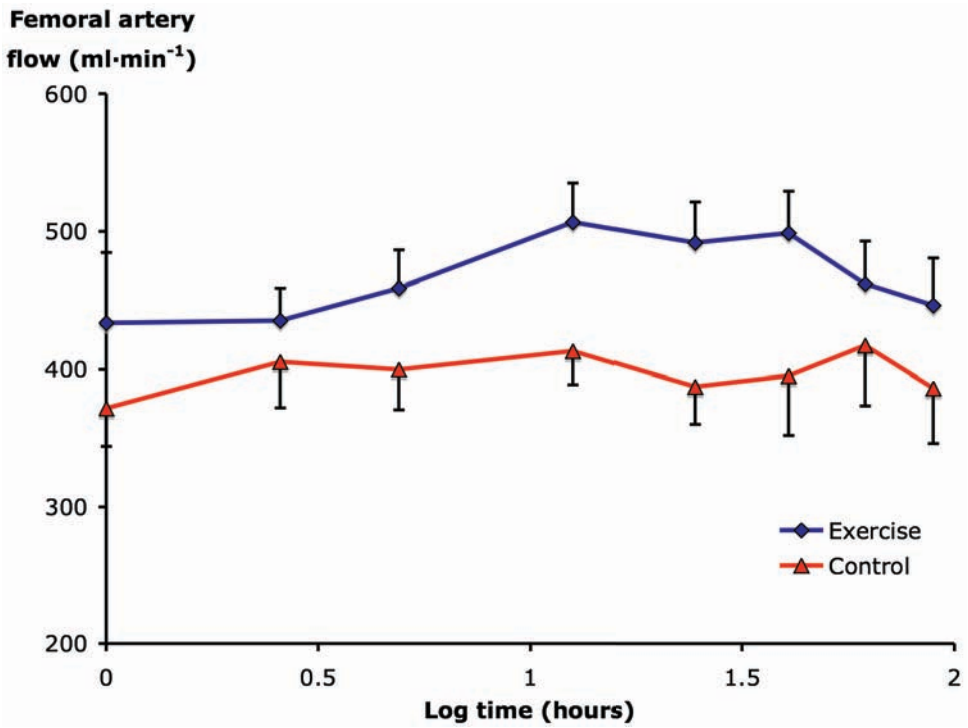




Metric used for analysis: Log transformed time.

