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ACUTE EXERCISE AND POSTPRANDIAL TRIGLYCERIDE METABOLISM:

**MECHANISMS FOR THE EXERCISE EFFECT
AND IMPLICATIONS FOR ENDOTHELIAL FUNCTION**

by

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School of Health and Human Performance

A Doctoral Thesis

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Declaration of Originality

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of Ph.D. is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

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ABSTRACT

MICHAEL HARRISON. SCHOOL OF HEALTH AND HUMAN PERFORMANCE.

ACUTE EXERCISE AND POSTPRANDIAL TRIGLYCERIDE METABOLISM: MECHANISMS FOR THE EXERCISE EFFECT AND IMPLICATIONS FOR ENDOTHELIAL FUNCTION

Acute exercise has consistently been shown to reduce postprandial lipemia, an independent risk factor for atherosclerosis. In a series of three studies, this thesis sought to

1. compare the influence of acute exercise on postprandial metabolism in normal weight, overweight and obese insulin resistant men
2. determine the influence of acute exercise on endothelial microparticles and soluble adhesion molecules, plasma biomarkers of endothelial dysfunction, in the fasted and postprandial states
3. investigate the mechanisms mediating the exercise attenuation of postprandial lipemia, and in particular the role of skeletal muscle lipoprotein lipase (LPL) and the importance of glycogen depletion.

In all three studies, recreationally active men (aged 22–45) consumed a high fat mixed meal on the morning following prolonged exercise at 70% VO_2max , or no exercise. One study involved two exercise trials, with the influence of exercise determined with and without post-exercise carbohydrate refeeding.

A 700kcal exercise bout reduced postprandial lipemia similarly (~20%) in normal weight, overweight and obese men, with the magnitude of the attenuation positively related to VO_2max . Exercise also reduced postprandial insulin though this was unrelated to the reduction in lipemia.

Endothelial microparticles increased postprandially, indicative of endothelial dysfunction, but this response was not attenuated by exercise. Soluble adhesion molecules did not change postprandially or following exercise.

The exercise attenuation of lipemia was largely reversed by post-exercise carbohydrate refeeding that repleted muscle glycogen. However, LPL activity was not significantly influenced by exercise or by carbohydrate refeeding. Considerable inter-individual variation was evident in the LPL response to exercise, with changes in LPL activity inversely related to changes in postprandial lipemia.

Taken together, these studies confirm that single bouts of exercise can reduce postprandial lipemia, but without enhancing endothelial function. This attenuation is likely to be mediated by changes in muscle LPL activity but also by unknown factors associated with glycogen depletion.

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*Dedicated to
Pamela, Ellen and Kate*

Chapter One

LITERATURE REVIEW

1.1 Atherosclerosis

Atherosclerosis is a vascular disease that occurs principally in the large and medium sized elastic and muscular arteries. Specific sites in the arterial tree including branch points and curvatures are particularly predisposed to atherosclerosis (Ross, 1999). Atherosclerotic lesions develop in the subendothelial space of the arterial intima. The earliest type of lesion, the fatty streak, consists of lipid-laden macrophages and lymphocytes. If hyperlipidemia persists and inflammatory conditions prevail, the *lipid rich core can grow and the artery* typically remodels and enlarges outwards, thus preserving lumen diameter. The inflammatory conditions can degrade the collagen matrix of the intima, weakening the fibrous cap and making the lesion vulnerable to rupture. Such ruptures bring blood in contact with tissue factor and result in a thrombus forming that can occlude the vessel resulting in infarction. Alternatively, if fibrinolytic mechanisms prevail, a limited thrombus results and the clot is resorbed into the lesion as the wound heals. The release of growth factors from thrombus platelets leads to smooth muscle migration from the media, smooth muscle proliferation and collagen production. This thickening of the lesion stabilises the cap but the growth is often inwards, decreasing lumen diameter. The evidence from clinical observations suggests that *atheromas grow discontinuously with sudden lesion progression, probably relating to lesion* disruption and thrombus formation (Libby, 2002).

The pathophysiology of atherosclerosis is closely linked to lipid and lipoprotein metabolism. Low density lipoprotein (LDL) is regarded as the principal atherogenic lipoprotein but chylomicron and very low density lipoprotein (VLDL) remnants are also rich in

cholesterol and have been shown to penetrate the endothelium (Mamo et al, 1998). The importance of dyslipidemia as a mediator of atherosclerosis is evident from a wealth of experimental trials using animal models of atherosclerosis, and more recently from clinical intervention trials using statins to lower plasma cholesterol (Steinberg, 2005). However LDL, even in high concentrations, does not lead to foam cell formation in vitro (Brown and Goldstein, 1983) whereas LDL oxidised by incubation with endothelial cells, smooth muscle cells or macrophages, is taken up by macrophages rapidly. Hypercholesterolemia is likely to be a permissive factor for atherogenesis. Although the majority of individuals with coronary artery disease (CAD) in the US have average levels of cholesterol, such average levels probably exceed by far normal levels for our species (Libby, 2002).

Atherosclerosis is an inflammatory disease. Modified lipoprotein particles in the arterial intima are a key trigger for inflammation. The mechanistic links between oxidised lipoproteins and inflammation are emerging. Endothelial cells in contact with blood normally resist leukocyte adhesion, but when subject to oxidative stress, express specific adhesion molecules including vascular cell adhesion molecule (VCAM-1) and intercellular adhesion molecule (ICAM-1). Chemoattractant cytokines (chemokines) specific for monocytes and T lymphocytes are released by activated endothelial cells, smooth muscle cells and leukocytes already resident in the atheroma, to attract additional monocytes and T lymphocytes into the arterial intima. Chemokines for monocytes include monocyte chemoattractant protein (MCP-1) and interleukin-8 (IL-8). Interferon γ (IFN- γ) inducible chemokines attract T lymphocytes into the intima. The activated endothelium also releases macrophage colony stimulating

factor (M-CSF) facilitating the conversion of the monocytes entering the subendothelial space to lipid-laden macrophages as well as macrophage proliferation (Libby, 2002). Together, these cytokines are responsible for the adhesion, migration, activation, accumulation and survival of monocytes and T lymphocytes in atheromas. Oxidised LDL (oxLDL) accumulates rapidly in macrophages via a scavenger receptor that is not down regulated with increasing lipid content, resulting in the lipid-laden macrophage or foam cell, characteristic of fatty streaks.

Macrophages release a variety of substances that result in lesion progression and complication. Pro-inflammatory cytokines including tumour necrosis factor- α (TNF- α) and IL-1 amplify the local inflammatory response in the lesion and on the endothelium. Reactive oxygen species lead to greater oxidation of atherogenic lipoproteins present in the intima. Proteolytic enzymes particularly matrix metalloproteinases (MMP) degrade the interstitial collagen matrix and the sub-endothelial basement membrane, weakening the fibrous cap of the lesion. Tissue factor creates a thrombogenic environment and in the event of lesion disruption, contact between tissue factor and circulating coagulation proteins leads to thrombus formation. Growth factors including vascular endothelial growth factor (VEGF), transforming growth factor β (TGF- β) and platelet derived growth factor (PDGF) result in smooth muscle migration into, and proliferation in the intima. Increased activity of these growth factors thickens the fibrous cap of the lesion.

Recent evidence suggests that acute phase inflammatory proteins of hepatic origin are not only markers of inflammation but may be direct mediators of atherosclerosis (Chart et

al , 2005). Both C-reactive protein (CRP) and serum amyloid A protein (SAA) are positive acute phase proteins that are secreted by the liver in response to IL-6, IL-1 and TNF- α . Evidence from cell culture studies suggests that CRP may increase synthesis of atherogenic molecules including cell adhesion molecules, MCP-1, IL-8, reactive oxygen species, tissue factor and MMPs (Chait et al , 2005) CRP is emerging as a risk factor for cardiovascular disease that is independent of traditional lipid risk factors. SAA is an apolipoprotein carried in the circulation primarily on the HDL particle and displaces apo A-I from HDL *in vitro* (Husebekk et al , 1987) It is predictive of cardiovascular disease in prospective and cross-sectional studies (Jousilahti et al, 2001) SAA present on HDL facilitates particle uptake by macrophages, transforming HDL from being an anti-atherogenic to a pro-atherogenic lipoprotein particle (Artl et al , 2000) It can trigger the expression of MMP in fibroblasts with implications for plaque stability (Migita et al., 1998) and act as a chemoattractant for monocytes and neutrophils (Xu et al , 1995)

A number of anti-inflammatory, antioxidant and lipid lowering mechanisms exist that are atheroprotective and relevant to an understanding of atherosclerosis A number of atheroprotective genes have been identified that appear to be upregulated by shear stress due to orderly laminar blood flow Endothelial nitric oxide synthase (eNOS) and superoxide dismutase are both expressed at higher levels in regions of laminar flow Superoxide dismutase combats oxidative stress and may limit VCAM-1 expression and other endothelial inflammatory pathways (Topper and Gimbrone, 1999). Nitric oxide (NO) has been shown to reduce VCAM-1, ICAM-1 and E-selectin expression on human endothelial cells and reduce

the release of IL-6 and IL-8 (DeCaterina et al , 1995) Genes for ICAM-1 (Nagel et al , 1994), PDGF (Resnick et al , 1993) and tissue factor (Lin et al , 1997) are also responsive to shear stress, resulting in reduced expression It is likely that the sites predisposed to atherosclerosis including the branch points of arteries are those that experience turbulent rather than laminar blood flow (Libby, 2002).

Serum levels of HDL-cholesterol have long been associated with a lower risk of cardiovascular disease One protective pathway involves the reverse transport of cholesterol from cells to the liver The HDL particle is small enough to penetrate the endothelium and apo A-I removes cholesterol from lipid-laden macrophages by a transport process mediated by ATP binding cascade A1 protein (ABCA1) However, the HDL particle may also have anti-inflammatory and anti-oxidant properties. It has been shown to increase NO bioavailability (Spieker et al , 2002), block the expression of adhesion molecules on the endothelium (Barter et al , 2002), inhibit LDL oxidation (Parthasarathy et al , 1990) and transport oxidised lipids from the periphery to the liver (Fluiter et al , 1996).

Many of the pathophysiological pathways previously outlined are further perturbed in obese, insulin resistant or diabetic states. The dyslipidemia central to these conditions typically involves higher levels of triglycerides (TG), TG-rich lipoprotein remnants, a preponderance of small dense LDL particles, and lower levels of HDL-C LDL-C can also be elevated Advanced glycation end products (AGE) seen in diabetes are known to increase modification of LDL and their uptake by macrophages. Free fatty acids and glucose are also elevated in these states As endothelial cells do not become insulin resistant, they have no

means of protecting themselves from free fatty acid and glucose overload (Ceriello and Motz, 2004). Nutrient-driven increases in Krebs's cycle activity are believed to fuel superoxide radical generation along the electron transport chain. The resultant oxidative stress is now considered to be a major source of macrovascular and microvascular damage (Brownlee, 2005). A number of inflammation-related proteins including TNF α , IL-6, IL-1, IL-8, IL-10, SAA and plasminogen activator inhibitor-1 (PAI-1) are secreted by adipose tissue (Trayhurn and Wood, 2004) contributing to chronic low grade inflammation. It is likely that the metabolic changes associated with obesity, the metabolic syndrome and diabetes, together induce changes in the vasculature that result not only in endothelial dysfunction, but also increased propensity to vascular injury and atherogenesis (Ritchie et al, 2004).

1.2 Lipoproteins

As lipids are virtually insoluble in water, their transport in the bloodstream requires their incorporation into amphipathic macromolecules of lipid and protein known as lipoproteins. These particles have a hydrophobic lipid core consisting of triglyceride and cholesterol ester and a surface of amphipathic molecules, namely apolipoproteins (apoproteins) and phospholipids. These surface molecules are arranged with their hydrophilic regions facing outwards and hydrophobic regions facing inwards, interacting with the lipid core. There are four general lipoprotein classes that differ in terms of origin, structure and function (Table 1.1). Collectively chylomicrons and VLDL are termed triglyceride-rich lipoproteins.

The apoprotein component is a vital part of the lipoprotein particle. Its amphipathic nature contributes to lipoprotein structural stability. Apoproteins also function as recognition sites for lipoprotein and lipoprotein remnant receptors and act as co-factors for various enzymes involved in lipoprotein metabolism (Table 1.2). The ability of a lipoprotein to interact with hepatic and extrahepatic receptors and enzymes is determined by specific apoproteins.

Table 1.1 *Composition and characteristics of plasma lipoproteins*

	Chylomicrons	VLDL	LDL	HDL
Major function	Transport of dietary fat	Transport of endogenous fat	Delivery of cholesterol to tissues	Reverse transport of cholesterol from tissues
Svedberg flotation (S_f)	>400	60 - 400	0 - 12	
Density (g/mL)	<0.95	0.95 - 1.006	1.019 - 1.063	1.063 - 1.210
Diameter (nm)	>70	30 - 90	18 - 22	5 - 12
Particle mass (Daltons*10⁶)	0.4 - 30	10 - 100	2 - 3.5	1.75 - 3.6
Apolipoproteins	A-I, B48, C-I, C-II, C-III, E	B100, E	B100	A-I, A-II
<i>Composition (% particle mass)</i>				
Triglyceride	83	50	10	8
Cholesterol (free and esterified)	8	22	48	20
Phospholipid	7	20	22	22
Protein	2	7	20	50

Adapted from Gurr et al. (2002) and Durrington (1989)

Table 1.2 Characteristics of the major apolipoproteins

Apoprotein	Function	Site of synthesis
A-I	Structural protein of HDL Activates LCAT	Liver and intestine
A-II	Activates hepatic lipase, Inhibits LCAT	Liver
B48	Structural protein of chylomicrons	Intestine
B100	Structural protein of VLDL, IDL and LDL Ligand for binding to LDL receptor	Liver
C-I	Activates LCAT	Liver
C-II	Activates LPL	Liver
C-III	Inhibits LPL, Activates LCAT	Liver
E	Ligand for binding of remnants to LDL receptor and LRP	Liver

Abbreviations. Lecithin Cholesterol Acyl Transferase (LCAT), Lipoprotein lipase (LPL), LDL receptor-related protein (LRP)

Adapted from Gurr et al. (2002) and Witztum and Steinberg (2004)

1.2.1 Chylomicrons and chylomicron remnants

Chylomicrons transport lipids of dietary origin in the bloodstream. They are the most lipid rich of the lipoprotein classes with over 80% triglyceride content by weight. The products of fat digestion are re-esterified in the enterocytes of the small intestine, and incorporated into a nascent chylomicron. The surface coat of this nascent chylomicron contains apo B48, apo A-I, apo A-II and apo A-IV (Witztum and Steinberg, 2004). Nascent chylomicrons are released into the lymphatic system and travel through the lacteals, entering the bloodstream via the thoracic duct. Chylomicrons can be identified by apo B48 (one per chylomicron particle) which remains with the particle throughout its metabolism and is not present on any other lipoprotein. Once in the circulation, chylomicrons acquire apo C-I, C-II, C-III and apo E from VLDL and HDL particles. They are not capable of interacting with the enzyme

lipoprotein lipase on the vascular endothelium until apo C-II is acquired. Lipoprotein lipase (LPL) is the key enzyme responsible for the hydrolysis of triglycerides to fatty acids and glycerol for uptake by the local tissue. LPL has a greater affinity for chylomicron than for VLDL particles and consequently, chylomicron particles have a relatively short half-life of less than 5 min (Durstine and Haskell, 1994). The appearance of chylomicrons in the circulation can almost abolish VLDL-TG clearance (Bjorkegen et al, 1996). As the core shrinks, surface materials become too crowded and there is a net transfer of apoproteins, phospholipid and free cholesterol to HDL (Durrington, 1989). HDL acts as a circulating reservoir for C apoproteins. These apoproteins are transferred to nascent chylomicrons from HDL and back again during chylomicron catabolism (Durrington, 1989). A cholesterol-enriched, potentially atherogenic chylomicron remnant remains, after 70-90% of the triglyceride content has been removed.

1.2.2 Very Low Density Lipoproteins and Low Density Lipoproteins

In the fasted state, chylomicrons are absent from the circulation. However, during this time, TG and cholesterol are synthesised endogenously by the liver and secreted in VLDL. All VLDL contain one apo B100 particle which remains with the lipoprotein throughout its metabolism. Triglyceride is synthesised in the liver from free fatty acid and glucose precursors. Free fatty acids (FFA) released from adipose tissue are the major source of substrate for hepatic lipogenesis. FFA not trapped by local tissues following the hydrolysis of circulating postprandial triglycerides by LPL, also contribute to circulating FFA concentrations (Frayn et al., 1994). VLDL-TG secretion depends on circulating FFA precursors but also on

the balance between FFA re-esterification and oxidation in the liver (Ruderman et al., 1999). Diets high in simple sugars (Hudgins et al , 1998) and carbohydrate overfeeding (Schwartz et al , 1995) can increase hepatic de novo lipogenesis and VLDL-TG secretion. Hepatic triglyceride synthesis does not cease in the postprandial period but is suppressed by insulin, at least in insulin sensitive individuals. Hepatic de novo lipogenesis is elevated in the insulin resistant state, even with high fat, low carbohydrate diets (Schwarz et al , 2003). In the insulin resistant state, insulin does not effectively inhibit Microsomal Triglyceride Transfer Protein (MTP) gene expression or effectively stimulate ER60 expression (Adeli et al., 2001). (MTP catalyses the transfer of triglyceride to nascent apo B particles. ER60 is a protease that degrades apo B prior to secretion)

VLDL are catabolised similarly to chylomicrons with the progressive removal of TG by LPL, the transfer of surface components to HDL and the generation of a cholesterol enriched, potentially atherogenic remnant particle. VLDL particles have a lower affinity for LPL and consequently have a longer half-life of 2-4 hours (Durstine and Haskell, 1994). The remnants are known as intermediate density lipoproteins (IDL). Evidence exists that VLDL are not a homogenous species with independent regulation of both the production and catabolism of the large triglyceride-rich VLDL₁ and small cholesterol-rich VLDL₂ subclasses (Packard and Shepherd, 1997). Future studies may need to consider the metabolism of each subclass separately.

IDL are either taken up by hepatic receptors or further catabolised to LDL. This latter pathway requires the further hydrolysis of IDL by hepatic lipase and LPL (Taskinen and Kuusi,

1987) Apo E acts as a ligand for interaction of IDL particles with hepatic receptors and is crucial for both the uptake of IDL and the conversion to LDL (Witztum and Steinberg, 2004) With most of the triglyceride core removed and surface components transferred to HDL, the LDL particle is cholesterol ester enriched, with apo B100 the only apoprotein now present Being small enough to penetrate the vascular endothelium and come in contact with nearly every cell in the body, LDL is responsible for the delivery of cholesterol to cells for membrane growth and repair, and to specialist tissues including the gonads, adrenals and skin, and for hormone and Vitamin D synthesis LDL concentration in extracellular fluid is approximately 10% of that in plasma (Durrington, 1989). Particles have a half-life of approximately 2.5 days

1.2.3 LDL and remnant removal

Chylomicron remnants, some IDL and LDL are removed from circulation principally by the liver, mediated by two key receptors, the LDL receptor (which recognises apo B100 and apo E) and the LDL receptor-related protein (which recognises apo E) Chylomicron remnants are rapidly taken up as a result of binding between apo E molecules, still present on the lipoprotein particle, and their affinity for both hepatic receptors Apo E is also crucial for the removal of IDL from the circulation Individuals with apo E isoforms that bind less efficiently to receptors can accumulate remnants in plasma (Witztum and Steinberg, 2004) The remaining IDL are further delipidised and converted to LDL, with apo B100 the sole surface component Approximately 75% of the uptake of LDL occurs via the LDL receptor pathway in the liver and in other cells with a cholesterol requirement Approximately two-thirds of LDL is cleared by the liver (Witztum and Steinberg, 2004) The expression of the

LDL receptor is downregulated in all cells by the accumulation of intracellular cholesterol. However, LDL can also enter cells by a non-receptor-mediated pathway that involves binding to the cell membrane and subsequent internalisation. This non-receptor-mediated but gradient-related pathway may be particularly important in adult humans who have higher circulating levels of LDL-C than animal species (Durrington, 1989).