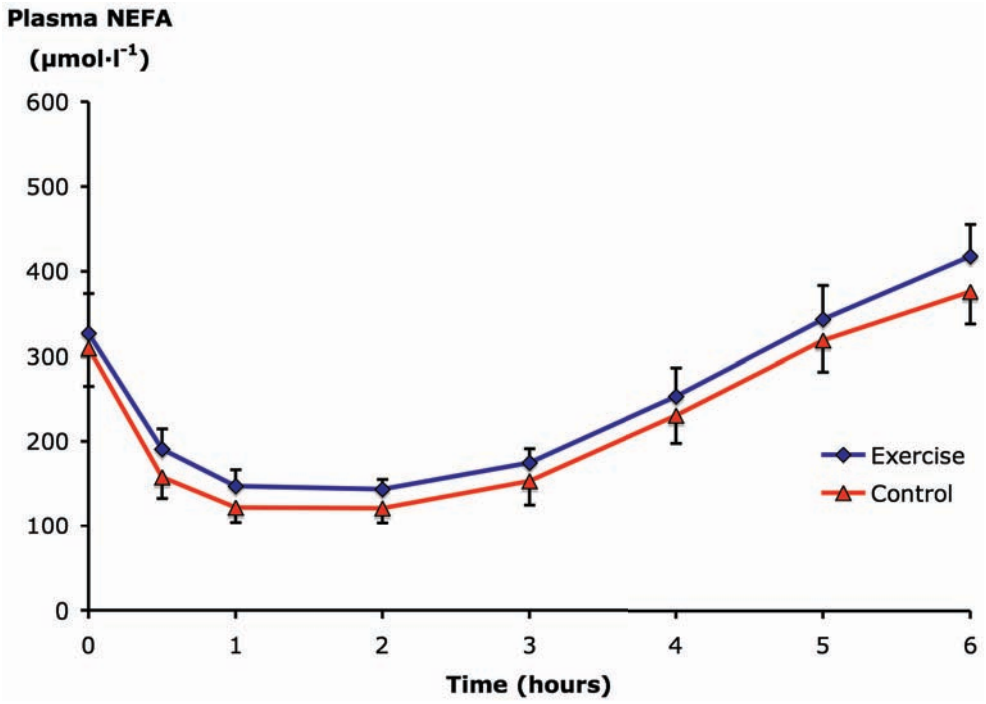
Chapter 6: Femoral artery blood flow

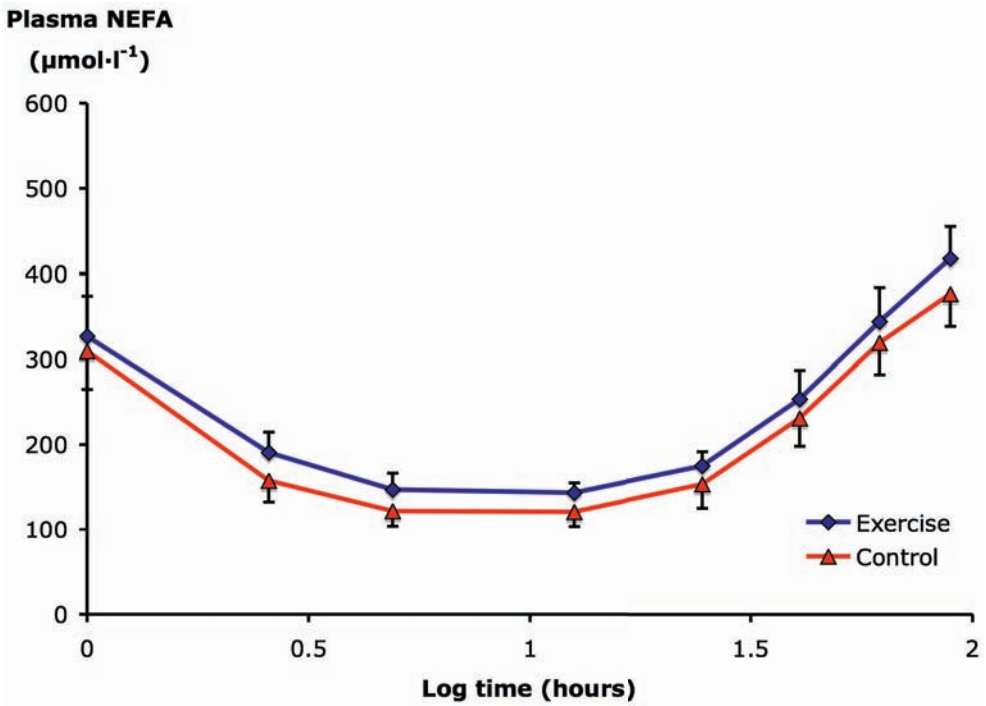




Metric used for analysis: Real time.

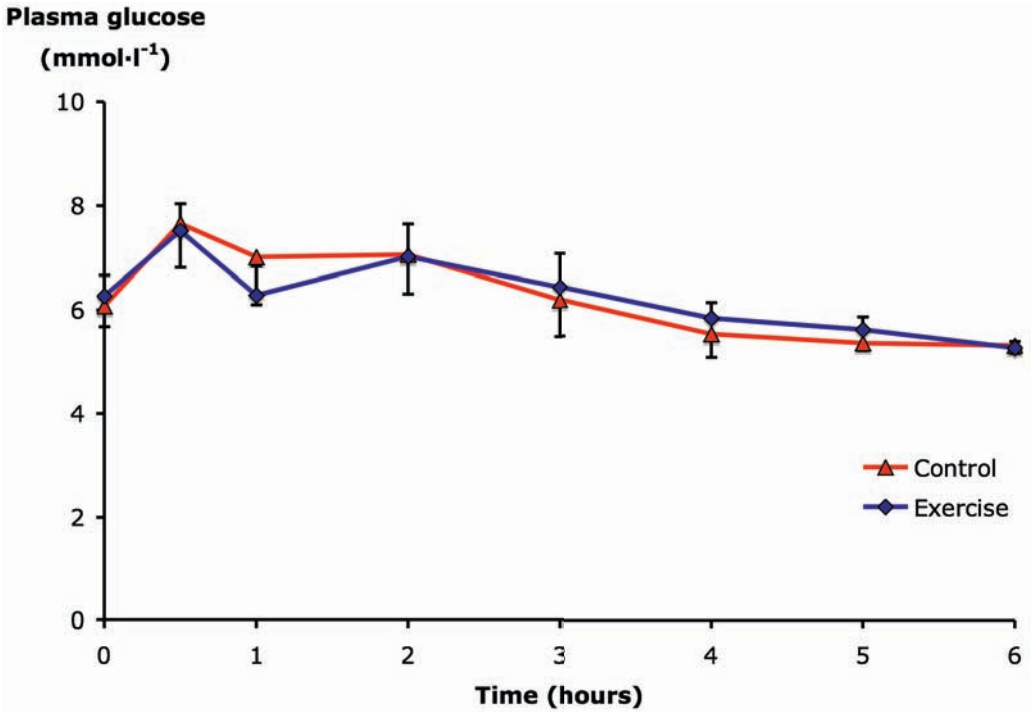
Chapter 6: Non-esterified fatty acids (NEFA)