1.2.4 High Density Lipoprotein

The HDL particle differs from chylomicrons, VLDL, IDL and LDL in a number of important respects. HDL is the smallest and most dense of the lipoprotein species. The nascent particle, secreted by the liver, increases in size as it travels in the circulation. Furthermore, it is associated with the removal of excess cholesterol from peripheral tissues and its transport back to the liver. The precursors of mature HDL particles are disc-shaped bilayers of phospholipid embedded with apo A-I and A-II. These are fully formed in the circulation by the transfer of surface components from chylomicrons and VLDL during their catabolism. Two distinct subspecies of HDL exist, HDL₃ and HDL₂. The enzyme lecithin cholesterol acyl transferase (LCAT) activated by apo A-I, catalyses the esterification of free cholesterol on the HDL surface. This enables it to move into the hydrophobic core, maintaining a gradient for the uptake of more cholesterol. The increasing lipid content changes the small HDL₃ particle to the larger less dense HDL₂. Despite a five-fold difference in serum HDL-cholesterol and LDL-cholesterol concentrations, particle concentrations are often similar. The SR-B1 receptor has been implicated in the hepatic binding of HDL, facilitating the transfer of cholesterol ester out of HDL₂ in the liver. The particle is converted back to

HDL₃, and released back into circulation (Witztum and Steinberg, 2004). The liver also removes complete HDL particles. HDL has a relatively long half-life of 3-5 days

1.2.5 Lipid exchange between lipoprotein species

In addition to the transfer of the surface components between lipoprotein species, there is also a transfer of cholesterol ester and triglycerides between the lipid rich lipoprotein cores. This exchange is mediated by cholesterol ester transfer protein (CETP). In particular, the exchange between the triglyceride-rich lipoproteins (chylomicrons and VLDL) and LDL, increases the triglyceride content of the latter, the cholesterol ester content of the former and the atherogenicity of both. The newly acquired triglyceride is quickly removed from LDL by the action of hepatic lipase and LPL, resulting in a small dense, highly atherogenic LDL particle. Similarly, there is an exchange of triglyceride and cholesterol ester between triglyceride-rich lipoproteins and HDL, resulting in cholesterol ester depleted HDL particle. These processes are exacerbated in a hyperlipidemic environment and during the postprandial period. Griffin et al (1994) has suggested that there is an abundance of small dense LDL particles when fasting triglycerides are above 1.5 mmol L⁻¹.

1.3 Triglycerides and risk of coronary artery disease

Elevated triglycerides constitute a risk factor for cardiovascular disease that was first described by Albrink and Man (1959). Since that time, numerous prospective and cross-sectional studies have confirmed a univariate association between TG and risk of CAD. Although this association was usually independent of total cholesterol and LDL-C, it was often

eliminated in multivariate analyses after adjustment for HDL-C (Austin, 1991). As a result, a US National Institutes of Health Consensus Panel (NIH, 1993) has questioned whether serum TG is a risk factor for CAD independent of HDL-C. Pharmacological treatment was only recommended when lifestyle modifications failed to reduce TG below 5.65 mmol L^{-1} .

In a meta-analysis of 17 population-based prospective studies, based on nearly 47,000 men and 11,000 women, Hokanson and Austin (1996) found the relative risk of cardiovascular disease to be 1.31 in men and 1.76 in women for each 1 mmol L^{-1} increase in TG. After adjustment for a variety of other risk factors including HDL-C, the relative risks had decreased to 1.15 for men and 1.37 for women, but were still significant. This finding suggests that the association of TG with CAD is only partially explained by HDL-C.

That accurate assessment of CAD risk, requires attention to TG in addition to or in conjunction with LDL-C and HDL-C, is demonstrated by the findings from the Prospective Cardiovascular Munster (PROCAM) study (Assmann, 2001) and the Copenhagen Male Study (Jeppesen et al., 1998). Analysis of PROCAM 10 year follow-up data in the cohort of middle-aged men and women, indicates that rates of myocardial infarction (fatal and non-fatal) rose with increasing LDL-C at baseline, and within each LDL-C quintile with increasing triglycerides. Data from the Copenhagen study are presented by TG and HDL-C tertiles and reveal a similar pattern. The incidence of CAD increased with increasing TG and decreasing HDL-C, but within each HDL-C tertile, the incidence of CAD and all cause mortality increased with increasing TG. Although there appears no relation between serum triglycerides and LDL-C, studies have shown that TG and LDL particle size are closely correlated ($\rho = -0.71$,

Stampfer et al , 1996) High triglycerides are associated with small dense LDL particles (sometimes termed LDL phenotype B) that are particularly atherogenic Recent prospective epidemiologic studies found that both TG and LDL particle size predicted future CAD (Stampfer et al , 1996, Gardner et al., 1996, Lamarche et al., 1997)

The level of fasting TG that should be regarded as desirable has been a matter of debate and revision The US National Cholesterol Education Programme Adult Treatment Panel III thresholds for normal, borderline high, high and very high TG concentrations have been revised downwards with 1.67 mmol L^{-1} (150 mg dL^{-1}) defined as normal (NCEP, 2002) Whether further refinement is needed is debatable. Results from the Baltimore Coronary Long-Term Study (COLTS) indicate increased risk of CAD events and reduced survival from CAD events with TG levels greater than 1.13 mmol L^{-1} (Miller et al , 1998) The Copenhagen Male Study (Jeppesen et al., 1998) also found an increased incidence of CAD and all cause mortality across TG tertiles with median tertile values of 0.88, 1.33 and 2.45 mmol L^{-1} Even at a value of 1.67 mmol L^{-1} approximately 60% of LDL particles will be small and dense (Austin et al , 1990)

In his interpretation of the epidemiological evidence linking TG to cardiovascular disease risk, McCarty (2004) adds a caveat. He argues that TG are only pathogenic insofar as they act as a marker for insulin resistance, a syndrome in which central obesity and elevated hepatic TG secretion are key components He points out that the frequently cited epidemiological meta-analysis of Hokanson and Austin (1996) does not include a valid surrogate for insulin sensitivity, and thus cannot demonstrate that TG increase risk

independent of insulin sensitivity. In contrast, he argues that carbohydrate-induced hypertriglyceridemia, that occurs mainly from reduced TG clearance, may not be pathogenic. A lifestyle intervention is cited (Ornish et al, 1998) in which a 10% fat (thus high carbohydrate) diet reduced risk of cardiac events in patients with moderate to severe atherosclerosis, despite increases in TG. Parks and Hellerstein (2000) also caution against drawing erroneous conclusion in relation to carbohydrate-induced hypertriglyceridemia. They argue that two types of hypertriglyceridemia exist with different mechanisms and thus different health risks.

1.4 Endothelial function and dysfunction

The endothelium has traditionally been considered as a layer of cells whose primary function is to facilitate smooth blood flow, and separate circulating blood from underlying tissue. Landmark studies in the 1980s demonstrated the obligatory role of endothelial cells in acetylcholine-mediated vasodilation (Furchgott and Zawadzki, 1980) and in the paradoxical acetylcholine-mediated vasoconstriction of atherosclerotic vessels (Ludmer et al, 1986). The endothelium is now recognised as having a crucial role in maintaining vascular integrity, releasing of a wide variety of substances that regulate leukocyte adhesion, migration and activation, thrombus formation and vessel vasomotion. A delicate balance between antagonistic factors is maintained by the healthy endothelium. A disturbance in this balance, termed endothelial dysfunction, is generally considered as an early event in atherosclerosis (de Koning and Rabelink, 2002).

1.4.1 Vasomotion

Although the endothelium regulates a number of physiological functions, the majority of studies have focused on endothelial dependent changes in vasomotion. Nitric oxide (NO) is one of the key factors released by the endothelium in both conduit and resistance vessels including coronary vessels and is a potent vasodilator. Activation of the endothelium by mechanical and chemical stimuli including shear stress (from laminar blood flow), acetylcholine, bradykinin, serotonin and catecholamines result in NO-mediated vasodilation, secondary to increases in endothelial nitric oxide synthase (eNOS) activity. Acetylcholine-induced vasodilation can be blocked with NOS inhibition by N-monomethyl-L-arginine (L-NMMA) (Quyyumi et al, 1995). The endothelium releases other vasodilating factors including prostacyclin and potent vasoconstricting factors such as endothelin-1, thromboxane A2 and angiotensin-II.

1.4.2 Inflammation and Coagulation

The anti-inflammatory properties of the healthy endothelium are important in the prevention of atherosclerosis. Endothelial cell activation encompasses the active response of cells to pro-inflammatory stimuli including pro-inflammatory cytokines and oxidative stress. Activation of the transcription factor nuclear factor kappa B (NF- κ B) by oxidative stress results in the co-ordinated upregulation of a number of genes encoding pro-inflammatory factors (Barnes and Karin, 1997). When activated, these factors are expressed or released including VCAM-1, ICAM-1, E-selectin, IL-6, IL-8, MCP-1 and M-CSF (Jimenez et al., 2005). Together, these chemokines, adhesion molecules, cytokines and growth factors are responsible for the

attraction, adhesion, migration, activation, accumulation and survival of monocytes and T lymphocytes in the subendothelial space. Activated platelets adhering to the endothelium can promote vasoconstriction (Golino et al., 1991), induce MCP-1 secretion and ICAM-1 surface expression (Gawaz et al., 1998). In addition to mediating endothelial dependent vasodilation, NO appears to have anti-inflammatory properties and has been shown to inhibit leukocyte adhesion (Kubes et al., 1991). NO also inhibits platelet aggregation at the vessel wall (Radomski et al., 1987). When NO bioavailability is reduced, endothelial dependent vasodilation is likely to be impaired and endothelial cell activation increased.

The endothelium also releases a number of factors that promote coagulation (thromboxane A₂, plasminogen activator inhibitor-1 (PAI-1) and von Willebrand factor (vWF)) and inhibit coagulation (prostacyclin). The endothelium also releases bioactive molecules that promote fibrinolysis including tissue plasminogen activator (tPA).

1.4.3 Assessment of endothelial function

Conduit vessel endothelial function is usually determined by assessing flow-mediated vasodilation (FMD) of the brachial artery. FMD measures the increases in brachial artery diameter in response to increased blood flow following 5 min of arterial occlusion. Resistance vessel endothelial function is usually determined by quantifying increases in forearm blood flow (FBF) using venous occlusion plethysmography (VOP), following infusion of endothelial dependent vasodilators (EDD) such as acetylcholine (ACh) or metacholine (Mch). Endothelial independent vasodilation (EID) is measured by administering glyceryl trinitrate sublingually in FMD studies or infusing sodium nitroprusside (SNP) in FBF studies. Although a direct

assessment of coronary conduit and resistance vessel endothelial function is also possible by infusing acetylcholine and measuring changes in conduit vessel diameter by angiography (Ludmer et al , 1986) or coronary blood flow using intravascular ultrasound (Doucette et al , 1992), endothelial function is normally assessed in the peripheral circulation. A close relation has been demonstrated between endothelial function in the human coronary and brachial arteries in CAD patients and healthy controls (Anderson et al , 1995; Takase et al , 2005)

FMD is non-invasive but expensive requiring a high-resolution ultrasound scanner, high frequency vascular transducer and internal ECG monitor. Some (Suwaidi et al , 2000) but not all (Palmer et al., 1987, Schachinger et al , 2000) laboratories have reported good accuracy and reproducibility using FMD. Ultrasonographic assessment of the brachial artery is uniquely challenging and must be performed by well-trained, skilled technicians (Alam et al., 2005). The quantification of vasodilator-mediated increases in FBF can be regarded as moderately invasive as it requires chemical agents, to be introduced directly into the brachial artery using a small (27G) cannula (Alam et al , 2005). However, authors report good reproducibility (Petrie et al, 1998, Roberts et al, 1986) and it should be regarded as the gold standard for resistance vessel endothelial function assessment (Alam et al , 2005).

A number of novel techniques for assessing endothelial function are appearing in the literature. With one such method, dilation of the cutaneous microcirculation is triggered by iontophoresis of Ach and SNP (ED and EID agonists). Iontophoresis involves the transdermal delivery of ionised drugs under the influence of an electrical field. Changes in skin perfusion are quantified using laser Doppler imaging. Whereas iontophoresis of ED and EID agonists

increases skin perfusion, iontophoresis of saline has been shown to be ineffective (Ramsay et al , 2002). This technique has been described as a well-tolerated and non-invasive method for assessing resistance vessel function (Gill et al , 2003a)

1.4.4 Adhesion molecules

Three groups of adhesion molecules have been identified, integrins, selectins (including P-selectin and E-selectin) and immunoglobulins (including VCAM-1 and ICAM-1). Accumulating data suggest that selectins are involved in the initial rolling and tethering of leukocytes on the endothelium whereas VCAM-1 and ICAM-1 are involved in their transendothelial migration (Schram and Stehouwer, 2005). Levels of soluble E-selectin, ICAM-1 and VCAM-1 in plasma are directly associated with their membrane-bound expression (Leeuwenberg et al , 1992, Schmidt et al, 1995). True soluble adhesion molecules are released from the extracellular matrix by proteolytic enzymes including metalloproteases and sheddases (Horstman et al., 2004a). However much of that detected using standard ELISA techniques is in fact membrane bound, released on endothelial microparticles as a result of membrane budding (Horstman et al , 2004a). The release of these molecules from the endothelium may serve to limit the inflammatory process. Soluble molecules are not simply the products of degradation but can inhibit leukocyte recruitment into the intima by binding with their ligands in the circulation (Blankenberg et al , 2003).

1.4.5 Adhesion molecule expression

Adhesion molecules have been shown to be upregulated by pro-inflammatory cytokines and in particular TNF- α , advanced glycation end products (AGE) and oxidised lipoproteins. Following incubation of endothelial cells with inflammatory cytokines, sICAM-1 and sE-selectin were expressed on endothelial cell membranes and were also found in supernatant (Blankenberg et al, 2003). Cell surface expression was directly correlated with supernatant concentrations. Maximal levels of sE-selectin and sICAM-1 were detected 6-12 h and 24 h respectively after activation by cytokines. Despite being linked to endothelial activation and inflammation, the relations between the various soluble makers are relatively weak. Correlations between sVCAM-1 and sICAM-1 ($r=0.17$, $p=0.004$), sE-selectin and sICAM-1 ($r=0.22$, $p=0.0001$) and sVCAM-1 and sE-selectin ($r=0.006$, $p=0.92$) were found in a subset of 316 healthy control subjects from the Atherosclerosis Risk In Communities (ARIC) study (Hwang et al., 1997).

1.4.6 Adhesion molecules in disease states

Soluble forms of adhesion molecules have been shown to be elevated in a range of disease states including CAD (Hwang et al, 1997, Ridker et al, 1998) hypertension (Cottone et al., 2002), and type II diabetes (Stenier et al, 1994). In addition, prospective cohort data suggests that elevated levels of adhesion molecules can predict cardiovascular mortality and all cause mortality in type II diabetics (Jager et al, 2000; Becker et al, 2002). From their review of large prospective studies, Blankenberg et al (2003) found sICAM-1, but not sVCAM-

1, to be a good predictive marker of cardiovascular risk in healthy individuals, correlating with levels of CRP. In contrast, sVCAM-1 emerges as a strong predictor of future events in individuals with pre-existing endothelial dysfunction or vascular disease. Studies in peripheral artery disease patients also suggest that sICAM-1 is a better predictor of disease presence, with sVCAM-1 predicting disease severity (Peter et al , 1997, Pradhan et al , 2002)

Cross-sectional studies generally show elevated levels of at least one soluble adhesion molecule in obesity (Ferri et al , 1999, Rohde et al., 1999, Weyer et al , 2002), hypertriglyceridemia (Hackman et al , 1996, Abe et al , 1998), hypercholesterolemia (Hackman et al , 1996, Kvasnicka et al , 2001) and insulin resistance (Chen et al., 1999, Weyer et al , 2002). However, some reports are conflicting. Weight loss results in a decrease in sICAM-1, sVCAM-1 and sE-selectin (Ferri et al , 1999, Ito et al , 2002, Pontiroli et al , 2004). This effect may be mediated by a decrease in TNF- α and IL-6 release from adipose tissue.

1.4.7 Adhesion molecules and endothelial dysfunction

Endothelial function is routinely assessed using soluble cell adhesion molecules. These biomarkers are easy to determine in large populations, are independent of observer variability and less time-consuming. However, the validity of this approach is based on a number of assumptions. First, cell types other than endothelial cells must not be an important source of the markers used. Second, synthesis of these adhesion molecules must be more important than clearance in determining plasma concentrations. Third, endothelial function in the microcirculation must parallel that in the large arteries as the microcirculation has a very

large surface area. Information on the validity of these assumptions is scarce (Schram and Stehouwer, 2005)

A number of studies (Table 1.3) have documented the relationship between soluble markers of arterial inflammation and a direct measurement of endothelial function, or between changes in these soluble markers (usually in response to pharmacological treatment) and changes in directly measured endothelial function. Case-control studies generally show a moderate relationship between conduit vessel endothelial function and soluble adhesion molecules (Lupattelli et al, 2000, Brevetti et al, 2001, Nawawi et al, 2003, Glowinska-Olszewska et al, 2005) though not always (Van Haelst et al, 2003). In addition, FMD and soluble adhesion molecules have been shown to change in parallel (inverse relation) during pharmacological treatment (Alonso et al, 2001, Marchesi et al., 2003, Nawawi et al, 2003, Ceriello et al, 2005, dietary supplementation (Lupattelli et al, 2004) and postprandial lipemia (Marchesi et al, 2003), further evidence of a common physiology.

The relation between resistance vessel endothelial function and soluble adhesion molecules is weaker and sometimes not evident, due in part to the variety of methodologies employed in these studies. In a study involving type II diabetics, Koga et al (2005) demonstrated a weak relation between sICAM-1 and acetylcholine-mediated increases in coronary vessel diameter ($r = -0.41$) and blood flow ($r = -0.46$). Interestingly, the relation between coronary endothelial function and endothelial microparticles was considerably stronger. Holmlund et al (2002) reported a weak relation between resistance vessel endothelial function and sICAM-1 but not with sVCAM-1 or sE-selectin. John et al (2000)

Table 1.3 Summary of studies that assess endothelial dependent dilation directly and using serum biomarkers

Study	Subjects	Main findings
John et al. 2000	52 HC patients and 43 healthy controls	FBF response to Ach infusion not related to adhesion molecules, FBF-Ach vs sICAM-1, $r = -0.02$; FBF-Ach vs sVCAM-1, $r = 0.16$, FBF-Ach vs sE-selectin, $r = -0.10$
Lupattelli et al. 2000	16 HTG patients and 16 controls	FMD vs sVCAM-1, $r = -0.61$, FMD vs sICAM-1, $r = -0.38$
Alonso et al. 2001	25 FH patients	Parallel increases in FMD and decreases in sE-selectin with pharmacological treatment
Brevetti et al. 2001	34 IC patients with and 14 controls	FMD lower in those with higher levels of both sICAM-1 and sVCAM-1
Holmlund et al. 2002	59 healthy adults	FBF-Mch/FBF-SNP vs sICAM-1 $r = -0.31$, No relationship with IMTc, sVCAM-1, hsCRP and sE selectin.
Gill et al. 2003a	8 endurance athletes	Skin perfusion in response to iontophoresis of Ach (microvascular EDD) decreased PP, without parallel changes in adhesion molecules
Marchesi et al. 2003	10 HTG patients	PP changes in FMD vs TG ($r = -0.34$), PP changes in FMD vs sICAM-1, $r = -0.66$ Increase in FMD in parallel with decreases in TG, sICAM-1 and sVCAM-1 following 3months pharmacological treatment
Nawawi et al. 2003	27 FH and 47 NFH patients	FH group FMD vs sICAM-1 $r = -0.58$ NFH group. FMD vs sICAM-1, $r = -0.47$, FMD vs IL-6, $r = -0.25$. Parallel changes in FMD and sICAM-1 during pharmacological treatment
Van Haelst et al. 2003	35 FH patients	No relationship between FMD and adhesion molecules, FMD vs CRP, $\rho = -0.14$, vs sICAM-1 $\rho = -0.21$, vs sE-selectin, $r = 0.15$.
Brown et al. 2004	23 FH patients	Changes in FMD with pharmacological treatment without parallel changes in sICAM-1, sVCAM-1, sE-selectin, hsCRP or IL-6.

Lupattelli et al 2004	28 HTG patients	Parallel increase FMD and decrease both sICAM-1 and sVCAM-1 following dietary supplementation with artichoke juice Δ FMD vs Δ sICAM-1, $r = -0.62$, Δ FMD vs Δ sVCAM-1, $r = -0.66$
Ceriello et al (2005)	20 T2D patients	Parallel increase in FMD and decreases in sICAM-1, NT, IL-6 and CRP following 4d pharmacological treatment in the absence of changes in any lipid parameters
Glowinska-Olszewska et al (2005)	76 T1D adolescents and 33 controls	FMD vs sE-selectin, $r = -0.33$ IMTc vs sE-selectin, $r = 0.32$

Abbreviations Hypercholesterolemia (HC), forearm blood flow (FBF), acetylcholine (Ach), hypertriglyceridemia (HTG), flow-mediated vasodilation (FMD), familial hypercholesterolemia (FH), non-familial hypercholesterolemia (NFH), high sensitivity C-reactive protein (hsCRP), metacholine (Mch), sodium nitroprusside (SNP), nitrotyrosine (NT), carotid artery intima media thickness (IMTc), postprandial (PP), type I diabetic (T1D), type II diabetic (T2D), soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), interleukin-6 (IL-6)

found no evidence of a relation between resistance vessel function and any adhesion molecule.

1.5 Interleukin-6

Interleukin-6 is generally described as a pleiotropic cytokine involved in a wide range of biological activities including inflammation, sepsis, oncogenesis, hematopoiesis, bone metabolism and fat metabolism (Song and Kellum, 2005). IL-6 has been shown to be elevated in aging (Dobbs et al, 1999), obesity and type II diabetes (Vgontzas et al, 2000). Levels of IL-6 are related to all cause mortality, cardiovascular mortality (Harris et al, 1997) and myocardial infarction (Ridker et al, 2000).

1.5.1 Interleukin-6 and inflammation

The role of IL-6 in inflammatory processes is controversial and it has been described as both a pro- and anti-inflammatory molecule. It acts alongside other cytokines and inflammatory markers including TNF- α and CRP. IL-6, TNF- α and CRP intercorrelate in healthy older adults (Ballou et al, 1996). IL-6 is best characterised as a pro-inflammatory cytokine. It is the key trigger for the production of CRP, the acute phase reactant that is a potent marker and mediator of endothelial inflammation (Szmitko et al, 2003). Recent clinical trials of tocilizumab, that functions to block the IL-6 receptor, have shown significant improvement in rheumatoid arthritis, an inflammatory condition, along with reductions in CRP (Maini et al., 2006).

Evidence is also available that IL-6 has anti-inflammatory properties. Although its production can be triggered by TNF- α , it appears to have a role in regulating levels of TNF- α in the circulation. Levels of TNF- α are elevated in IL-6 knockout mice and IL-6 inhibits lipopolysaccharide induced TNF- α production in human monocytes in vitro (Mizuhara et al, 1994, Schindler et al., 1990). The infusion of recombinant IL-6 has also been shown to inhibit endotoxin-induced increases in TNF- α in healthy humans (Starkie et al, 2003). In addition, IL-6 stimulates the release of anti-inflammatory cytokines including IL-10, IL-1 receptor antagonist (IL-1ra) and soluble TNF- α receptors but not TNF- α (Tilg et al, 1997).

Both TNF- α and IL-6 are produced in adipose tissue. The high levels associated with the metabolic syndrome are associated with visceral adipose tissue. Given that TNF- α can trigger IL-6 release in adipose tissue, it has been suggested that TNF- α is actually the main driver of the metabolic syndrome with locally produced TNF- α causing systemic increases in IL-6 (Petersen and Pedersen, 2005). Consequently, chronically elevated levels of IL-6, IL-1ra and CRP are likely to reflect ongoing local TNF- α production, even though an elevation in systemic TNF- α is not always detectable.

1.5.2 Exercise and substrate metabolism

Unlike during sepsis where TNF- α and IL-1 are the first cytokines to be produced in the cytokine cascade, IL-6 is the first cytokine to appear in the circulation during exercise (Petersen and Pedersen, 2005). Levels of IL-6 in the circulation can increase up to 100 fold during exercise depending on exercise intensity, duration and muscle mass recruited (Petersen and Pedersen, 2005). These increases can occur in the absence of muscle

damage. Low pre-exercise muscle glycogen has been shown to further enhance IL-6 gene transcription (Steensberg et al , 2001), indicating an energy sensing role for muscle-derived IL-6 (Petersen and Pedersen, 2005) Such an effect might be mediated via an effect on AMP-activated protein kinase (AMPK), another putative sensor of cellular energy status Incubation of skeletal muscles with IL-6 increases the phosphorylation of AMPK (an indicator of its activation) and that of its target molecule acetyl CoA carboxylase (ACC) The authors suggest that the activation of AMPK may be dependent on the presence of IL-6 (Kelly et al , 2004). The phosphorylation of ACC by AMPK ultimately serves to inhibit malonyl CoA synthesis, facilitating greater entry of fatty acids into the mitochondria for oxidation (Ruderman et al , 2003) Direct evidence exists of a role for IL-6 in regulating substrate metabolism. The infusion of recombinant IL-6 infusions has been shown to increase lipolysis and fat oxidation in humans without causing hypertriglyceridemia (VanHall et al , 2003) Although TNF- α also increases lipolysis, only IL-6 has been reported to also increase fat oxidation (Petersen and Pedersen, 2005)

Although the precise biological action of muscle-derived IL-6 has yet to be fully elucidated, it appears to act during exercise in a hormone like manner to increase adipose tissue lipolysis, hepatic glucose production and muscle fat oxidation Consequently it has been suggested that muscle could be regarded as an endocrine organ and the term myokine applied to IL-6 and other cytokines released by skeletal muscle (Pedersen et al , 2003)

1.6 Microparticles

The existence of subcellular vesicles in the *bloodstream* has been known since the 1960s. However, only recently has the role of these microparticles in a range of physiological processes, and their elevation in a range of disease states, been appreciated. Microparticles were first described by Wolf (1967) using electron microscopic techniques when studying a subcellular factor originating from platelets. They are small membranous vesicles or blebs (<1.5µm in diameter) that are shed from a variety of cells. The majority of microparticles in the circulation originate from platelets, with smaller quantities from endothelial cells, smooth muscle cells, leukocytes and erythrocytes.

1.6.1 Microparticle release and composition

Microparticles are shed in response to two cellular processes, cell activation and apoptosis. A variety of agonists can result in cell activation and microparticle formation. Human umbilical vein endothelial cells (HUVEC) release microparticles *in vitro* when stimulated by cytokines including TNF-α and IL-1β (Mutin et al., 1997) and by PAI-1 (Brodsky et al., 2002). An early sign of cell activation, permissive for microparticle release, is an increase in cytosolic calcium concentration (Van Wijk et al., 2003). Increases in intracellular calcium activates the proteolytic enzyme calpain and the degradation of the membrane skeleton (Miyoshi et al., 1996). Apoptosis is characterised by cell contraction, DNA fragmentation and membrane blebbing. Apoptotic microparticles may therefore contain fragments of DNA (Van Wijk et al., 2003). The membrane blebbing is related to contraction of actin-myosin structures in the membrane skeleton, following activation of the effector

molecule rho-associated kinase (ROCK 1) by caspase-3 in the apoptotic cascade (Coleman et al , 2001)

Microparticles consist of a phospholipid bilayer embedded with proteins, consistent with their origin as cell membrane blebs. These proteins serve as biomarkers and can indicate the parent cell of origin. Present on microparticles are constitutive proteins typically found in the parent cell membrane under resting conditions, and inducible proteins that are translocated to the membrane during cell activation. The phospholipid bilayer of cell membranes is arranged asymmetrically with negatively charged phospholipids such as phosphatidylserine (PS) located on the inner surface. A prominent manifestation of cell apoptosis is the reversal of this asymmetry. Negatively charged phospholipids can therefore be found on microparticles shed in response to apoptosis, permitting microparticle binding with annexin V, a phospholipid-binding protein that can be labelled with fluorescent dyes. It has been argued that the need for annexin V positivity favoured by some research groups, when enumerating or indeed defining microparticles is unwarranted, as this approach excludes a large proportion of microparticles formed as a result of cell activation (Horstman et al , 2004a). Evidence exists of heterogeneity even among microparticles shed from the same parent cell. Endothelial cells have been shown in vitro to release microparticles that are phenotypically different in response to activation and apoptosis (Jimenez et al , 2003). Endothelial cell activation resulted in increases in microparticles expressing inducible markers, including CD62E, CD54 and CD106. Endothelial cell apoptosis resulted in increases in microparticles expressing the constitutive marker CD31 and in annexin V binding. The

response to cell activation and apoptosis was also different in renal microvascular, brain microvascular and coronary artery macrovascular endothelial cell lines. Thus the lipid and protein composition of microparticles can indicate the parent cell of origin and the cellular processes and/or agonists triggering their formation (Ahn, 2005).

1.6.2 Quantification

The majority of studies to date have quantified microparticles using flow cytometry. With this method, sizing beads (either 1.0 or 1.5 μm) are used to set the upper microparticle diameter limit and to gate out larger non-microparticle material from the analysis. Microparticles from different parent cells are then differentiated using monoclonal antibodies labelled with fluorescent dyes against cell-specific biomarkers. As a biomarker may be co-expressed on microparticles shed from different parent cells, two colour flow cytometry can be employed to enumerate microparticles carrying two specific biomarkers (+/+) or one biomarker but not another (+/-). Annexin V, a phospholipid-binding protein also labelled with fluorescent dyes, has been used to enumerate total microparticle number as it binds to all negatively charged microparticle membranes. Possible shortcomings of this approach have already been highlighted.

As collection and assay protocols vary considerably between laboratories, direct comparisons are difficult (Horstman et al., 2004a). Differences exist in terms of how blood is collected, centrifuged and processed. Some laboratories only assay fresh plasma while others freeze samples. Some groups isolate microparticles by pelleting and washing while others assay microparticles in platelet poor plasma. Assay protocols also vary, with no

agreement on a definition of microparticle size, the need for annexin V positivity or the combination of antibodies to be employed to differentiate microparticles of endothelial, platelet and leukocyte origin. In a number studies, endothelial microparticles (EMP) have been defined as those with CD31 but not CD42b present on their surface (CD31+/CD42b-). Platelet endothelial cell adhesion molecule (CD31) is expressed on both platelets and endothelial cells with CD42b specific to platelets only. VE-Cadherin (CD144), CD146, CD62E, CD51, ICAM-1 (CD54) and VCAM-1 (CD106) have also been employed as antigens to distinguish EMP from other microparticle species

1.6.3 Biological Functions

Although the physiological and pathophysiological roles of microparticles are not fully understood, it is likely that they are involved in the transfer of biological information between cells (Martinez et al., 2005). Most of the exchange of information takes place with endothelial cells (Martinez et al , 2005). There is evidence that microparticles play a role in inflammation, coagulation and endothelial dysfunction, key features of atherosclerosis (Van Wijk et al , 2003). These functions are linked to molecules on the microparticle surface and within their cytoplasm (Martinez et al , 2005).

1.6.3.1 Inflammation

Microparticles shed from activated endothelial cells can amplify inflammatory processes. Microparticles shed from TNF- α activated endothelial cells have been shown to activate cultured monocytes in vitro, with only weak activation from apoptotic EMP (Jy et al ,

2004) The interaction of CD54 (ICAM-1) on EMP with its monocyte receptor may be crucial for monocyte activation, as activation was largely inhibited by anti-CD54. As EMP-monocyte complexes were more easily able to cross an endothelial monolayer, the authors concluded that at least one function of EMP is to increase leukocyte activation and transendothelial migration.

Leukocytes microparticles are upregulated by inflammatory stimulation and behave as inflammatory mediators for their parent cells (Mesri and Altieri, 1999). Microparticles shed from monocytes are a major secretory pathway for the pro-inflammatory cytokine IL-1 β (MacKenzie et al., 2001). Few inflammatory effects have been described for platelet microparticles. However, prior incubation of endothelial cells with platelet microparticles (PMP) has been shown to result in the upregulation of ICAM-1 and increased monocyte adhesion (Barry et al., 1998). Although the precise pathways are unclear, the inflammatory effects of microparticles on endothelial cells and leukocytes may be mediated by oxidised phospholipids present on the microparticle membrane or via the delivery of arachidonic acid to these cells (VanWijk et al., 2003). Oxidised phospholipids exert their actions via platelet activating factor receptors exposed on endothelial cells and leukocytes (Patel et al., 1992) while arachidonic acid sits at the top of the arachidonic acid inflammatory cascade and can increase cytokine transcription.

1.6.3.2 Coagulation

A major role of microparticles, particularly PMP is pro-coagulant activity. The potential of PMP to initiate coagulation was recognised at the outset by Wolf (1967). PMP

generated in vitro and harvested in vivo have been shown to initiate and support thrombin generation in vitro (Berckmans et al , 2001). Tissue factor (CD142) is a key activating protein in the coagulation cascade and is present on platelet and other microparticles. Membranes exposing negatively charged phospholipids especially PS also play an important role in coagulation, facilitating factor VIIa - tissue factor binding and the formation of prothrombinase complexes. Thus, PMP both actively initiate and passively support coagulation (Horstman et al., 2004b). Microparticles expressing tissue factor are present in atherosclerotic plaques and are a primary source of procoagulant activity within a lesion (Mallat et al , 1999). There is evidence that these microparticles are shed from plaque leukocytes (Mallat et al , 1999) and from apoptotic smooth muscle cells (Brisset et al , 2003).

1.6.3.3 Endothelial dysfunction

Microparticles from acute myocardial infarction patients (Boulanger et al , 2001) and women with pre-eclampsia (van Wijk et al , 2002) have been shown to impair endothelial dependent relaxation in isolated arteries. Although the mechanisms and pathways by which EMP impair endothelial function are unclear, there is some evidence that oxidative stress may be a culprit. Oxidised phospholipids are present on microparticles released from endothelial cells subject to oxidative stress (Huber et al , 2002) and oxidised phosphatidylcholine has been shown to induce endothelial dysfunction in isolated arteries (Rikitake et al , 2000). Brodsky et al (2004) also demonstrated an impairment of endothelial dependent relaxation in rat aortic rings that were incubated with EMP generated in vitro. This was accompanied by increased superoxide production in the aortic ring endothelium and a decrease in NO

production. The authors attributed the effect on endothelial function to decreased NO production or bioavailability.

Oxidative stress and apoptosis are well-recognised features in many cardiovascular diseases including atherosclerosis. The occurrence of oxidised phospholipids in apoptotic microparticles or microparticles formed in the presence of oxidative stress may be an important mechanism in the pathogenesis of the disease (VanWijk et al, 2003). These studies indicate that EMP may not only be a marker but also a mediator of endothelial dysfunction.

1.6.4 Disease states

Circulating numbers of microparticles have been shown to be elevated in a variety of vascular and other disease states including atherosclerosis, acute myocardial infarction (MI), stable and unstable angina, vasculitis, hypertension, metabolic syndrome, pre-eclampsia, lupus, multiple sclerosis, thrombotic thrombocytopenic purpura and sepsis (Jiménez et al, 2005, VanWijk et al, 2003). The evidence (above) suggests however that microparticles play a causal role in inflammation, coagulation and endothelial dysfunction and are not simply a consequence of disease (VanWijk et al, 2003). Microparticle combinations may also be able to distinguish disease stage and severity. In a study of coronary patients, CD51-EMP were elevated in all patients but only CD31-EMP could distinguish first MI patients from unstable angina patients (Bernal-Mizrachi et al, 2003). The authors concluded that CD31-EMP are mainly a marker of acute events whereas CD51 are a better marker of chronic endothelial stress. As a general rule, EMP carrying inducible rather than constitutive endothelial

membrane proteins are typically elevated in the acute phase of disease (Jimenez et al , 2005), with CD31 (a constitutive protein) the notable exception

1.7 Postprandial period and atherosclerosis

The concept of atherosclerosis as a postprandial phenomenon was first proposed by Zilversmit (1979). In his landmark paper, he proposed that cholesterol-enriched chylomicron remnant particles were atherogenic and that the metabolism of chylomicrons at the arterial wall, facilitated the movement of remnants into the arterial intima. In an extension of this hypothesis, Miesenbock and Patsch (1992) postulated that postprandial triglyceride (TG) transport was critical for the development of atherosclerosis and more revealing of risk than fasting values. Since then, research interest in postprandial metabolism has developed in parallel with advances in our understanding of the pathophysiology of atherosclerosis. Whereas earlier hypotheses focused on the role of lipids and lipoproteins, current research emphasises postprandial oxidative stress, endothelial inflammation and impaired endothelial function.

1.7.1 Lipids and Lipoproteins

Evidence exists from animal and human studies that the remnants of triglyceride-rich lipoproteins (TGRL) are atherogenic. Apo E knockout mice have been shown to experience TGRL remnant accumulation and severe atherosclerosis (Zhang et al , 1994). Remnant uptake and retention has been demonstrated in rabbit coronary arteries when perfused in situ with radio- or fluorescently labelled chylomicron remnants (Mamo and Wheeler, 1994; Proctor

and Mamo, 1998) Rapp et al (1994) found direct evidence that TGRL remnants are deposited in human atherosclerotic lesions by examining lesions removed during aortic surgery. One third of the apo B associated cholesterol came from VLDL and IDL. However, apo B48 was not detected

In addition to the generation of atherogenic remnants, TGRL may contribute indirectly to the development of atherosclerosis via an influence on other lipoprotein species. When compared to the fasted state, the postprandial state is dynamic and nonsteady, with rapid remodelling of lipoproteins (Hyson et al, 2003). Cholesterol ester transfer protein (CETP) mediates the reciprocal transfer of cholesterol ester and TG between lipoproteins. In the postprandial triglyceride-rich environment, TG is transferred from TGRL to HDL and LDL with cholesterol ester exchanged in the opposite direction, resulting in cholesterol-enriched chylomicrons and VLDL, and triglyceride-enriched, cholesterol-depleted LDL and HDL. The TG content of LDL and HDL is rapidly hydrolysed by hepatic lipase resulting in small dense LDL and HDL.

The inverse association consistently found between TG and HDL-C, in particular with HDL₂-C, can be explained by this neutral lipid exchange. One research group has previously suggested that high HDL-C may not be protective per se, but simply reflect an efficient metabolism of TGRL (Miesenbock and Patsch, 1991). More recent evidence suggests that the HDL particle is protective per se (Parthasarathy et al, 1990, Fluiters et al, 1996, Barter et al, 2002; Spieker et al, 2002). The cholesterol enrichment of the TGRL is pro-atherogenic as cholesterol ester cannot be metabolised by lipoprotein lipase. The reduction in LDL

particle size is also pro-atherogenic, allowing it to more easily cross the endothelium, and increasing its susceptibility to oxidation (Diwadkar et al., 1999). Small dense LDL are highly predictive of CAD and present in 40-50% of all patients with CAD, despite normal LDL-C (Griffin et al , 1994)

The postprandial nature of atherosclerosis is supported by epidemiological studies (Patsch et al., 1992, Sharrett et al , 1995, Groot et al , 1991) showing higher postprandial lipemia and remnant lipoproteins in CAD patients compared to matched controls. Triglyceride concentrations in the late postprandial period have been shown to distinguish CAD cases from controls with an accuracy of 68% and to remain significant in a multivariate model after the elimination of fasting TG and HDL-C (Patsch et al , 1992). Similar results were found in the ARIC study (Sharrett et al , 1995) where higher postprandial increases in TG and TGRL remnants were observed in those with asymptomatic atherosclerosis (based on IMTc measurements) compared to matched controls. A surprising finding however, confirmed by Ginsberg et al (1995) was the lack of association of postprandial markers with disease status in obese men and women. It was suggested that the marker function of postprandial TG for TGRL remnants may be abolished in the obese state, when TGRL are particularly large and less dense.

A shortcoming of all cross-sectional studies is their inability to determine causality. It could be argued that the presence of atherosclerosis in some way impairs postprandial TG metabolism. Prospective studies of those with established CAD indicate that disease progression is related to TGRL and their remnants. Karpe et al (1994) found an association

($r = 0.51$, $p < 0.05$) between the progression of atherosclerosis over a 5-year period determined by coronary angiography, and postprandial levels of small chylomicrons at baseline. In the Montreal Heart Study, hepatic TGRL remnants predicted the progression of atherosclerosis (Havel, 1994). A cross-sectional study comparing healthy offspring of CAD patients with matched controls, demonstrated higher TG levels in the late postprandial period in the CAD offspring, despite similar fasting TG (Uiterwaal et al., 1994). The authors suggested that delayed clearance of TG might be associated with familial risk of CAD.

1.7.2 Atherogenic component of postprandial lipoproteins

Evidence from numerous in vitro studies suggests that TGRL and/or their lipolysis products (TGRL remnants and free fatty acids) are toxic to endothelial cells. Studies have shown increased endothelial permeability from TGRL extracted from postprandial but not fasting serum, and incubated with lipoprotein lipase (Hennig et al., 1992; Chung et al., 1998). The components of postprandial lipoproteins that increase endothelial permeability, and trigger the expression of adhesion molecules are not fully clear. However, the lipolysis products of oxidised chylomicrons have been identified as potential culprits. Chylomicrons obtained from postprandial serum and oxidised in vitro have been shown to be cytotoxic to endothelial cells and stimulate adhesion molecule expression (Jagla and Schrezenmeir, 1998; Mabile et al., 1995; Kurtel et al., 1995). Non-oxidised chylomicrons did not stimulate endothelial monocyte adhesion in rat venules and the cytotoxicity of oxidised chylomicrons was blunted by pre-treatment with the antioxidant enzyme superoxide dismutase (Kurtel et al., 1995). Chylomicron cytotoxicity has also been shown to relate to lipoprotein fatty acid

composition Chylomicrons obtained following meals rich in polyunsaturated fats were more easily oxidised in vitro and consistently induced higher levels of adhesion molecule expression (Jagla and Schrezenmeir, 1998, Mabile et al , 1995) Similarly, Chung et al (1998) found FFA released lipolytically from postprandial serum to be more injurious to endothelial cells and macrophages following a meal high in polyunsaturated fatty acids compared to saturated fatty acids.