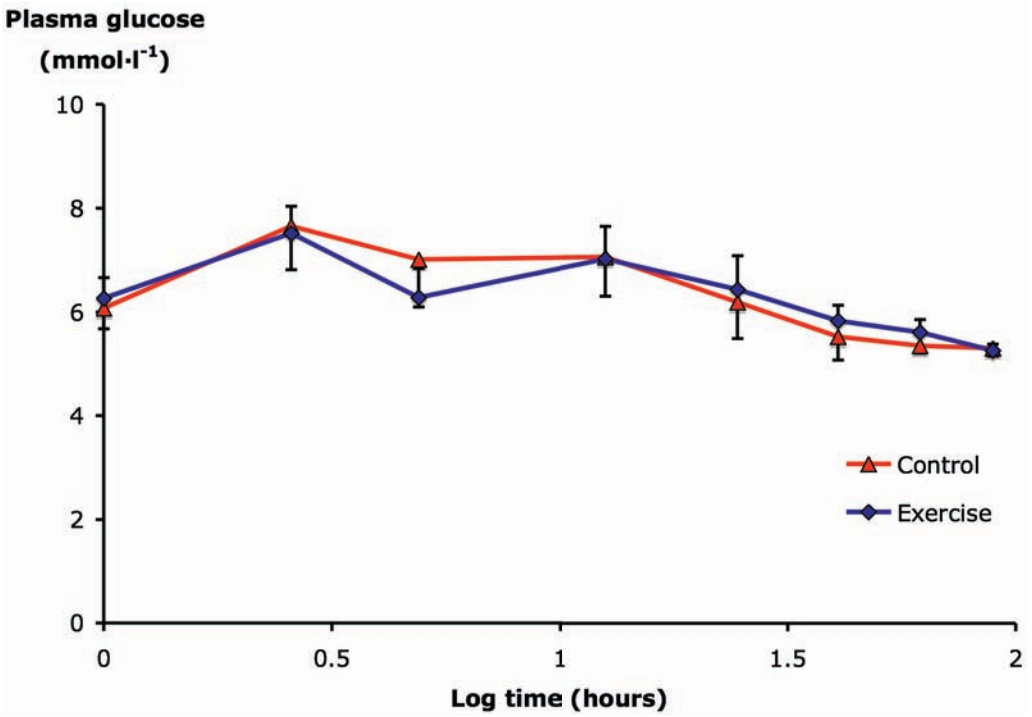




Metric used for analysis: Log transformed time.