Individual fatty acids have been shown to stimulate and to protect against endothelial cell activation in vitro Linoleic acid, the most abundant polyunsaturate in the diet, has been shown to increase cellular oxidative stress, activate NF κ B, increase IL-8 production and ICAM-1 expression (Young et al , 1998). Its hydroxy- or hydroperoxy- derivatives, have been shown to stimulate E-selectin, ICAM-1 and VCAM-1 expression (Sultana et al , 1996) Prior incubation of endothelial cells with linoleic acid has been shown to increase cytokine-induced adhesion molecule expression (Toborek et al , 1996). In contrast, inhibitory (protective) effects have been shown for the omega-3 docasahexaenoic acid (Weber et al , 1995) and the monounsaturate oleic acid (Carluccio et al , 1999) In summary, evidence from a number of in vitro studies indicate that the lipolysis products of postprandial TGRL increase oxidative stress in endothelial cells and are therefore cytotoxic Individuals fatty acids, and in particular linoleic acid may also activate inflammatory pathways via increased oxidative stress or other unknown mechanisms

1.7.3 Postprandial endothelial activation and dysfunction

Early proponents of the link between postprandial metabolism and atherosclerosis identified the postprandial period as a critical time of heightened risk. However, precise mechanistic explanations were lacking. Since then, advances in our understanding of atherosclerosis have revealed a key role for the endothelium in regulating vascular function, with endothelial dysfunction an early indicator of disease. Studies of postprandial endothelial function provide evidence of a threat to vascular health by the metabolites of high fat meals. This evidence comes from studies that have shown

1. endothelial dependent dilation (EDD) to be impaired during the postprandial period
2. postprandial increases in circulating adhesion molecules, cytokines and endothelial microparticles
3. evidence of endothelial cell activation in vitro when incubated with postprandial serum

In 25 of 30 studies reviewed, EDD of the conduit vessel was impaired following a high fat or high glucose load. This finding was evident in a variety of populations including young healthy, older healthy and clinical cohorts (Table 1.4). The maximum impairment in endothelial function typically occurred postprandially at the 4 h timepoint, coinciding with the peak TG concentration. During oral glucose tolerance tests (OGTT), the maximum impairment occurred earlier, coinciding with the peak glucose concentration. Other frequent observations in these studies were the interrelationships in flow-mediated dilation (FMD), postprandial lipemia and oxidative stress. A number of studies reported inverse correlations between the postprandial changes in FMD and the changes in TG ($r = -0.34$ to -0.70 , median

$r = -0.47$) The postprandial changes in oxidative stress were also associated with the changes in FMD ($r = -0.784$) and TG ($r = 0.664$). Collectively, these studies suggest that the postprandial impairment of EDD is related to postprandial oxidative stress, and varies in proportion to postprandial hypertriglyceridemia. However, postprandial endothelial function can be improved without reductions in lipemia. The postprandial impairment of FMD is consistently prevented when high doses of antioxidant vitamins are administered with the fat and glucose challenges (Plotnick et al., 1997; Title et al., 2000; Ling et al., 2002; Bae et al., 2003). Short-term treatment with statins can also attenuate endothelial dysfunction before changes in blood lipids occur (Ceriello et al., 2005), evidence of a lipid-independent mechanism of action. However, endothelial function is further improved with changes in TG and HDL-C following months of statin or fibrate treatment (Marchesi et al., 2003; Ceriello et al., 2005).

Hypertriglyceridemia and hyperinsulinemia have both been shown to increase conduit vessel diameter and forearm blood flow (Gokce et al., 2001; Lind et al., 2002). The increases in triglycerides and insulin that occur postprandially may therefore pose methodological problems when assessing postprandial endothelial function. Gokce et al. (2001) observed an *apparent decrease in FMD following a high fat meal* (FMD calculated as the *percentage difference between pre- and post-occlusion diameters*). However, pre-occlusion brachial artery diameters were higher in the postprandial state with post-occlusion diameters similar in the fasted and postprandial states. The authors concluded that EDD was consequently not impaired postprandially and suggested that the changes reported by others may be an

artefact. Verification of this observation in the studies reviewed here is not possible as many only report the pre- to post-occlusion percentage change.

The influence of high fat and high glucose challenges on resistance vessel function has been less frequently studied. In addition, the studies that exist have employed a wider range of methodologies (Table 1.5). Of 3 studies that assessed changes in FBF in response to infusion of Ach or Mch, only one (Steer et al., 2003a) was able to demonstrate a postprandial impairment, and only at the 1 h postprandial timepoint. It has therefore been suggested that endothelial function may only be impaired postprandially in conduit vessels (de Koning and Rabelink, 2002). More research is clearly needed given the relatively small number of studies that have examined resistance vessel function. Postprandial impairments in resistance vessel function have been shown using other methodologies (Gill et al., 2003a; Schinkovitz et al., 2001). However, the extent to which increases in Ach-mediated skin perfusion and increases in reactive hyperemia reflect resistance vessel EDD is uncertain.

Postprandial changes in endothelial function have also been investigated using circulating markers of endothelial activation and inflammation including soluble adhesion molecules, cytokines, leukocytes and more recently endothelial microparticles (Table 1.6). Postprandial increases in soluble adhesion molecules have consistently been shown in clinical populations, including cohorts with type II diabetes and hypertriglyceridemia (Nappo et al., 2002, Marchesi et al., 2003, Ceriello et al., 2004, Ceriello et al., 2005). Small increases have been shown in some studies involving apparently healthy individuals (Nappo et al., 2002, Ceriello et al., 2004), but not in others (Gill et al., 2003a, Tsai et al., 2004).

Table 1.4 *Influence of postprandial lipemia and glycemia on conduit vessel endothelial function*

Study	Subjects	Main findings
Plotnick et al 1997	20 healthy adults,	FMD lower 2, 3 and 4 h PP, 21% (baseline) vs 8% (4hrsPP) No change in FMD when Vit C and E given with meal
Vogel et al 1997	10 healthy adults	FMD lower at 2, 3, 4 h PP, 21% (baseline) vs 11% Δ FMD vs Δ TG, $r = -0.47$
Djousse et al 1999	13 healthy adults	No PP change in FMD, 9.5% (baseline) vs 6.8% (P=NS)
Williams et al 1999	10 middle-aged healthy adults	FMD lower PP (5.8 vs 0.8%) only after meal cooked in used fat
Marchesi et al 2000	10 young healthy men	FMD lower 2 and 4 h PP Δ FMD vs Δ TG, $r = -0.70$
Raitakari et al. 2000	12 young healthy adults	No change in 3 h PP FMD, 4.2 vs 3.2%, $p = 0.11$ PP increase in pre-occlusion arterial diameter For changes in resistance vessel function see table 5
Title et al 2000	10 young healthy adults	FMD lower 2 h PP following 75g glucose load, 6.5-3.7% No PP change in FMD if Vit C and E administered with glucose load
Vogel et al 2000	10 healthy adults	FMD lower PP only following meal rich in olive oil rich (1 of 5 test meals) FMD decreased 14.3% to 9.9% Δ FMD vs Δ TG, $r = -0.47$
Wilmink et al, 2000	20 young healthy adults	FMD lower PP, 10.6% (baseline) vs 5.8% (4hr PP), No PP change in FMD following 2wks folic acid treatment
Anderson et al 2001	12 T2D patients and 12 healthy controls	FMD decreased 4hr PP, in healthy controls (6.3 vs 4.77%) and T2D patients (2.65 vs 1.45%) Δ FMD related to baseline lipids and Δ lipids
Wilmink et al 2001	15 young healthy men	FMD lower PP, 9.1% (baseline) vs 4.3% (4 h PP) Changes in FMD related to RLP-C rather than TG 3 wks treatment with statins but not fibrates reversed the PP impairment of FMD

Bae et al 2001a	20 middle-aged healthy men	FMD lower PP, 13.7% (baseline) vs 8.2% (2 h PP) Leukocyte superoxide anion ($O_2^{\cdot -}$) production 34% higher PP Δ FMD vs Δ TG, $r = -0.65$, Δ FMD vs Δ $O_2^{\cdot -}$, $r = -0.784$
Bae et al 2001b	11 CAD patients	No PP change in FMD Δ TG vs Δ $O_2^{\cdot -}$ production, $r = 0.664$
Gaenger et al 2001	17 healthy men,	FMD lower PP PP FMD vs PP TG, $r = -0.81$ Diurnal variation in FMD observed, with lowest values at 8am
Gokce et al 2001	14 healthy adults	FMD lower PP, 14.7% (baseline) vs 10.6% (PP) No difference between fasting and PP post-occlusion artery diameter, PP % decrease due to increase in pre-occlusion artery diameter
Katz et al 2001	50 healthy adults	Brachial artery reactivity (ultrasound assessment of brachial artery blood flow post-occlusion) lower 3 h PP No PP changes in BAR following meal ingested with Vit E
Schillaci et al. 2001	10 young healthy women	No PP change of FMD Conclusion: Gender effect evident (repeat of Marchesi et al , 2000 study)
Williams et al 2001	14 adults	No PP change in FMD following olive or safflower heat-modified oil meals
Marchesi et al. 2001	7 young healthy men	FMD lower 2-4 h PP Baseline FMD vs TG ($r = -0.426$), Baseline FMD vs GSH ($r = 0.48$) Effect partly reversed by 10d L-arginine supplementation
Zhao et al 2001	50 CAD patients & 25 healthy controls	FMD lower PP 3% vs 1.7% in patients, 6.6% vs 4.9% in controls
Cerello et al 2002	30 T2D and 20 healthy controls	FMD lower PP following high fat and glucose loads in T2D and healthy controls Biggest PP decrease following combined fat-glucose load Short term (3-6 d) statin therapy improved fasting and PP FMD in T2D without changes in lipids Long term statin therapy reduced lipids with further improvements in FMD
De Roos et al 2002	21 healthy men	No PP change in FMD following meals rich in saturated FA and trans FA

Funada et al. 2002	24 adults	No PP change in FMD Baseline FMD vs Δ RLP-C, $r = -0.588$
Lee et al. 2002	12 T2D	FMD lower PP following with high fat (13.1 vs 7.3% 4 h) or glucose load (13.2 vs 7.3% 1 h) Increase in PP $O_2^{\cdot -}$ production No PP changes in fat and glucose loads administered with Vit E
Ling et al. 2002	74 CAD patients and 50 controls	FMD lower 4 h PP in CAD patients and healthy controls Δ FMD vs Δ TG, $r = -0.545$ No PP change FMD in either group if Vit C administered with test meal
Siepi et al. 2002	10 postmenopausal women	FMD lower 2 h PP, 7.7 vs 2.3% GSH also decreased PP in parallel with FMD Δ FMD vs Δ TG, $r = -0.37$
Bae et al. 2003	10 young healthy adults	FMD decreased PP, 13.3% (baseline) vs 6.6% (2 h PP), 7.1% (4 h PP) No PP change in FMD if Vit E administered with meal Δ FMD vs Δ TG $r = -0.54$ For changes in resistance vessel function see table 5
Marchesi et al. 2003	10 HTG patients	FMD lower 2-6 h PP, 4.3% (baseline) vs 1.3% (6 h) Δ FMD vs Δ TG $r = -0.34$, Δ FMD vs Δ sICAM-1, $r = -0.66$. Baseline FMD higher following 3 months fibrate treatment
Tsai et al. 2004	16 healthy adults	FMD lower 2-6 hrs PP Increase in PP oxidative stress (from assessments of 8-PGF _{2α} and GSH) No PP change in adhesion molecules
Cerello et al. 2005	20 T2D patients	FMD lower PP following high fat and glucose loads, biggest PP decrease following combined fat-glucose load Baseline FMD higher and baseline sICAM-1, IL-6, NT and CRP lower following 4d combined atorvastatin-irbesartan treatment prior to changes in lipids

Abbreviations: Flow-mediated vasodilation (FMD), postprandial (PP), type II diabetic (T2D), remnant lipoprotein particle-cholesterol (RLP-C), superoxide anion ($O_2^{\cdot -}$), reduced glutathione (GSH), coronary artery disease (CAD), 8-epi-prostaglandin F_{2 α} (8-PGF_{2 α}), soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), interleukin-6 (IL-6)

Table 1.5 *Influence of postprandial lipemia and glycemia on resistance vessel endothelial function*

Study	Subjects	Main findings
Gudmundsson et al 2000	15 young healthy adults	EDD evaluated as Δ FBF from resting in response to Ach and bradykinin No difference in EDD 2-4 h PP following consumption of high and low fat meals. Changes in EDD pre- to postprandial not determined Increase in plasma glucose PP following low fat meal
Raitakari et al 2000	12 young healthy adults	Reactive hyperemia (post-occlusion FBF) higher at 3 & 6 h PP Baseline blood flow (pre-occlusion FBF) higher 3 & 6 h PP Δ FBF vs Δ TG $r=0.77$, Δ FBF vs Δ insulin, $r=0.80$ For changes in conduit vessel function see table 1.4
Muntwyler et al. 2001	12 young healthy adults	EDD evaluated as absolute Ach-FBF and Δ Ach-FBF from resting values No change EDD 4 h PP
Schinkovitz et al 2001	11 healthy adults	Reactive hyperemia (increase in FBF following arterial occlusion) lower at 2 h PP No change in 2 h resting FBF
Bae et al 2003	10 young healthy adults	Reactive hyperemia (FBF following venous occlusion) higher PP but baseline blood flow (FBF pre-occlusion) also higher PP Effects on EDD thus inconclusive For changes in conduit vessel function see table 1.4
Gill et al 2003a	8 endurance-trained men	Skin perfusion following iontophoresis of Ach (EDD) 17-22% lower 4hrs PP No PP change in skin perfusion of SNP (EID). IL-6 increased at 4 & 6 h PP
Steer et al 2003a	26 young healthy adults	EDD evaluated as (1) absolute FBF during Mch infusion and (2) Mch-FBF/ SNP-FBF ratio during infusion EDD measured by both indices decreased 1 h PP following consumption of 34% fat meal but not 20% fat meal
Gill et al 2004	10 lean and 10 obese middle-aged men	Skin perfusion following iontophoresis of Ach (EDD) 15% lower PP Skin perfusion following iontophoresis of SNP (EID) also lower Thus evidence of decreased skin perfusion PP but not EDD

Abbreviations: Postprandial (PP), endothelial dependent vasodilation (EDD), endothelial independent vasodilation (EID), acetylcholine (Ach), metacholine (Mch), sodium nitroprusside (SNP), forearm blood flow (FBF), carotid artery intima media thickness (IMTc)

Table 1.6 Influence of postprandial lipemia and glycemia on soluble adhesion molecules, cytokines, microparticles and other inflammatory markers in the circulation

Study	Subjects	Main findings
Nappo et al 2002	20 OW T2D (aged 46±5 y) and 20 matched controls	PP increases in sVCAM-1, sICAM-1, IL-6 and TNF-α in T2D and controls Baseline values and PP increases more pronounced in T2D
Gill et al. 2003a	8 endurance-trained athletes	No PP changes in sVCAM-1, sICAM-1 or TNF-α PP increases in IL-6
Marchesi et al. 2003	10 HTG patients	PP increases in sVCAM-1 and sICAM-1
Van Oostrom et al 2003	8 healthy young men	PP increases in leukocytes (mainly neutrophils), IL-6 and IL-8
Ceriello et al 2004	30 OW T2D (aged 54±3 y) and 20 matched controls	PP increases in sICAM-1, sVCAM-1 and sE-selectin following high fat and high glucose test meals PP increases more pronounced following combined fat-glucose meal. PP increases more pronounced in T2D
Tsai et al 2004	16 young healthy adults	No PP changes in sICAM-1, sVCAM-1 or CRP
Van Oostrom et al 2004	10 middle-aged healthy men	PP increase in neutrophil counts PP increase in activation of monocytes and leukocytes (increased CD11b expression) Δ CD11b monocyte expression vs Δ TG r= 0.64
Ferreira et al. 2004	18 young healthy adults	PP increases in EMP defined as CD31+/CD42- Δ EMP vs Δ TG r= 0.77
Ceriello et al. 2005	20 T2D patients	PP increases in sICAM-1, IL-6, CRP following high fat and high glucose meals PP increases more pronounced following combined fat-glucose meal
Tushuizen et al 2006	17 healthy adults	PP increases in total MP (mainly platelet MP) EMP were not detectable in fasting or postprandial samples using CD62E, CD106 and CD144 biomarkers

Abbreviations: Overweight (OW), type II diabetic (T2D), hypertriglyceridemia (HTG), soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), interleukin-6 (IL-6), interleukin-8 (IL-8), tumour necrosis factor-α (TNF-α), C-reactive protein (CRP), microparticle (MP), endothelial microparticle (EMP), cluster of differentiation molecule (CD)

Although increases in adhesion molecules have been demonstrated in type II diabetics following a high fat and a high glucose challenge, the greatest increase occurred following a combined high fat-glucose challenge (Ceriello et al , 2004). Peak values coincided with peak TG during the OFTT and with peak glucose during the OGTT. In some studies, the postprandial increases in soluble adhesion molecules have mirrored the impairment of EDD (Marchesi et al, 2003, Ceriello et al, 2004) though not in others (Gill et al , 2003a). Postprandial increases in IL-6 (Nappo et al., 2002, Gill et al , 2003a, Van Oostrom, 2003) leukocyte counts (Van Oostrom et al , 2003, Gill et al , 2004, Van Oostrom et al , 2004) and leukocyte activation (Van Oostrom et al , 2004) have also been demonstrated. An increase in TNF- α has been shown in one study (Nappo et al , 2002) but not another (Gill et al , 2003a).

Only two studies have examined postprandial changes in circulating microparticles. Ferreira et al (2004) reported a postprandial increase in EMP defined as CD31+/CD42-, in young healthy volunteers following a high fat but not a low fat test meal. The increase in serum TG was related to the increase in EMP ($r= 0.77$). Tushuizen et al (2006) demonstrated a postprandial increase in total microparticles (annexin V positive events) and in markers of oxidative stress, along with a decrease in FMD. However, EMP were not detectable in the fasting or postprandial samples of these young healthy volunteers, when CD62E, CD106 and CD144 were employed as biomarkers.

In summary, FMD in conduit vessels has consistently been shown to be impaired following high fat and high glucose challenges, in young healthy and older clinical populations. This change in endothelial function is accompanied by an increase in

inflammatory cytokines, leukocyte counts and leukocyte activation. Postprandial increases in adhesion molecules occur in clinical populations. Only small increases if any, occur in young healthy populations. Postprandial increases in EMP have been shown in one study involving young healthy volunteers but not another. EMP may prove to be a more sensitive marker of postprandial endothelial activation than soluble adhesion molecules in healthy volunteers, though more work is needed to confirm this.

1.7.4 Postprandial oxidative stress

A postprandial increase in oxidative stress is another consistent feature of the literature whether determined by changes in nitrotyrosine (Ceriello et al, 2004, Ceriello et al, 2005), lipid peroxides (Andersen et al., 2001; Van Oostrom et al, 2003), malondialdehyde (Wilmink et al, 2000), secondary lipid radicals (Andersen et al, 2001), 8-epi-prostaglandin $F_{2\alpha}$ (Tsai et al, 2004), glutathione peroxidase (Siepi et al, 2002, Tsai et al, 2004) or leukocyte superoxide anion production (Bae et al, 2001a, Bae et al, 2001b, Lee et al., 2002,) Most authors attribute postprandial endothelial dysfunction to the postprandial increase in oxidative stress. Nappo et al, (2002) were not able to demonstrate increases in soluble adhesion molecules or cytokines, when the test meals were administered with high doses of vitamin E. Similarly Ceriello et al (2004) demonstrated a decrease in all soluble adhesion molecules following 3-6 days of simvastatin treatment before any reduction in lipids occurred. These early decreases in adhesion molecules were attributed a reduction in oxidative stress, as there was a concurrent reduction in nitrotyrosine. Postprandial oxidative stress also

increases the oxidation and atherogenicity of circulating and subendothelial LDL particles (Ursini and Sevanian, 2001).

Although its existence is well documented, the source of postprandial oxidative stress is still unclear. McCarty (2003) has proposed that the exposure of endothelial cells to FFA and insulin results in the accumulation of an intracellular TG pool that has a detrimental effect on EDD. Recent landmark papers (Brownlee, 2005, Ceriello and Motz, 2004) also point to nutrient-driven increases in mitochondrial Krebs's cycle activity, and the generation of reactive oxygen species along the electron transport chain, as the major source of oxidative stress in endothelial cells. It is likely that nutrient uptake increases considerably in endothelial cells during the postprandial period. Unlike adipose tissue and muscle, endothelial cells cannot limit postprandial nutrient uptake as they do not become insulin resistant. Studies generally show that the maximum impairment of EDD and the maximum increase in endothelial cell activation markers coincides with peak TG and glucose concentrations (Ceriello et al, 2002, Ceriello et al, 2005).

Evidence of a specific role for FFA in oxidative stress-mediated endothelial dysfunction comes from a number of infusion studies. Intralipid-heparin infusions that resulted in a seven-fold increase in FFA have been shown to impair resistance vessel endothelial function (Steinberg et al, 1997; Steer et al, 2003b; Steer et al, 2003c), but this impairment was abolished by the concomitant infusion of vitamin C, L-arginine or cyclooxygenase (COX) inhibitors (Steer et al, 2003c). Although serum FFA generally decrease in the early postprandial period, later rising above fasting values, local endothelial

concentrations will be largely determined by serum TG, which release FFA at the endothelial surface (Jagla and Schrezenmeir, 2001)

1.7.5 Summary

In summary, cholesterol-enriched remnants of TG-rich lipoproteins are generated during the postprandial period that in addition to LDL, are known to be directly atherogenic. A postprandial triglyceride-rich environment is known to exert an atherogenic influence on other lipoprotein species increasing the preponderance of small dense LDL and decreasing serum HDL-C. Postprandial oxidative stress is a consistent finding in the literature, increasing the oxidation and atherogenicity of LDL and other remnant lipoproteins. Postprandial oxidative stress in endothelial cells, possibly mediated by *nutrient overload*, also results in endothelial cell activation and a series of pro-inflammatory changes that increase leukocyte attraction, adhesion, transendothelial migration, proliferation and survival. These postprandial perturbations may be exacerbated in centrally obese, insulin resistant individuals, who experience a greater degree of lipemia, have higher levels of pro-inflammatory cytokines and *a reduced ability to limit endothelial cell nutrient overload*. Twenty-five years on from Zilversmit's original paper, the proposal that postprandial lipemia (and indeed glycemia) plays a role in the development of atherosclerosis, has been borne out by multiple mechanisms.

1.8 Exercise and postprandial triglycerides

Physical activity and physical fitness are associated with a lower incidence of CAD. In a meta-analysis of prospective studies, the relative risk of death from CAD in the most

active individuals was approximately half that of their sedentary counterparts (Berlin and Colditz, 1990). The cardioprotective effect of exercise may be mediated in part by an influence on triglyceride metabolism

1.8.1 Effect of a single exercise bout

There is clear evidence that a single bout of prolonged exercise that occurs prior to feeding can reduce postprandial TG concentrations. This finding is consistent in young adult (Aldred et al., 1994), older male (Gill et al , 2001a, Gill et al , 2004), postmenopausal female (Gill and Hardman, 2000), sedentary (Tsetsonis et al , 1997), trained (Tsetsonis et al , 1997), overweight (Murphy et al , 2000, Gill et al , 2004) and hypertriglyceridemic (Zhang et al , 2006) populations. Acute exercise typically results in a 20-30% decrease in the area under the TG-time curve, with reductions in the chylomicron-TG, VLDL₁-TG and VLDL₂-TG subfractions (Malkova et al , 2000, Gill et al , 2001b, Gill et al , 2006). Decreases in postprandial lipemia have been shown with exercise bouts of only 30 min duration at 60% VO₂max (Murphy et al , 2000). A small number of studies employing low to moderate intensity exercise bouts of short duration, have failed to demonstrate any influence of acute exercise on postprandial lipemia (Pfeiffer et al , 2005, Pfeiffer et al , 2006, Teixeira et al , 2006).

Some (Gill et al , 2001b; Malkova et al , 1999) but not all (Malkova et al , 2000, Herd et al , 2001) studies have reported a decrease in postprandial insulin following acute exercise. Reductions in postprandial insulin have been demonstrated in centrally obese but not in lean middle-aged men (Gill et al , 2004) and in trained but not in untrained women (Tsetsonis et

al., 1997). The test meals employed in these studies were high in fat and carbohydrate, resulting in a pronounced insulin response that peaked at 30-60 min postprandially. Postprandial lipemia was reduced similarly in all studies regardless of the exercise effect on postprandial insulin

The timing of the exercise bout in relation to the oral fat tolerance test (OFTT) may be an important consideration. Sixty minutes of exercise at 60% $\text{VO}_{2\text{max}}$ has been shown to reduce postprandial lipemia when undertaken 12 h but not 24 h prior to feeding (Zhang et al. 2004). Katsanos and Moffatt (2004) reported similar decreases in postprandial lipemia when exercise was completed 30 min before or commenced 90 min after meal ingestion. In contrast, Zhang et al (1998) found decreases in postprandial lipemia from exercise bouts that commenced 1 h and 12 h pre-feeding but not 1 h post-feeding. In the majority of studies in this review, exercise sessions were conducted on the day prior (16 h approximately) to the test meal

In a retrospective examination of the data from 7 previous studies, 90 min exercise at 60% $\text{VO}_{2\text{max}}$ reduced fasting TG, postprandial TG and the postprandial TG increment (AUC above baseline) by $18.2 \pm 2.2\%$, $21.5 \pm 1.5\%$ and $21.9 \pm 3.1\%$, respectively (Gill et al, 2002a). The attenuation of fasting and postprandial TG was similar in men and women and in apo E3/2, E3/3 and E3/4 genotypes. In these studies, approximately 10% of subjects experienced no change or even an increase in postprandial TG as a result of exercise. A strong relationship existed between the exercise-induced changes in fasting and postprandial triglycerides ($r = 0.82$, $p < 0.01$) with no significant relationship between the changes in fasting

TG and the postprandial TG increment ($r=0.16$, $p=0.16$). A relationship was also evident between the exercise-induced changes in postprandial TG and VO_2max ($r=0.33$, $p<0.01$), though the authors suggest that this may reflect differences in energy expenditure during the experimental exercise bout. Acute exercise attenuated fasting insulin in men but not women. However, postprandial insulin was reduced similarly in men (13%) and women (17%).

The available evidence suggests that energy expenditure may be more critical than exercise intensity in influencing postprandial triglyceride metabolism. Gill et al (2002b) demonstrated a dose-dependent decrease in postprandial lipemia of 9.3% and 22.8% respectively from 60 min and 120 min walking at 50% VO_2max . Similarly, Tsetsonis et al (1996a) found the attenuation of postprandial lipemia to be greater following 90 min at 60% VO_2max compared to 90 min at 30% VO_2max . In a related study, isocaloric exercise bouts at two different intensities (90 min at 60% VO_2max vs 3 h at 30% VO_2max) resulted in similar decrease in postprandial lipemia (Tsetsonis et al, 1996b). In contrast, Zhang et al (2006) found postprandial lipemia to be attenuated similarly in 10 hypertriglyceridemic men following 60 min exercise at 40%, 60% and 70% VO_2max .

The available evidence also suggests that exercise benefits can be accumulated over the course of a day in two or three shorter bouts. Studies have demonstrated similar reductions in lipemia following one 90 min and three 30 min bouts at 60% VO_2max (Gill et al, 1998), one 30 min and three 10 min bouts at 60% VO_2max (Murphy et al, 2000) and one 30 min and ten 3 min bouts at 70% VO_2max (Miyashita et al, 2006). In one study, only intermittent exercise resulted in significant reductions in lipemia (Altena et al, 2004). The

possibility of accumulating benefit with multiple short bouts has major public health implications

The majority of the research studies have employed aerobic exercise, typically treadmill walking, jogging or cycle ergometry. However, a recent study has demonstrated a reduction in postprandial TG in young adults following four 15 min bouts of intermittent games activity, that was comparable to that achieved with four 15 min bouts of uphill treadmill walking (Barrett et al, 2006). The influence of resistance exercise is less clear. Single sessions of resistance exercise have been shown to result in a decrease (Petitt et al, 2003), no change (Burns et al., 2005, Shannon et al, 2005) and even an increase (Burns et al, 2006) in postprandial lipemia.

Two recent studies have documented qualitative changes in postprandial TGRL following acute exercise, with potential pro- and anti-atherogenic effects. Gill et al, (2006) reported a lower postprandial ratio of apo CIII/apoB in the VLDL₁ fraction following acute exercise, indicating fewer apo CIII apoproteins per VLDL particle. The apo CIII content of apo B lipoproteins has been shown to be an independent predictor of coronary lesion progression (Hodis et al, 1994). This reduction in apo CIII content is likely to inhibit CETP-mediated neutral lipid exchange and facilitate LPL-mediated lipolysis, as apo CIII activates CETP and inhibits LPL action (Wang et al, 1985; Jong et al, 1999). Accordingly, favourable changes were observed post-exercise in the CE/TG ratios of the VLDL₁ and HDL₂ fractions. In contrast, Magkos et al, (2006) demonstrated an increase in IDL on the morning after 2 h cycling at 60% VO₂max. A decrease in VLDL-apo B secretion was observed but in the

absence of changes in VLDL-TG secretion. This points to VLDL particles that are fewer in number but richer in TG content and this qualitative change may account for the potentially pro-atherogenic increase in IDL remnants.

1.8.2 Effects of endurance training

The effect of endurance training on postprandial triglyceride metabolism is not so clear. Cross-sectional studies have shown postprandial lipemia to be lower in endurance-trained individuals compared to non-exercisers (Merill et al, 1989, Cohen et al, 1989). However, cross-sectional studies cannot prove cause and effect. The results of training and detraining studies are conflicting. When assessments are made at least 48 h after the last training bout, some studies report a decrease in fasting and/or postprandial TG (Altekruse and Wilmore, 1973, Wirth et al, 1985). In contrast others have found postprandial lipemia to be unchanged following 4-13 weeks of training (Aldred et al, 1995, Herd et al, 1998, Altena et al, 2006). Hardman et al (1998) examined the effect of seven days *de-training* on postprandial triglyceride metabolism, with OFTTs conducted 15 h, 60 h and 6.5 d after the last training bout. Most of the benefit was lost between the 15 h and 60 h trial, suggesting an acute effect of exercise on postprandial triglyceride metabolism lasting less than 3 d. This conclusion is supported by the results of a cross-sectional study of trained and untrained young adults (Herd et al., 2000). When OFTTs were undertaken two days after the last exercise bout, postprandial lipemia was not affected by training status. Although the majority of studies underline the importance of acute over chronic exercise, training will undoubtedly be of indirect benefit as increases in fitness will enable acute bouts of greater intensity,

duration and frequency to be completed Tsetsonis et al (1997) compared the effects of a single exercise bout of equal relative intensity and duration in trained and untrained women As a result of differences in fitness, energy expenditure during exercise was 48% higher in the trained women with postprandial lipemia attenuated by 30% and 16% respectively in the trained and untrained groups

1.8.3 Interaction with diet

Studies that examine the interaction of dietary manipulations and exercise have important implications for public health but can also shed light on the mechanisms by which acute exercise influences triglyceride metabolism Gill and Hardman (2000) compared the effect on postprandial lipemia of an energy deficit induced by previous day exercise, and an equivalent energy deficit induced by previous day dietary restriction The effect of exercise was considerably greater suggesting that the hypotriglyceridemic effects of acute exercise are largely independent of the overall energy deficit However, the authors point out that energy deficits achieved were likely to have occurred in different stores

High carbohydrate (CHO) diets are known to increase serum TG (Abbasi et al., 2000) In a three trial study, Koutsari et al. (2001) compared postprandial lipemia in volunteers who on the 3 d previous followed either a low CHO diet with no exercise, a high CHO (isocaloric) diet with no exercise or a high CHO diet accompanied by 60 min exercise Postprandial lipemia was similar in the low CHO no exercise trial and the high CHO exercise trial but was 33% higher in high CHO no exercise trial These results suggest that the exercise can prevent the hypertriglyceridemia associated with high CHO - low fat diets

It is plausible that the exercise effect on serum TG may relate to changes in muscle and/or liver glycogen, given that exercise typically decreases and carbohydrate feeding increases glycogen stores. It might therefore be expected that the attenuation of postprandial lipemia would be greater following exercise bouts that maximise the contribution of CHO to exercise metabolism. Although Malkova et al (1999) successfully manipulated substrate utilisation during exercise by administering either placebo or acipimox, a nicotinic acid analog and powerful inhibitor of adipose tissue lipolysis, prior to exercise, postprandial lipemia was similar in the placebo and acipimox trials. Whereas, the magnitude of the additional fat oxidation was considerable in the acipimox trial (+76%), the magnitude of the additional CHO oxidation in the placebo trial (+16%) was small. Differences in post-exercise glycogen stores following the two exercise trials are therefore likely to have been small and may not have been sufficient to significantly influence lipemia.

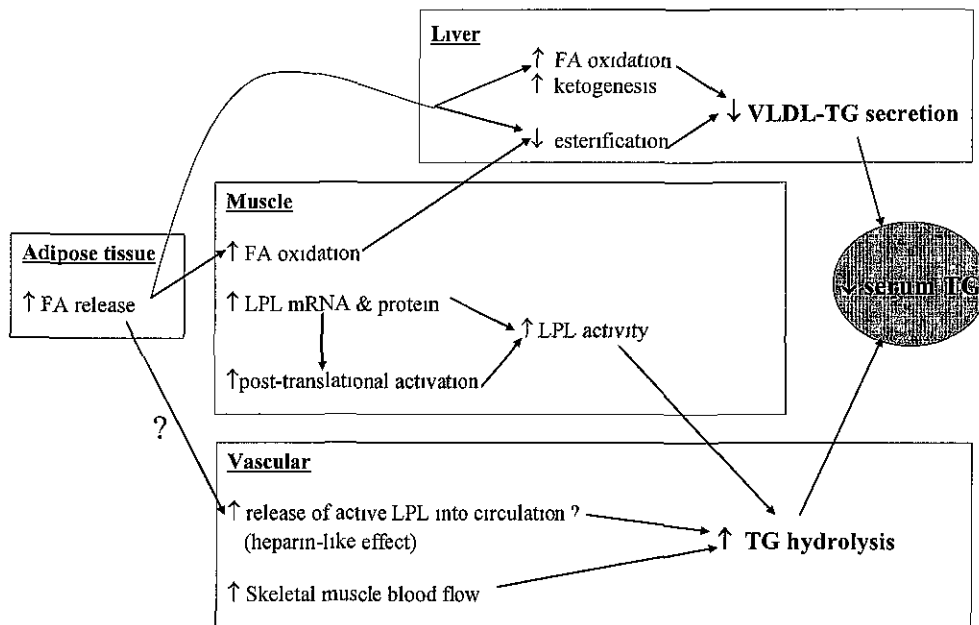
1.8.4 Influence on postprandial endothelial function

To date, only two studies have tested the hypothesis that the exercise attenuation of postprandial lipemia might also enhance postprandial endothelial function. In a study involving endurance-trained men, Gill et al, (2003a) found the postprandial impairment of microvascular endothelial function to be similar before and after 7d of detraining, even though postprandial lipemia was 53% higher in the detrained state. There were no changes in sVCAM-1 or sICAM-1 in response to the test meals or following detraining. In another study (Gill et al., 2004), a single session of exercise reduced fasting and postprandial TG in middle-aged men, and enhanced microvascular endothelial function in the fasted state. The

influence of acute exercise on postprandial microvascular function was unclear as postprandial changes were observed in both EDD and EID. More work is clearly needed examining the influence of acute exercise on directly measured endothelial function and biomarkers of endothelial activation, with reference to changes in postprandial lipemia. Postprandial increases in white blood cell count and IL-6 were also documented in these studies but values were not influenced by acute exercise or training status.

1.8.5 Mechanisms for exercise effect

Fig 1.1 Influence of acute exercise on muscle, adipose, hepatic and vascular tissue: putative mechanisms by which serum triglycerides are reduced



The mechanisms mediating the effect of acute exercise on TG metabolism are still unclear. One possibility is that TG are cleared faster from the circulation following acute exercise mediated by an acute increase in the activity of skeletal muscle lipoprotein lipase (LPL). This enzyme is located in every capillary bed, but particularly in muscle and adipose tissue. Increases in LPL mRNA and LPL protein have been demonstrated in muscle, 4-8 h post-exercise (Seip et al, 1997). Results from short-term training and detraining studies provide evidence of pre-translational (Seip et al, 1995) and post-translational (Simsolo et al, 1993) regulation of skeletal muscle LPL activity.

The influence of acute exercise on skeletal muscle LPL (SM-LPL) activity, post-heparin LPL (PH-LPL) activity and triglyceride clearance rate is controversial. Increases in SM-LPL activity lasting up to 3-5 days have been shown in young, trained volunteers following prolonged bouts of exercise (Lithell et al, 1979; Taskinen and Nikkila, 1980; Lithell et al, 1981; Lithell et al, 1984; Kiens et al., 1989; Kiens and Richter, 1998). Increases in PH-LPL activity have been demonstrated 24 h post-exercise but not at earlier timepoints (Zhang et al., 2002). Early studies have shown the clearance rate of an exogenous triglyceride infusion to be increased following prolonged bouts of exercise that included a marathon race (Sady et al, 1986), a 4 h orienteering race (Brandt et al, 1984) and a 3 h running test (Dufaux et al., 1981). In one study, the increase in triglyceride clearance rate was accompanied by an increase in PH-LPL activity.

In contrast, Gill et al (2001a) found no effect of 90 min exercise at 60% $\text{VO}_{2\text{max}}$ on the rate of clearance of an intralipid infusion in untrained middle-aged men, even though the

same exercise bout attenuated postprandial lipemia by 18%. Annuzzi et al. (1987) reported increases in intralipid clearance rate following 3 h but not 90 min exercise at 77% HRmax in untrained men. Three studies have demonstrated decreases in postprandial lipemia following 90 min of moderate intensity exercise in the absence of changes in SM-LPL or PH-LPL activity (Herd et al, 2000, Gill et al, 2003b, Katsanos et al, 2004). In two of these studies, considerable increases in LPL activity were evident in some subjects, with strong correlations between the exercise-induced changes in LPL activity and postprandial lipemia (Herd et al, $\rho = -0.79$, Gill et al, $r = -0.77$). These data suggest that increases in LPL activity and triglyceride clearance rate are not permissive factors for reductions in lipemia but where increases occur, the attenuation of lipemia will be of greater magnitude.

Malkova et al (2000) observed a 34% decrease in postprandial lipemia on the day following a prolonged bout of exercise. Postprandial TG uptake across leg muscle, determined from arterio-venous differences, was not significantly increased by exercise, though leg TG clearance (TG uptake / arterial concentration) increased two-fold. The clearance statistic was considered a fairer reflection of the efficiency of TG removal, as TG values were lower in the exercise trial. The authors suggested that the observed increase in TG clearance might serve to maintain a supply of FFA to the exercised tissue during the post-exercise period, in the presence of lower arterial TG concentrations.

The exercise attenuation of fasting and postprandial TG may also be mediated by a reduction in hepatic TG synthesis and VLDL-TG secretion. Early training studies in rats can provide some valuable insights into the changes in triglyceride metabolism that accompany

exercise, as all post-training assessments were conducted within 48 h of the last training bout. In three studies, exercise training has been shown to reduce VLDL-TG secretion in rats by 32-50% with similar reductions in serum TG (Simonelli and Eaton, 1978, Mondon et al, 1984, Fukuda et al, 1991). Evidence from these studies supporting an increase in TG clearance post-training was not as conclusive. Mondon et al. (1984) attributed the lower rates of hepatic TG secretion in the exercising rats to lower concentrations of insulin. Hepatic TG secretion was similar in the trained and sedentary rats, when livers were perfused with similar concentrations of insulin. Simonelli and Eaton (1978) suggested that the ratio of insulin/glucagon should be investigated further as a potential mediator of the difference in TG secretion. In this study, differences in insulin were only observed between obese trained and obese sedentary rats whereas differences in glucagon (but not insulin) were observed between lean trained and lean sedentary rats. In another study, the exercise attenuation of hepatic triglyceride secretion was accompanied by an increase in the hepatic production of ketones (Fukuda et al, 1991), indicative of increased hepatic fatty acid oxidation. The authors attributed the hypotriglyceridemic effect of exercise to altered partitioning of long chain fatty acids with a decrease in esterification and increase in oxidation.

Limited indirect evidence in humans supports this view. In an ultracentrifugation study 79±10% of the reduction in lipemia following acute exercise occurred in the VLDL fraction (Gill et al., 2001b). This study also documented exercise trial increases in whole body fat oxidation, exogenous fatty acid oxidation (quantified by incorporating labelled fatty acids into the test meal) and ketogenesis. The authors attributed the exercise attenuation of

lipemia to the hepatic repartitioning of circulating fatty acids toward oxidation and ketogenesis and away from esterification and secretion.

To date, only two studies have examined the influence of acute exercise or exercise training on VLDL kinetics in humans. In a six month training study involving type II diabetics, Alam et al (2004), observed a decrease in VLDL-apo B secretion rate but not VLDL-apo B fractional catabolic rate, with the changes in apo B secretion related to the changes in serum VLDL-TG ($r = 0.64$). Fasting values for TG, FFA, insulin, glucose, HbA1c and the HOMA_{IR} also decreased with training, indicating an increase in sensitivity. The authors attributed the reduction in VLDL-apo B secretion to an increase in insulin sensitivity and the reduced availability of FFA for hepatic TG synthesis. One important difference must be noted however between acute and chronic exercise. Chronic exercise training typically results in a decrease in serum FFA (Alam et al., 2004) whereas FFA release from adipose and serum FFA have both been shown to be elevated on the morning after acute exercise (Gill et al, 2001b, Magkos et al, 2006). Given that FFA are the substrate for hepatic TG synthesis, decreases in VLDL-TG secretion may be more easily demonstrated following chronic exercise.