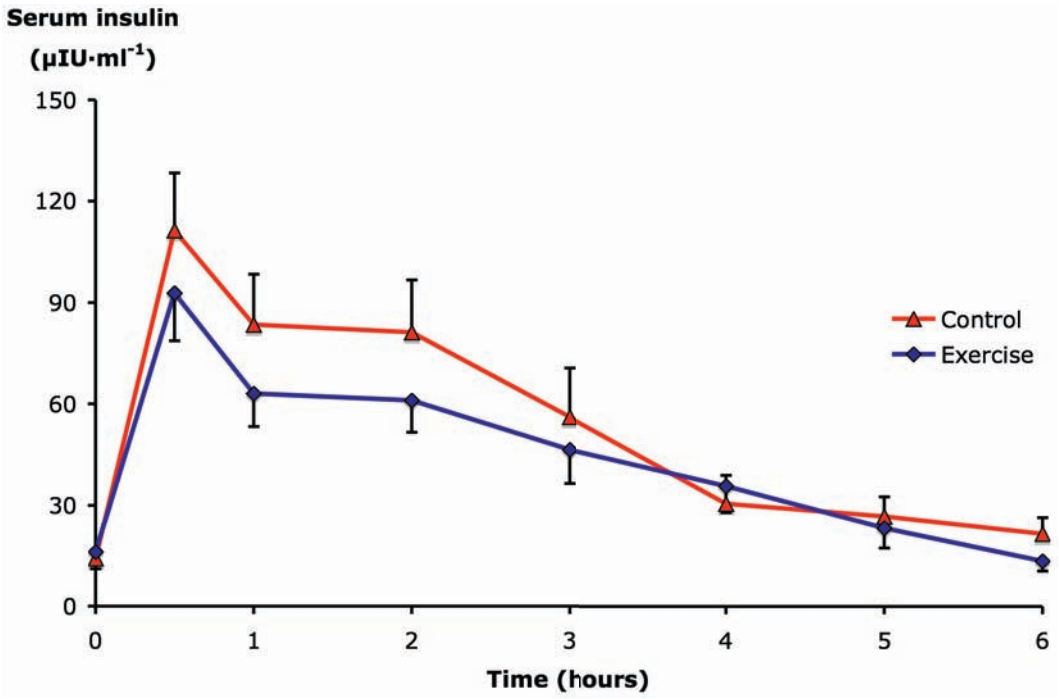
Chapter 6: Glucose

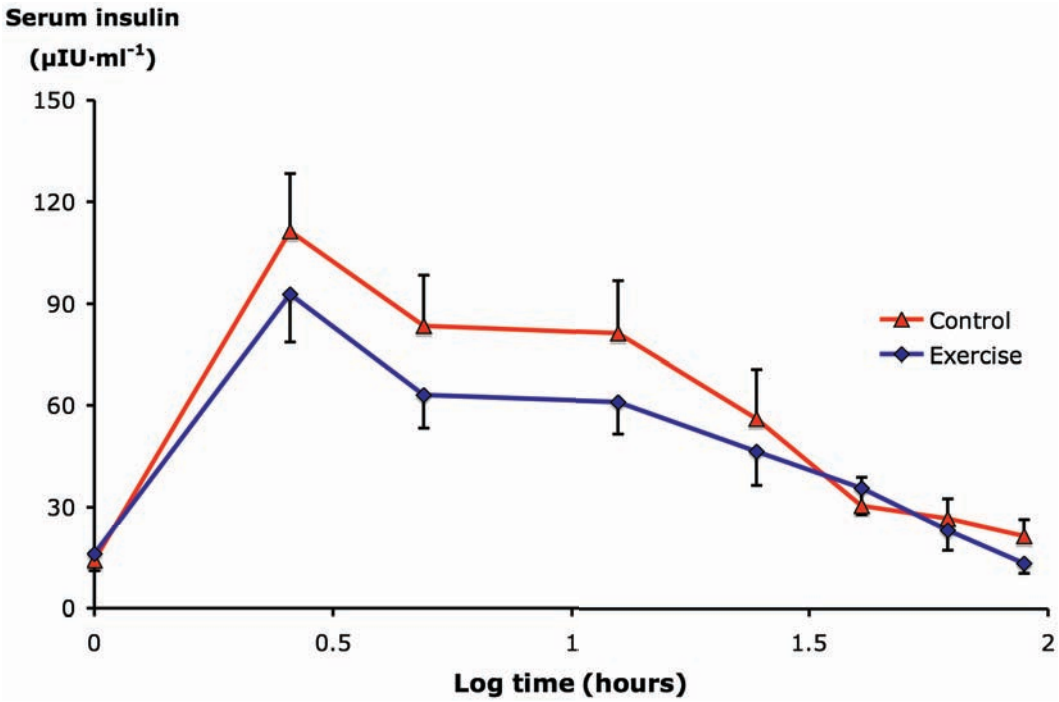




Metric used for analysis: Log transformed time.

Chapter 6: Insulin





Metric used for analysis: Log transformed time.