In contrast, Magkos et al, (2006) found VLDL-TG fractional catabolic rate to be 40% higher in young men on the morning after prolonged acute exercise, with VLDL-TG secretion rate unchanged. This increase in clearance occurred in the absence of a change in LPL protein in either the heparin releasable or heparin non-releasable muscle fractions. However, serum LPL concentrations were 20% higher following exercise. In this study, the hypotriglyceridemic influence of acute exercise was attributed to LPL upregulation, not

detectable in muscle protein due to the release of newly synthesised LPL into the circulation, but detectable from increases in serum LPL mass. Oscai et al (1992) has suggested that acute exercise can have a heparin-like effect on LPL, at least in rats, displacing it from the vascular endothelium into the circulation. In this study, exercise resulted in large increases in pre-heparin LPL activity and reductions in post-heparin LPL activity, with the majority of LPL activity still residing in pre-heparin plasma 24 h post-exercise. The post-exercise decreases in serum TG and increases in FFA mirrored the changes in pre-heparin LPL activity. Any release of functional LPL into the circulation following acute exercise, could account for the decreases in lipemia and increases in FFA that are frequently reported in humans, in the absence of changes in SM-LPL or PH-LPL activity.

Although acute exercise has been shown to reduce postprandial TG and postprandial insulin concentrations, it is unclear if these changes are related. Insulin is known to play a key role in regulating postprandial lipid metabolism, inhibiting the release of FFA from adipose tissue (hormone sensitive lipase inhibition) and inhibiting hepatic VLDL-TG secretion, (Lewis et al, 1995), decreasing LPL activity in skeletal muscle and activating LPL in adipose tissue (Lithell et al., 1978). Consequently, hypertriglyceridemia is a key component of the insulin resistant (metabolic) syndrome. It is plausible that increases in the sensitivity of these processes to postprandial insulin following exercise might result in altered rates of TG secretion and/or clearance. However, in their retrospective examination of seven studies, Gill et al (2002a) found no relationship ($r=0.04$, $p=0.70$) between the exercise-mediated changes in postprandial lipemia and postprandial insulin. It should be noted that the cohorts in these

studies differed considerably in terms of age, sex and fitness. It has been suggested that different mechanisms might explain the exercise attenuation of postprandial lipemia in young healthy populations and in older obese populations (Miesenboch and Patsch, 1992). Separate studies to elucidate these mechanisms in insulin-resistant and insulin-sensitive populations are justified.

Collectively, these studies suggest that an increase in triglyceride clearance can occur in response to acute exercise, mediated by an increase in SM-LPL activity. However such increases are most likely to be evident following prolonged, vigorous exercise bouts in well-trained individuals. It is equally clear that exercise bouts of a more moderate intensity and duration can reduce postprandial lipemia in the absence of increases in triglyceride clearance or LPL activity. Thus other mechanisms must exist. Gill and Hardman (2003) conclude that acute exercise is likely to attenuate postprandial lipemia by two mechanisms, decreased TG secretion and increased TG clearance, and that the relative importance of each is likely to depend on factors such as age, sex, degree of body fatness and training status.

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Chapter Two

INFLUENCE OF ACUTE EXERCISE ON POSTPRANDIAL LIPEMIA AND ENDOTHELIAL DYSFUNCTION IN NORMAL WEIGHT, OVERWEIGHT AND OBESE MEN

2.1 INTRODUCTION

Since Zilversmit first postulated that atherosclerosis was a postprandial phenomenon (1979), research interest in postprandial metabolism has developed in parallel with advances in our understanding of the pathophysiology of atherosclerosis. Whereas earlier hypotheses focused on the importance of postprandial lipoprotein remnants (Zilversmit, 1979) and the remodelling of lipoprotein species (Miesenbock and Patsch, 1992), current research emphasises a postprandial environment that is pro-inflammatory and pro-coagulant (Hyson et al, 2002; van Oostrom et al, 2004) in which endothelial dependent vasodilation is impaired and oxidative stress is increased (Bae et al, 2001).

The expression of adhesion molecules by endothelial cells is a critical step in atherogenesis, facilitating the adhesion and transendothelial migration of leukocytes. Concentrations of soluble adhesion molecules have been shown to correlate with membrane expression (Leeuwenberg et al, 1992, Schmidt et al., 1995), and are routinely employed in research studies as biomarkers of endothelial function. Studies generally show postprandial increases in sICAM-1 and sVCAM-1 in diabetic or hypertriglyceridemic populations (Nappo et al., 2002; Marchesi et al, 2003, Ceriello et al, 2004, Ceriello et al, 2005). Some studies have shown small postprandial increases in sICAM-1 and sVCAM-1 in healthy volunteers (Nappo et al, 2002, Ceriello et al., 2004) with others showing no change (Gill et al, 2003, Tsai et al., 2004). The influence of high fat meals on soluble adhesion molecules in obese, insulin resistant populations is unclear.

Insulin plays a key role in regulating postprandial triglyceride metabolism, activating lipoprotein lipase (LPL) in adipose tissue and inhibiting LPL in skeletal muscle (Lithell et al , 1978), inhibiting the release of FFA from adipose tissue and inhibiting hepatic apo B synthesis and VLDL-TG secretion (Lewis et al., 1995) These actions may be impaired in centrally obese insulin resistant individuals, contributing to postprandial hypertriglyceridemia (Jeppesen et al , 1995) Acute exercise is known to reduce postprandial lipemia (Gill et al., 2002a). It has been suggested that exercise may regulate triglyceride metabolism differently in young lean highly active and older obese individuals (Miesenbock and Patsch, 1992, Gill and Hardman, 2003). As the majority of studies of acute exercise and postprandial lipemia have been conducted in young, lean and active populations, comparative studies with obese and more clinical groups are justified, given the increasing prevalence of obesity worldwide. It is also unclear if the exercise attenuation of postprandial lipemia is sufficient to reduce sICAM-1 and sVCAM-1. Such a finding would contribute to an understanding of the mechanisms by which regular exercise lowers cardiovascular disease risk. Gill et al (2003) found no changes in soluble adhesion molecules following 7 d of detraining, despite a 53% increase in lipemia. However, the subjects in this study were young, lean, highly trained and presumably insulin sensitive individuals.

Although the mean attenuation of postprandial lipemia following prolonged acute exercise is ~20%, considerable interindividual variation has been shown to exist in this regard (Gill et al , 2002a). A weak correlation has been demonstrated between maximum oxygen uptake and the exercise attenuation of lipemia (Gill et al , 2002a). However, as subjects in

this study exercised for a set period (90 min) at a fixed intensity (60% VO_2max), the correlation was attributed to higher levels of energy expenditure in the high fit individuals. The magnitude of the exercise attenuation of lipemia has been shown to increase with exercise of increasing energy expenditure (Gill et al , 2002b). To our knowledge, no studies have been conducted examining the influence of an isocaloric exercise bout on postprandial lipemia

The purpose of this study was to examine the influence of a high fat mixed meal on biomarkers of endothelial function and inflammation in normal weight overweight and obese middle-aged men, and to examine the influence of a single 700 kcal exercise bout on these processes

2.2 METHODOLOGY

Subjects

Thirty men (10 normal weight, 10 overweight and 10 obese) aged 30-45 y volunteered for the study (table 2 1). Volunteers were excluded if they engaged in endurance training or competitive sports, were smokers, had cardiovascular disease or diabetes or were taking medication known to influence carbohydrate or lipid metabolism. Ethical approval was granted by the Dublin City University Ethics Committee and written informed consent was obtained (Appendix A1)

Study design

Subjects made four visits to the laboratory. During a preliminary visit, maximum oxygen uptake was determined and anthropometric measurements made, following a medical examination. Subjects subsequently underwent two oral fat tolerance tests (OFTT) in a randomised design, separated by 7 d approximately (Fig 2.1). On the evening prior, subjects either rested at home (control trial) or completed a 700 kcal bout of treadmill exercise at 70% VO_2max (exercise trial).

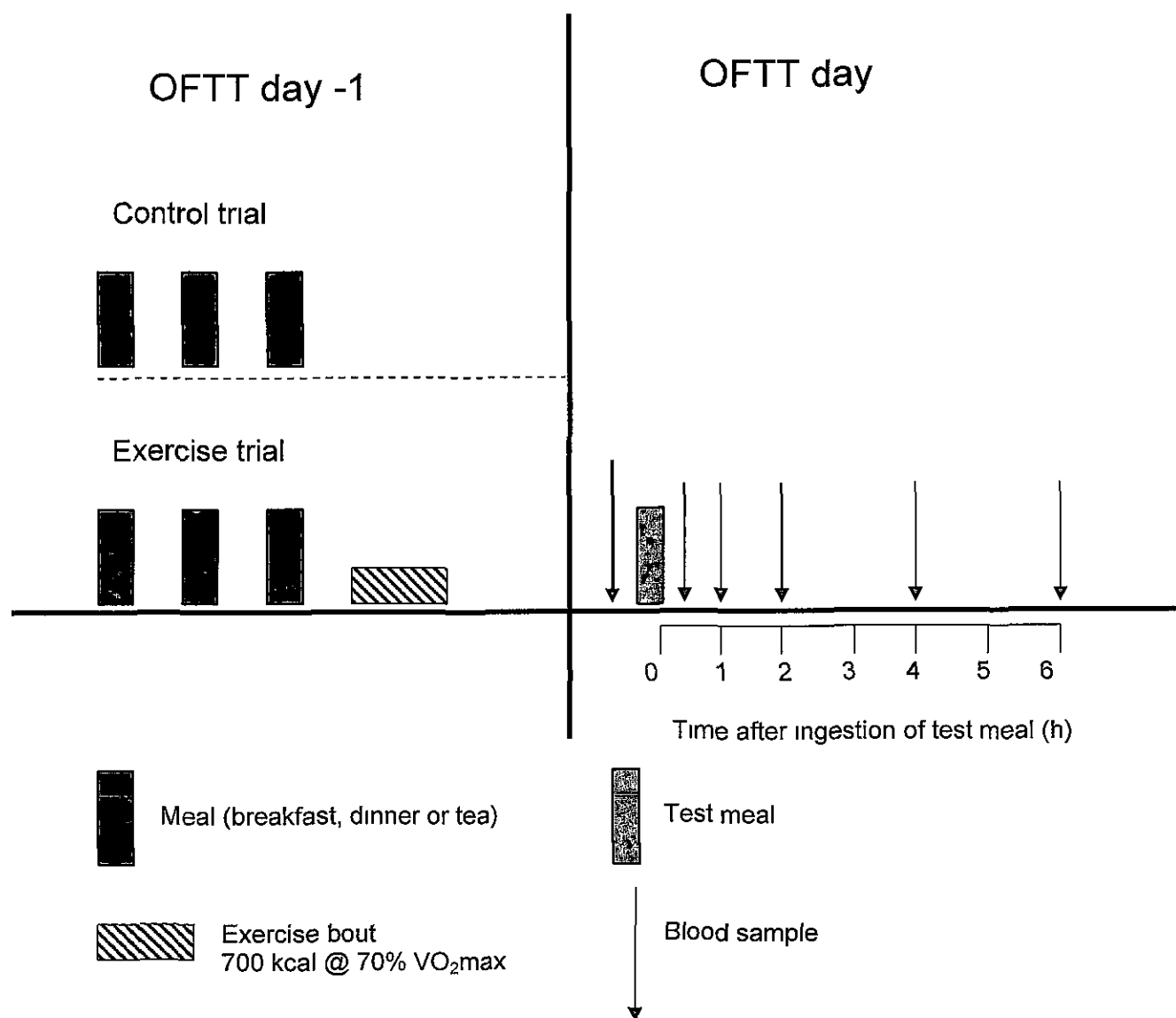
Table 2.1 Description of normal weight, overweight and obese groups

	Normal weight (n=10)	Overweight (n=10)	Obese (n=10)	Combined group (n=30)
Age (y)	38.4±1.5	36.7±1.6	37.1±1.6	37.4±0.9
Height (cm)	175.4±1.5	176.2±1.4	177.5±2.4	176.4±1.0
Weight (kg)	71.7±1.5 a	84.0±1.8 b	105.1±2.3 c	86.8±2.7
BMI (kg m^{-2})	23.3±0.4 a	27.0±0.4 b	33.4±0.7 c	27.9±0.8
Body surface area (m^2)	1.88±0.03 a	2.01±0.03 b	2.22±0.04 c	2.04±0.04
Waist circumference (cm)	82.5±1.1 a	88.7±5.4 a	113.9±2.4 b	94.6±3.3
Waist-hip ratio	0.86±0.01 a	0.92±0.01 b	1.02±0.02 c	0.93±0.01
VO_2max ($\text{ml kg}^{-1} \text{min}^{-1}$)	48.6±3.5 a	42.0±1.2 b	37.6±1.9 b	42.8±1.5
VO_2max (L min^{-1})	3.31±0.21	3.53±0.13	3.92±0.15	3.59±0.10

Values are mean ± SEM

a, ab, b Different letters denote values that are significantly different ($p < 0.05$). Values denoted as ab are not significantly different to values denoted a or b.

Fig 2.1 Schematic representation of the experimental design



Exercise testing

Maximum oxygen uptake was determined during a maximal incremental treadmill walk / run test, using a modified Astrand protocol. The initial workload and increments were individualised so that subjects would reach exhaustion between 8 and 12 min. Heart rate was monitored continuously using 12-lead electrocardiography (CASE, GE Healthcare, UK). The

experimental exercise bout was conducted between 17 00 and 20 00 h on the evening prior to the OFTT. Subjects ran/walked on a motorised treadmill at 70% $\text{VO}_{2\text{max}}$ until 700 kcal had been expended. Expired air was collected continuously during the exercise using a breath by breath metabolic system (Vmax 229 Sensormedics, USA). Total energy expenditure was estimated using the equation of Weir (1949). Carbohydrate and fat oxidation were estimated using the equation of and Peronnet and Massicotte (1991). Heart rate was continuously recorded by short range telemetry (Polar Electro Oy, Finland) and Rate of Perceived Exertion (RPE) was recorded every 10 min using the 16 pt Borg scale (Borg, 1973). Water was consumed *ad libitum* following exercise. Food intake was not permitted.

Oral fat tolerance tests

For 3 d prior to each OFTT, subjects were required to abstain from alcohol and not to engage in exercise or heavy physical work. Subjects consumed their normal diet for 3 d prior to the first OFTT. Food items consumed along with portion size were recorded on sheets provided. This diet was then replicated in advance of subsequent OFTTs. Subjects arrived at the laboratory on the morning of each OFTT following an overnight (12 - 14 h) fast. A 20 G intravenous catheter was inserted into a forearm vein. This catheter was kept patent during the 6 h postprandial follow-up period by flushing regularly with a 0.9% saline solution. The test meal consisted of croissants, butter, high fat ice-cream, chocolate and potato crisps with a macronutrient composition per 2m^2 body surface area of 97 g fat, 124 g carbohydrate and 1450 kcal. This was well tolerated by all. Water intake was *ad libitum* during the first trial.

This pattern of water intake was recorded and repeated during the second trial. Subjects rested quietly in a room adjacent to the laboratory during the 6 h observation period.

Blood sampling and analysis

Blood samples were obtained in a seated position for 5 min with legs uncrossed in order to minimise plasma volume shifts. Serum vacutainers were allowed to stand at room temperature for 30 min before centrifugation. Vacutainers were centrifuged at 3000 rpm (1600 g) for 15 min at 4°C. Serum was stored at -80°C.

Spectrophotometric and immunoturbidimetric analyses were performed using an automated bench-top clinical chemistry system (ACE[®], Alfa Wassermann B.V., Netherlands) with appropriate reagents, calibrators and controls (Randox Laboratories, UK). Serum triglycerides, total cholesterol, HDL-cholesterol, LDL-cholesterol, 3-hydroxybutyrate, free fatty acids and glucose were determined using spectrophotometric assays. Immunoturbidimetric assays were used to determine concentrations of apo A-I, apo B and apo E. Serum insulin was determined by fluoroimmunoassay using a commercially available kit (PerkinElmer, Wellesley MA, USA). Serum concentrations of sVCAM-1, sICAM-1 and IL-6 were determined in duplicate using a quantitative sandwich enzyme immunoassay technique and commercially available kits (R&D Systems, Minneapolis, USA). Samples for each subject were analysed in the same run. The intra-assay coefficients of variation were < 3% for all spectrophotometric and immunoturbidimetric analyses, and 3%, 8%, 8% and 20% for immunoassays of insulin, sVCAM-1, sICAM-1 and high sensitivity IL-6, respectively. Haematocrit values, haemoglobin concentrations and counts of leukocytes, lymphocytes, monocytes and granulocytes were

determined from a fresh EDTA whole blood sample using an automated haematology analyser (AcTdiff2, Beckman Coulter, USA)

Data analysis

Summary postprandial responses to the test meal are reported as time-averaged postprandial values using the approach of Gill et al (2006). Time-averaged postprandial values represent the total area under the concentration vs time curve (AUC), calculated using the trapezium rule, divided by the length of the postprandial period (Appendix B). Fasting and time-averaged postprandial values were compared using a two-way (trial (2) x BMI subgroup (3)) repeated measures analysis of variance, followed by the Bonferroni post-hoc procedure. When the significance of changes during the postprandial period was of interest (sICAM-1, sVCAM-1, IL-6 and leukocyte counts), a three way repeated measures ANOVA was performed (trial x BMI subgroup x time). When the data from the BMI subgroups were combined, the significance of control and exercise trial differences (n=30) were determined using paired t-tests. The relationship between selected variables was determined using a bivariate or a partial correlation. Data are reported as mean \pm SEM unless otherwise stated. Significance was set at $p < 0.05$.

2.3 RESULTS

The 700 kcal exercise bout was completed in (mean \pm SD) 58.4 \pm 8.8 min. Carbohydrate and fat oxidation during the bout were (mean \pm SD) 164.1 \pm 11.9 g and 10.2 \pm 4.9 g respectively. Mean heart rate was (mean \pm SD) 82 \pm 6.9% HRmax. As haemoglobin

(14.3 ± 0.3 vs 14.2 ± 0.2 g dl⁻¹, $p=0.68$) and haematocrit (42.3 ± 0.7 vs $41.7 \pm 0.6\%$, $p=0.31$) were similar in the control and exercise trials respectively, values were not adjusted for changes in plasma volume

The influence of experimental trial and BMI subgroup on a range of fasting and postprandial variables is shown in tables 2.2 and 2.3. Fasting and postprandial values of TG, glucose, insulin and sICAM-1, and the HOMA index of insulin resistance (HOMA_{IR}), increased with increasing BMI in both the control trial and exercise trials (significant linear trend, $p<0.05$). Fasting and postprandial values of HDL-C decreased with increasing BMI in both the control and exercise trials (significant linear trend, $p<0.05$). However, significant differences were generally only evident when the normal weight and obese groups were compared. Exercise reduced postprandial TG and increased postprandial FFA in all BMI subgroups ($p<0.05$). Exercise reduced postprandial insulin, sICAM-1 and sVCAM-1 and increased postprandial HDL-C, LDL-C, FFA, IL-6, apo A-I and apo E in single BMI subgroups ($p<0.05$). However no experimental trial x BMI subgroup interaction was evident for any fasting or postprandial variables. The remainder of this section details the combined group ($n=30$) response to the test meal and to the exercise intervention

The combined group control and exercise trial responses to the test meal are presented below (table 2.4, fig 2.2 - 2.11). Exercise reduced fasting TG ($p<0.05$) by $19.2 \pm 5.2\%$ (range, 75% decrease to 68% increase) (fig 2.2). Exercise reduced postprandial TG by $19.9 \pm 4.0\%$ (range, 55% decrease to 24% increase) (table 2.4, fig 2.2). Fasting and postprandial insulin were also lower ($p<0.05$) in the exercise trial (table 2.4, fig 2.3). Although

fasting glucose was lower in the exercise trial ($p < 0.05$), postprandial glucose values were similar between trials (table 2.4, fig 2.4). $HOMA_{IR}$ was lower ($p < 0.05$) in the exercise trial (table 2.4). Fasting and postprandial FFA were higher ($p < 0.05$) in the exercise trial (table 2.4, fig 2.5).

Combined group values of sICAM-1 and sVCAM-1 did not change during the postprandial period (fig 2.6). Values were similar in the control and exercise trials at the 0 h and 4 h timepoints (table 2.4, fig 2.6). IL-6 increased during the postprandial period in both the control and exercise trials ($p < 0.05$) (fig 2.7). Postprandial IL-6 values were higher ($p < 0.05$) in the exercise trial (table 2.4). Control trial counts of leukocytes ($p < 0.05$), lymphocytes ($p < 0.05$) and granulocytes ($p < 0.05$) increased postprandially and counts of monocytes decreased ($p < 0.05$) postprandially (fig 2.8). Leukocytes, granulocytes and monocytes were higher at 0 h in the exercise trial ($p < 0.05$) but not at 6 h postprandially. There was a weak positive relation between BMI and sICAM-1 ($r = 0.38$, $p < 0.05$) and between IL-6 and sICAM-1 ($r = 0.34$, $p = 0.07$) but sVCAM-1 did not correlate with any body composition, lipid or inflammatory marker.

Total cholesterol did not change postprandially. Cholesterol values were similar in the control and exercise trials (table 2.4). HDL-C and LDL-C decreased postprandially ($p < 0.05$) from 0 h to 6 h in both the control and exercise trials (fig 2.9). There were no postprandial changes in apo A-I or apo B. Consequently, the ratio of HDL-C/apo A-I decreased postprandially in the control trial ($p < 0.05$) and the ratio of LDL-C/apo B decreased postprandially in both the control and exercise trials ($p < 0.05$) (fig 2.10). HDL-C and LDL-C

were higher in the exercise trial at the 0 h and 6 h timepoints ($p < 0.05$) Apo A-I was higher at 0 h in the exercise trial ($p < 0.05$) but not at 6 h. There was no difference in apo B between trials at any timepoint. Apo E decreased ($p < 0.05$) from 0 to 6 h postprandially (fig 2.11). Apo E values were higher in the exercise trial at the 0 h and 6 h timepoints ($p < 0.05$) (fig 2.11).

There were strong correlations between fasting TG and insulin ($r = 0.80$, $p < 0.05$) and between postprandial TG and insulin ($r = 0.67$, $p < 0.05$). TG and insulin were also related to BMI, waist circumference and waist-hip ratio with the strongest relationships existing with waist-hip ratio (table 2.5). Postprandial concentrations of TG ($r = -0.45$, $p < 0.05$) and insulin ($r = -0.53$, $p < 0.05$) were related to $VO_{2\max}$ ($\text{ml kg}^{-1} \text{min}^{-1}$). However, these relationships were no longer evident after controlling for BMI (TG, $r = -0.18$, $p = 0.36$, insulin $r = -0.30$, $p = 0.10$). The percentage change in postprandial TG was related to $VO_{2\max}$ ($r = -0.55$, $p < 0.05$) and this relationship was still significant after controlling for BMI ($r = -0.51$, $p < 0.05$). There was no relation between the decrease in postprandial TG and the decrease in postprandial insulin as a result of acute exercise ($r = -0.16$, $p = 0.42$). Postprandial TG, postprandial insulin and waist-hip ratio were related to fasting HDL-C, fasting apo A-I and fasting apo E but not to fasting cholesterol, fasting LDL-C or fasting apo B (table 2.6).

Table 2.2 Influence of acute exercise on a range of fasting variables in normal weight, overweight and obese men

	Normal weight (n=10)		Overweight (n=10)		Obese (n=10)	
	Control	Exercise	Control	Exercise	Control	Exercise
TG (mmol L ⁻¹)	1.15±0.11	0.88±0.10	1.77±0.19	1.42±0.21	3.17±0.79 a	1.98±0.31* a
sICAM-1 (ng mL ⁻¹)	191.4±5.9	182.1±5.8	214.1±7.5	213.7±10.4	221.7±14.0	224.7±11.3 a
sVCAM-1(ng mL ⁻¹)	604.0±549.1	605.5±58.5	547.5±39.1	517.8±41.6	558.4±34.2	566.0±45.0
IL-6 (pg.mL ⁻¹)	0.70±0.12	0.98±0.28	1.38±0.48	1.77±0.69	1.10±0.15	1.62±0.37*
Insulin (μIU mL ⁻¹)	3.8±0.7	2.9±0.5	7.0±0.8	5.6±1.4	11.7±2.8 a	10.1±1.9 a
Glucose (mmol L ⁻¹)	4.62±0.07	4.20±0.08*	4.89±0.10	4.60±0.11*	5.17±0.29	4.73±0.17* a
HOMA _{IR}	0.79±0.15	0.55±0.10	1.52±0.17	1.08±0.31	2.94±0.83 a	2.21±0.46* a
FFA (mmol L ⁻¹)	0.60±0.05	1.13±0.13*	0.77±0.11	1.02±0.10*	0.68±0.03*	0.89±0.06
Total cholesterol (mmol L ⁻¹)	5.46±0.36	5.55±0.30	5.52±0.35	5.79±0.35	5.46±0.20	5.59±0.27
HDL-C (mmol L ⁻¹)	1.24±0.07	1.27±0.07	1.06±0.04	1.14±0.06	0.96±0.08 a	1.05±0.07
Apo A-I (mg dL ⁻¹)	123.2±5.3	125.4±4.2	113.9±4.3	117.9±4.8	112.7±4.1	120.9±4.6*
LDL-C (mmol L ⁻¹)	3.26±0.35	3.41±0.34	3.28±0.37	3.63±0.31*	3.43±0.22	3.71±0.26
Apo B (mg dL ⁻¹)	79.8±8.1	77.3±6.6	80.1±7.6	85.9±5.4	78.5±6.0	89.3±5.0*
Apo E (mg dL ⁻¹)	3.66±0.34	4.39±0.41*	4.96±0.46	5.30±0.57	4.59±0.51	5.01±0.48

**p*<0.05 compared to control trial a *p*<0.05 compared to normal weight group (same trial) Bonferroni adjustment for all multiple comparisons
Values are mean ± SEM

Table 2.3 Influence of acute exercise on a range of time-averaged postprandial variables in normal weight, overweight and obese men

	Normal weight (n=10)		Overweight (n=10)		Obese (n=10)	
	Control	Exercise	Control	Exercise	Control	Exercise
TG (mmol L ⁻¹)	2.10±0.26	1.54±0.24*	3.18±0.39	2.61±0.46*	4.00±0.45 a	3.32±0.47* a
sICAM-1 (ng mL ⁻¹)	188.5±6.6	175.8±9.7*	216.4±6.4	220.2±8.4 a	219.2±12.1 a	223.3±12.4 a
sVCAM-1 (ng mL ⁻¹)	585.5±54.4	590.5±58.0	556.2±44.9	522.2±39.9*	556.4±48.4	556.3±43.7
IL-6 (pg mL ⁻¹)	1.34±0.24	1.63±0.40	2.11±0.46	2.55±0.74	1.54±0.13	2.29±0.40
Insulin (μIU mL ⁻¹)	19.0±4.4	17.5±4.0	29.2±3.8	24.3±3.3	65.1±21.3 a	50.4±13.7* a
Glucose (mmol L ⁻¹)	4.65±0.13	4.81±0.15	5.35±0.17 a	5.18±0.17	5.68±0.29 a	5.46±0.30
FFA (mmol L ⁻¹)	0.85±0.05	1.05±0.13*	0.85±0.11	0.98±0.10*	0.56±0.03	0.79±0.06*
HDL-C (mmol L ⁻¹)	1.21±0.07	1.26±0.07	1.05±0.03	1.11±0.05	0.93±0.09 a	1.01±0.08* a
Apo A-I (mg dL ⁻¹)	123.3±5.6	123.9±5.4	115.2±3.8	116.2±4.0	114.4±5.4	119.8±5.5*
LDL-C (mmol L ⁻¹)	3.19±0.37	3.31±0.37	3.26±0.34	3.48±0.29	3.26±0.30	3.61±0.31*
Apo B (mg dL ⁻¹)	79.7±8.2	77.4±6.6	80.1±7.6	86.0±5.4	78.5±6.0	89.2±5.0
Apo E (mg dL ⁻¹)	3.11±0.34	3.65±0.43*	3.69±0.27	4.03±0.42	3.80±0.48	4.09±0.58

*p<0.05 compared to control trial. a p<0.05 compared to normal weight group (same trial) Bonferroni adjustment for all multiple comparisons.

Values are mean ± SEM

Time-averaged postprandial values represent the total area under the concentration vs time curve, divided by the length of the postprandial period. Units are mmol L⁻¹

Table 2.4 Influence of acute exercise on a range of time-averaged postprandial variables in the combined group (n=30)

	CON trial	EX trial
Postprandial TG (mmol L ⁻¹)	3.09±0.28	2.49±0.29*
Postprandial sICAM-1 (ng mL ⁻¹)	208.7±5.2	207.5±6.6
Postprandial sVCAM-1 (ng mL ⁻¹)	565.4±25.3	555.2±24.6
Postprandial IL-6 (pg mL ⁻¹)	1.67±0.19	2.18±0.31*
Postprandial insulin (mmol L ⁻¹)	37.8±7.4	30.7±5.0*
Postprandial glucose (mmol L ⁻¹)	5.23±0.13	5.15±0.12
HOMA _{IR}	1.75±0.32	1.28±0.22*
Postprandial FFA (mmol L ⁻¹)	0.76±0.06	0.94±0.06*
Postprandial total cholesterol (mmol.L ⁻¹)	5.52±0.18	5.64±0.16
Postprandial HDL-C (mmol L ⁻¹)	1.06±0.04	1.13±0.04*
Postprandial LDL-C (mmol L ⁻¹)	3.23±0.18	3.46±0.17*

**p*<0.05 compared to CON trial

Values are mean ± SEM

Time-averaged postprandial values represent the total area under the concentration vs time curve, divided by the length of the postprandial period. Units are mmol L⁻¹

Table 2.5 Correlation coefficients for the association of postprandial TG, postprandial insulin with indices of adiposity and aerobic fitness

	BMI	Waist circumference	Waist-hip ratio	VO ₂ max (ml kg ⁻¹ min ⁻¹)	VO ₂ max (L min ⁻¹)
Postprandial TG	0.57*	0.57*	0.77*	-0.45*	-0.14
Postprandial insulin	0.53*	0.56*	0.72*	-0.53*	-0.16

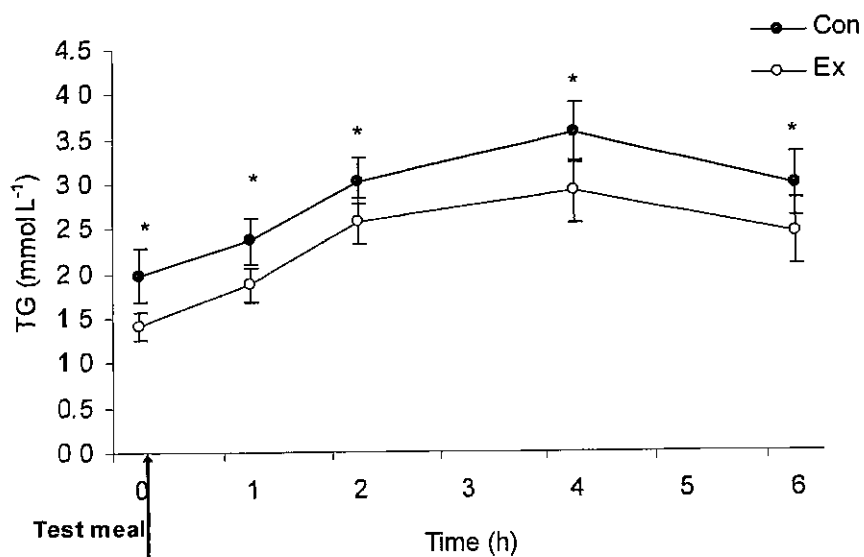
* p<0.05

Table 2.6 Correlation coefficients for the association of postprandial TG, postprandial insulin and waist-hip ratio with various fasting values of various lipids and apoproteins

	Total cholesterol	LDL-C	HDL-C	Apo A-I	Apo B	Apo E
Postprandial TG	0.23	0.33	-0.59*	-0.38*	0.11	0.63*
Postprandial insulin	0.09	0.19	-0.49*	-0.29	0.11	0.38*
Waist-hip ratio	0.25	0.34	-0.63*	-0.39*	0.13	0.54*

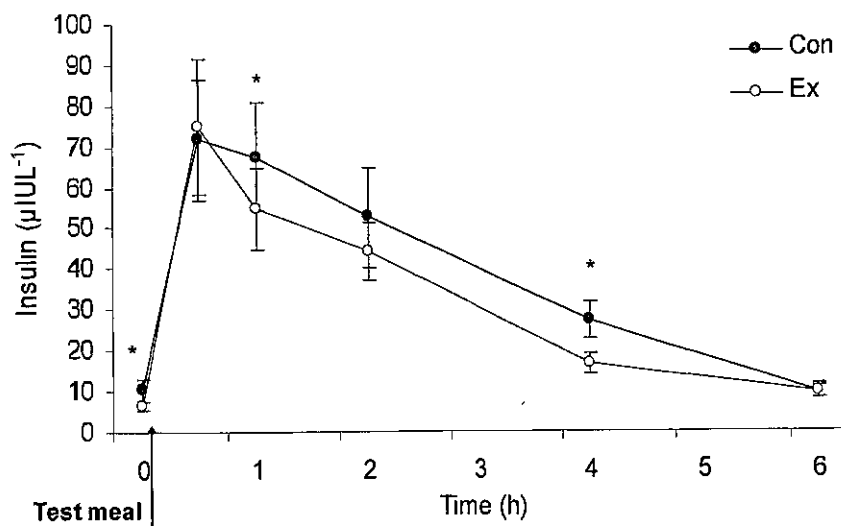
* p<0.05

Fig 2.2 Influence of acute exercise on the triglyceride response to a high fat mixed meal (n=30)



**p<0.05 compared to same timepoint in exercise trial*

Fig 2.3 Influence of acute exercise on the insulin response to a high fat mixed meal (n=30)



**p<0.05 compared to same timepoint in exercise trial*

Fig 2.4 Influence of acute exercise on the glucose response to a high fat mixed meal (n=30)

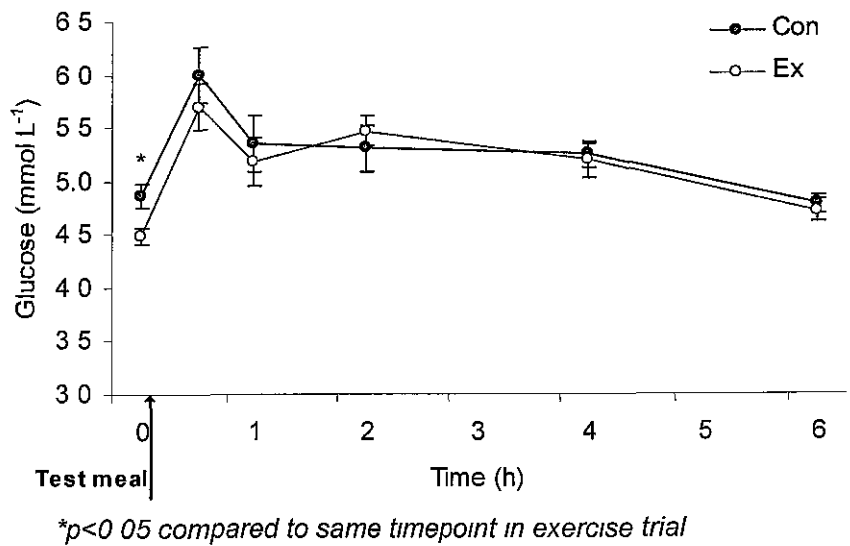


Fig 2.5 Influence of acute exercise on the free fatty acid response to a high fat mixed meal (n=30)

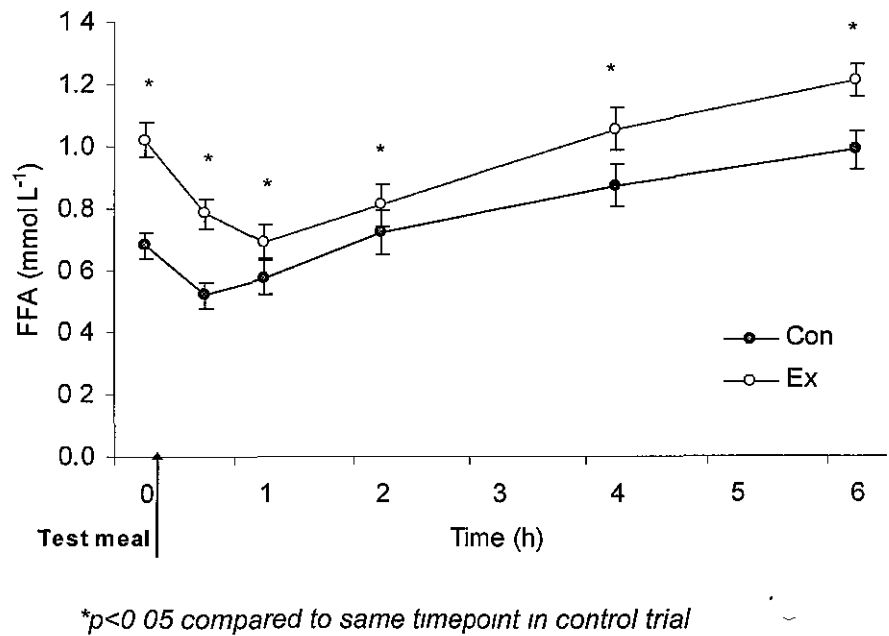


Fig 2.6 **Influence of acute exercise on the (A) sICAM-1 and (B) sVCAM-1 responses to a high fat mixed meal (n=30)**

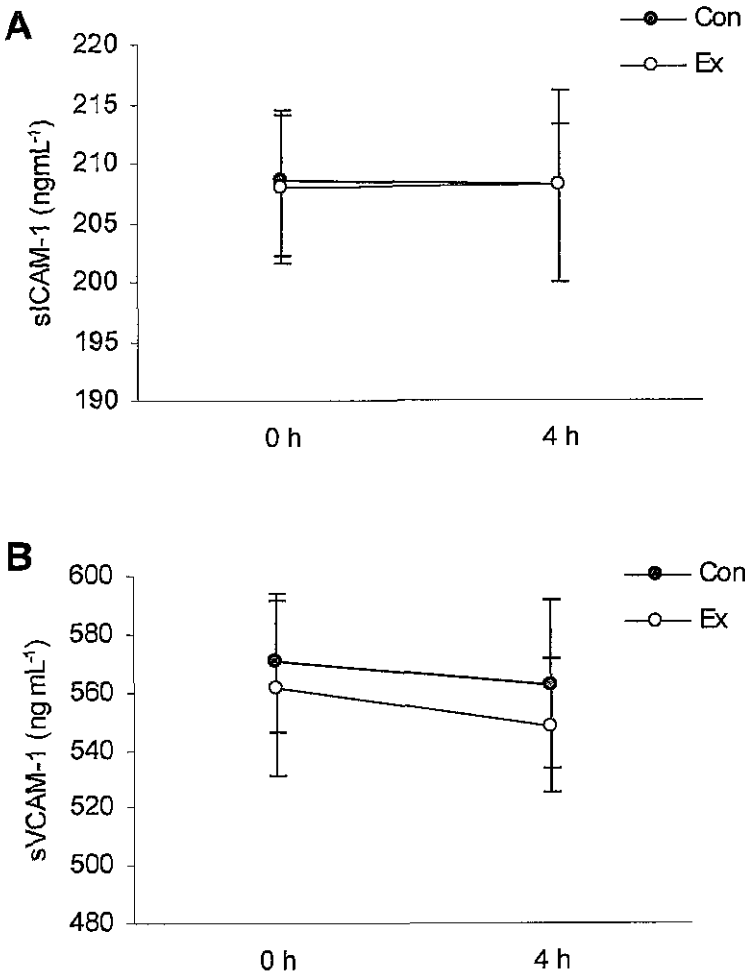
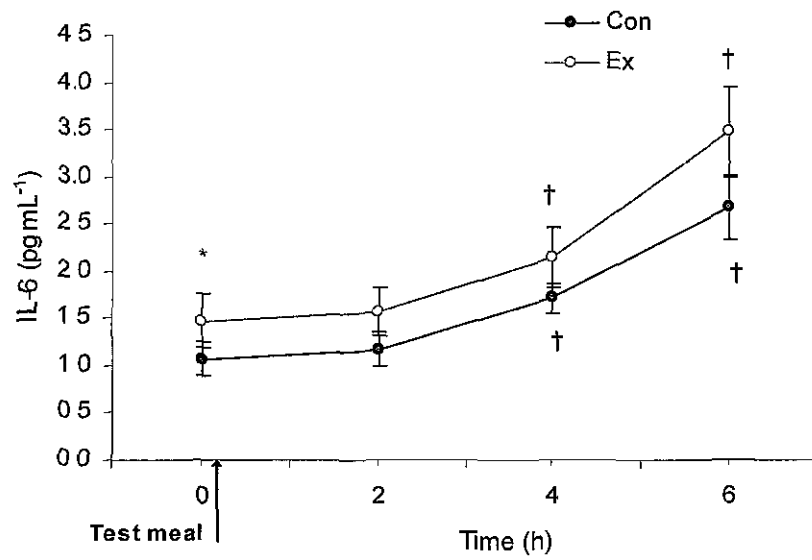


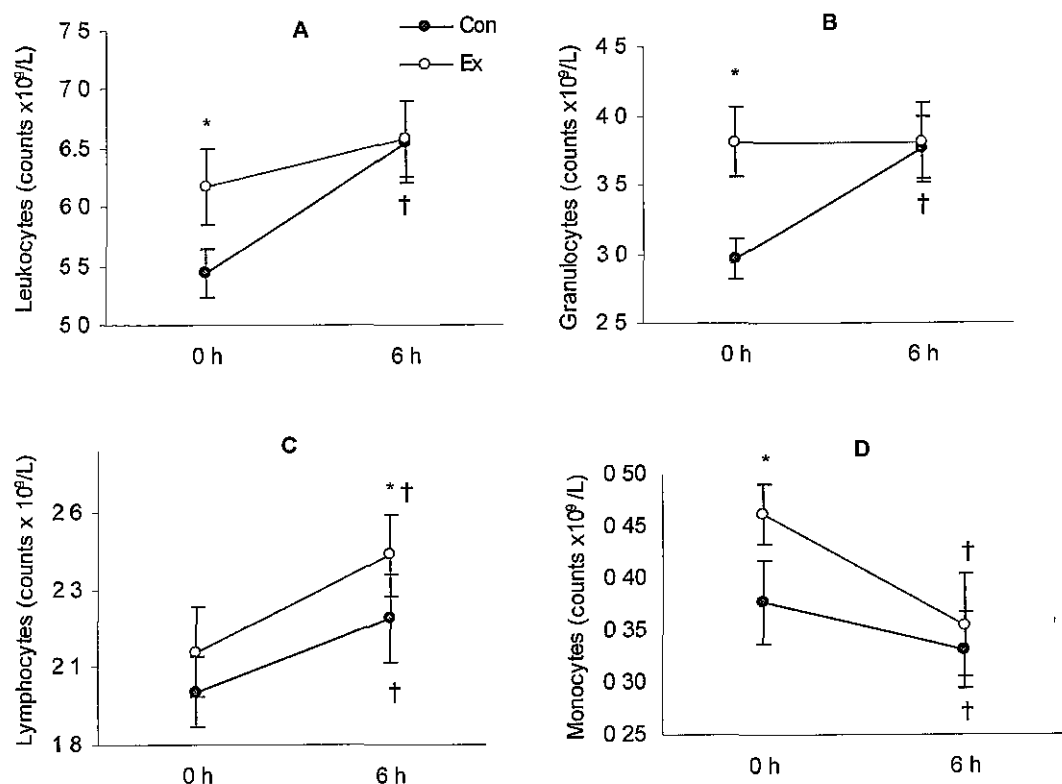
Fig 2.7 Influence of acute exercise on the IL-6 response to a high fat mixed meal (n=30)



* $p < 0.05$ compared to same timepoint in control trial

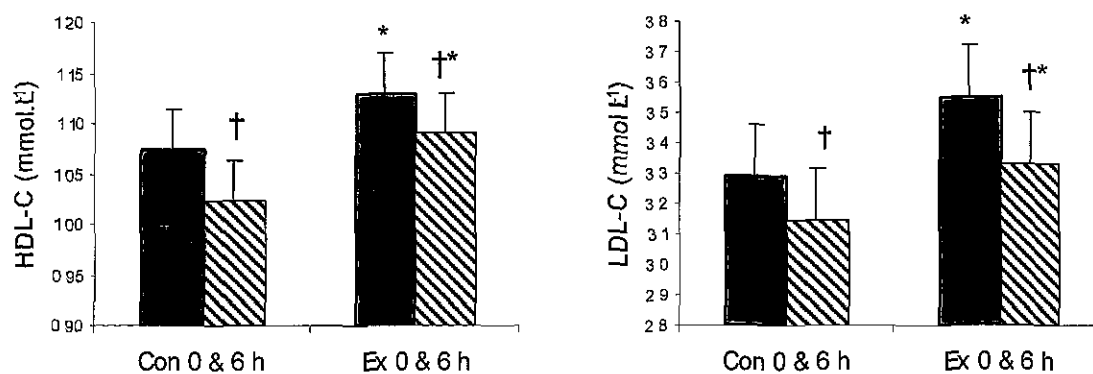
† $p < 0.05$ compared to 0 h timepoint in same trial

Fig 2.8 Counts of total (A) leukocytes, (B) granulocytes, (C) lymphocytes and (D) monocytes at the 0 h and 6 h timepoints in the control and exercise trials (n=30)



* $p < 0.05$ compared to same timepoint in control trial
† $p < 0.05$ compared to 0 h timepoint in same trial

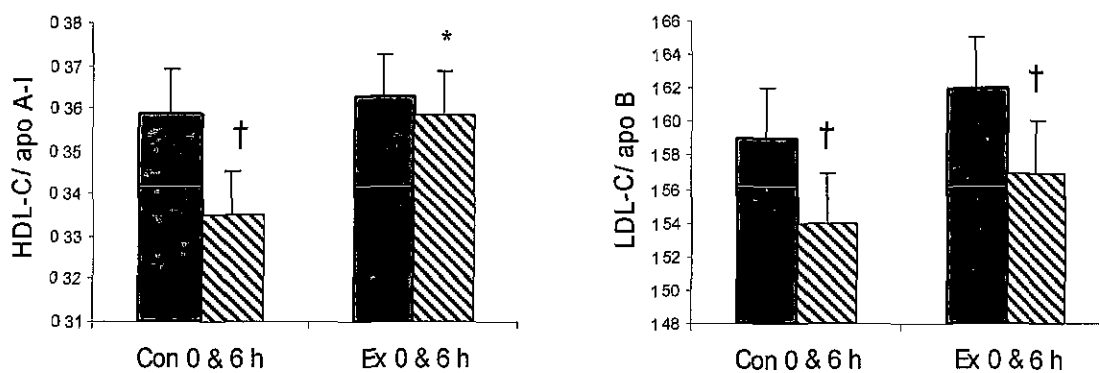
Fig 2.9 Concentrations of HDL-C and LDL-C in the control and exercise trials at the 0 h and 6 h timepoints (n=30)



^{*} $p < 0.05$ compared to same timepoint in control trial

[†] $p < 0.05$ compared to 0 h timepoint in same trial

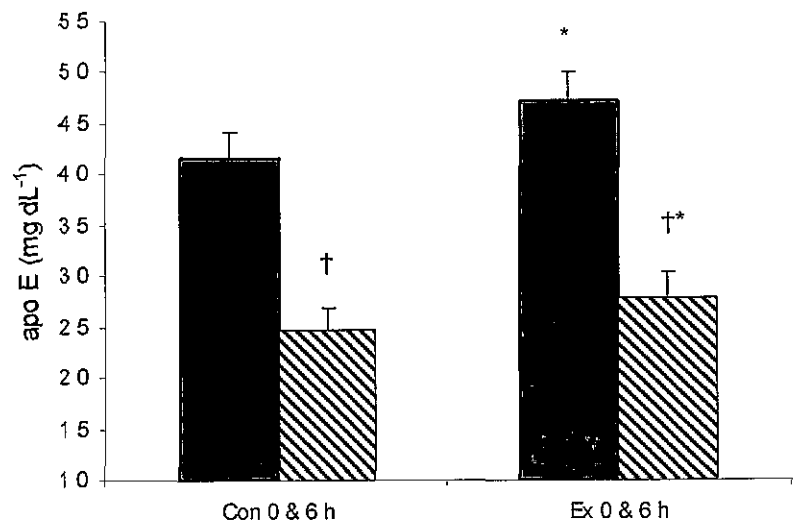
Fig 2.10 Ratios of HDL-C/ apo A-I and LDL-C/ apo B in the control and exercise trials at the 0 h and 6 h timepoints (n=30)



^{*} $p < 0.05$ compared to same timepoint in control trial

[†] $p < 0.05$ compared to 0 h timepoint in same trial

Fig 2.11 Concentrations of apo E in the control and exercise trials at the 0 h and 6 h timepoints (n=30)



* $p < 0.05$ compared to same timepoint in control trial
† $p < 0.05$ compared to 0hr timepoint in same trial

2.4 DISCUSSION

The influence of exercise on indicators of metabolic and vascular health is frequently studied in the postprandial period, when the endothelium is being challenged by the lipolysis products of triglyceride-rich lipoproteins. In this study, the fat and energy content of the test meal were towards the upper end of that typically used in postprandial research, resulting in pronounced lipemia. The results confirm that acute exercise can reduce postprandial lipemia and insulinemia and increase HDL-C. However, there is no evidence that these metabolic changes are accompanied by a reduction in endothelial activation in either the fasted or postprandial states.

A key objective of this study was to compare the influence of acute exercise on a range of metabolic variables and markers of endothelial activation, in normal weight, overweight and obese men. Although control trial differences were evident in postprandial TG, insulin, glucose, HDL-C and sICAM-1 between the normal weight and obese men, any exercise effect on these variables was similar across BMI subgroups. Similar findings were reported by Gill et al (2004), when comparing lean and centrally obese middle-aged men. Although the magnitude of the exercise-induced changes in postprandial lipemia differed considerably between individuals, this effect appears not to be related to BMI. Thus, the benefits of acute exercise to postprandial metabolism frequently observed in lean individuals, also accrue to overweight and obese men, at least when bouts of equivalent energy expenditure are undertaken. As no experimental trial x BMI subgroup interaction was evident in the analyses, the remainder of this discussion will focus on the combined group data.

To our knowledge, this is the first study that has demonstrated a correlation between the change in postprandial lipemia from isocaloric exercise bouts and VO_2max . This suggests that the benefits of acute exercise to triglyceride metabolism are greater in high fit individuals. In contrast, no relationship existed between control trial values of postprandial lipemia (unexercised state) and VO_2max , after controlling for BMI. This latter finding is in line with a cross-sectional study showing postprandial lipemia to be similar in trained and untrained individuals, when compared 60 h after the last training bout (Herd et al., 2000). Collectively, these results suggest that factors associated with aerobic fitness such as muscle oxidative capacity and capillary density do not influence postprandial lipemia, but may potentiate the effects of acute exercise on lipemia. Similar conclusions were reached by Tsetsonis et al (1997) who found postprandial lipemia to be similar in trained and untrained women in the absence of acute exercise, but lower in the trained women following acute exercise. Future studies should investigate the influence of a single isocaloric exercise bout on postprandial lipemia, before and after endurance training. The mechanisms underpinning this apparent interaction of acute and chronic exercise are open to speculation. FFA concentrations have been shown in this study and by others (Gill et al, 2001, Gill et al, 2004) to be higher following acute exercise. The high oxidative capacity that is characteristic of trained muscle may facilitate increased oxidation of these fatty acids, reducing the fraction available for re-esterification in the liver. Alternatively, the high capillary density that is characteristic of trained muscle could provide additional binding sites for lipoprotein lipase after upregulation by acute exercise.

Endothelial dependent dilation (EDD) has been shown to be impaired postprandially (Plotnick et al , 1997, Vogel et al , 1997) In this study, sICAM-1 and sVCAM-1 were employed as markers of endothelial activation There was no postprandial change in sICAM-1 or sVCAM-1, despite considerable increases in serum TG and IL-6 Postprandial triglyceride-rich lipoproteins (Moers et al , 1997) and IL-6 (Watson et al , 1996) have been shown to induce adhesion molecule expression in vitro Previous studies have consistently shown postprandial increases in adhesion molecules in diabetic cohorts (Nappo et al , 2002, Ceriello et al., 2004, Ceriello et al., 2005), with only very small (Nappo et al., 2002, Ceriello et al., 2004) or no increases (Gill et al., 2003, Tsai et al , 2004) in healthy populations Endothelial dysfunction and endothelial cell activation have been postulated to occur during the postprandial period via increased oxidative stress Nutrient overload has been proposed as a major source of endothelial cell oxidative stress (Brownlee, 2005, Ceriello and Motz, 2004). It is possible that anti-oxidant defences are capable of limiting endothelial cell activation postprandially in an obese mildly hypertriglyceridemic population but not in a diabetic population, for whom hyperglycemia may be an additional source of nutrient overload

Alternatively, soluble adhesion molecules may not be a sufficiently sensitive biomarker to detect postprandial changes in endothelial cell activation in healthy individuals EDD and soluble adhesion molecules have been shown to change in parallel (inverse relationship) during pharmacological treatment (Alonso et al , 2001, Marchesi et al., 2003, Nawawi et al , 2003, Ceriello et al., 2005) and dietary supplementation (Lupattelli et al , 2004),

evidence of a common physiology. However, case-control studies generally show only a moderate relationship (Koga et al., 2005, Lupattelli et al., 2000, Brevetti et al., 2001, Holmlund et al., 2002, Nawawi et al., 2003, Glowinska-Olszewska et al., 2005) between EDD and soluble adhesion molecules or no relationship (Van Haelst et al., 2003, John et al., 2000). True soluble forms of adhesion molecules are released from the extracellular matrix by proteolytic enzymes including metalloproteases and sheddases. However, much of the adhesion molecules detected by the ELISA methodology are in fact microparticle bound (Horstman et al., 2004). The inability of current methodologies to distinguish these soluble forms may limit their ability to act as sensitive biomarkers of endothelial dysfunction.

Unexpectedly, IL-6, leukocytes and leukocyte subfractions were elevated in the exercise trial. In a similar study design involving lean and obese men, Gill et al. (2003) found the postprandial response of these inflammatory markers to be unchanged following acute exercise. However, the experimental exercise bout employed by Gill et al. involved moderate intensity treadmill walking. It is possible that muscle damage occurred in the present study in individuals unaccustomed to strenuous weight bearing exercise, resulting in an inflammatory response. However, no physiological or self-report index of muscle soreness was utilized in either study.

Soluble adhesion molecules were also unaffected by acute exercise conducted 12-14 hours previously. Although exercise training has been shown to reduce adhesion molecules (sICAM-1) in older clinical patient groups (Zoppini et al., 2006; Adamopoulos et al., 2001), others have failed to find changes with exercise training when studying non clinical

populations (Hammett et al , 2006, Ostergard et al , 2006) In some instances, soluble adhesion molecules have been shown to be elevated when assessed one day after a bout of prolonged strenuous weight bearing exercise, with these increases once again attributed to *muscle damage and immune activation* (Akimoto et al , 2002) It is possible that any anti-inflammatory effects of acute exercise mediated by shear stress or changes in blood lipids could be negated by the pro-inflammatory effects of muscle damage and immune activation Although the acute exercise model is useful when studying changes in lipids and insulin action, attempts to elucidate the influence of vigorous exercise on endothelial activation may require short-term training or detraining studies

Postprandial triglycerides and insulin were closely related to each other and to all *indices of adiposity*. We found postprandial triglycerides and insulin to correlate more strongly with waist-hip ratio than with BMI or waist circumference Although waist circumference has become the preferred measure for abdominal obesity and is endorsed by the WHO (1998), a number of large studies suggest that waist-hip ratio has the strongest correlations with CVD risk factors (Dalton et al , 2003) and is superior in predicting CVD mortality (Welborn et al , 2003)

The decreases in fasting insulin, postprandial insulin and HOMA_{IR} following acute exercise, suggest an increase in insulin sensitivity As exercise resulted in parallel decreases in postprandial triglycerides and insulin, and these parameters were closely related in the absence of exercise, it could be postulated that the exercise attenuation of each were mechanistically linked In theory insulin could more efficiently suppress postprandial VLDL-

TG secretion and activate adipose tissue LPL in the insulin sensitive, exercised state. However, in this study and in one other (Gill et al , 2002a), no relationship existed between the exercise attenuation of postprandial triglycerides and insulin. It appears that the exercise attenuation of postprandial lipemia does not depend on changes in insulin sensitivity, at least in healthy men. It is also possible however that the insulin response to a high fat mixed meal is not a sufficiently sensitive measure of insulin sensitivity to detect such a relationship.

In this study, the postprandial increase in serum TG was accompanied by small reductions in HDL-C and LDL-C, but not in apo A-I or apo B. Consequently, there was a small postprandial decrease in the ratios of HDL-C /apo A-I and LDL-C/ apo B. These ratios have been described as crude indicators of HDL and LDL particle size (Leroux et al , 2000). The Quebec Cardiovascular Study observed decreases in these ratios across increasing TG quintiles (Leroux et al., 2000). The authors suggested that relatively modest increases in TG may rapidly alter the cholesterol content of HDL and LDL particles. There is evidence that a remodelling of lipoprotein species can occur in lipid-rich postprandial environments with neutral lipid exchange of cholesterol ester and TG between the TG-rich lipoproteins and HDL and LDL, mediated by cholesterol ester transfer protein (Miesenbock and Patsch, 1992).

Apo E has a key role in the metabolism of triglyceride-rich lipoproteins, mediating the clearance of these lipoproteins and their remnants from circulation. Serum apo E has been shown to be related directly to fasting and postprandial TG in this study and by others (Huang et al , 1998, Salah et al , 1997). Surprisingly, changes in apo E during the postprandial period and following acute exercise were inversely related to changes in TG. Apo E decreased

considerably from 0 - 6 h postprandially with values higher in the exercise trial. Previous studies have generally shown apo E to be unchanged postprandially (Blum, 1982, Mero et al , 1998a, Mero et al , 1998b, Krimbou et al , 2003), although a redistribution of apo E between lipoprotein species has been documented (Blum, 1982).

In the present study, FFA, IL-6 and leukocytes all increased postprandially (after initial suppression of FFA during acute insulinemia) and all were higher in the exercise trial. The parallel nature of these changes warrants further attention. FFA released lipolytically from postprandial serum have been shown to activate lymphocytes and endothelial cells in vitro (Chung et al , 1998) and consequently these cells must be considered as a potential source of IL-6 during the postprandial period. However, an alternative explanation of these FFA – IL-6 similarities is also possible. Considerable research interest exists in the hormone-like actions of IL-6 and its influence on substrate metabolism (Pedersen et al , 2003). Recombinant IL-6 infusions have been shown to increase both lipolysis and fat oxidation in humans without causing hypertriglyceridemia (Van Hall et al , 2003). Thus, increases in FFA (either postprandially or following prolonged acute exercise) could in theory act as a pro-inflammatory stimulus, triggering the release of IL-6 or conversely, increases in IL-6 could stimulate lipolysis. These parallel increases are likely to be interpreted differently, depending on one's prevailing view of IL-6 as either a marker of inflammation or a mediator of lipolysis. It should be noted that van Oostrom et al (2003) dismissed the postprandial changes in IL-6 and lymphocytes as a diurnal variation. In their study, similar increases were documented following ingestion of various test meals and water.

In summary, we have shown that acute exercise attenuates postprandial lipemia similarly in normal weight, overweight and obese men. The magnitude of this attenuation was related directly to VO_{2max} but not to the change in postprandial insulin. Despite pronounced increases in serum TG and IL-6, there was no evidence of changes in endothelial function in the postprandial period or following acute exercise, even in the obese men. None of the men in the obese category were morbidly obese with only half meeting the criteria for the metabolic syndrome. This may be considered a limitation in a study designed to compare the effects of acute exercise in lean and obese insulin resistant men. The absence of a direct measure of endothelial dependent dilation may be considered another limitation of this study. The findings need to be considered in the light of similar studies that employ other methods to assess endothelial function. However, it is possible that postprandial lipemia does not impair endothelial function and acute exercise does not enhance endothelial function in men under 45 years that are free of metabolic and vascular disease.

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Chapter Three

INFLUENCE OF ACUTE EXERCISE ON POSTPRANDIAL LIPEMIA AND CELLULAR MICROPARTICLES

3.1 INTRODUCTION

Endothelial cells play a crucial role in ensuring vascular integrity, maintaining a delicate balance between a wide variety of antagonistic substances that regulate leukocyte adhesion and transendothelial migration, thrombus formation and vessel vasomotion. A disturbance in this balance, towards a pro-inflammatory, pro-coagulant, anti-fibrinolytic state is termed endothelial dysfunction, and is generally considered an early event in atherosclerosis (de Koning and Rabelink, 2002). Endothelial function has consistently been shown to be impaired following high fat meals in healthy (Plotnick et al, 1997) and clinical (Bae et al, 2001b) populations. The lipolysis products of postprandial triglyceride-rich lipoproteins have been shown to activate endothelial cells in vitro (Chung et al, 1998, Kurtel et al., 1995).

Soluble forms of cell adhesion molecules including intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are regularly employed in research settings as biomarkers of endothelial function. Studies generally show postprandial increases in sICAM-1 and sVCAM-1 in diabetic and hypertriglyceridemic populations (Nappo et al., 2002, Marchesi et al., 2003; Ceriello et al, 2004; Ceriello et al, 2005). Some studies have shown small postprandial increases in sICAM-1 and sVCAM-1 in healthy volunteers (Nappo et al, 2002, Ceriello et al., 2004) with others showing no change (Gill et al., 2003, Tsai et al, 2004). However, the ELISA methods commonly used to quantify these biomarkers do not distinguish true soluble adhesion molecules from membrane-bound forms in the circulation (Horstman et al, 2004).

Microparticles are membranous vesicles shed from formed elements such as circulating platelets, leukocytes, and vascular endothelial cells, in response to cell activation and apoptosis. They serve not only as markers but also as potential mediators of inflammation, coagulation and endothelial dysfunction, key features of atherosclerosis (Van Wijk et al., 2003). Endothelial microparticles (EMP) are elevated in a number of vascular disease states including angina (Mallat et al., 1999), acute myocardial infarction (Mallat et al., 1999), hypertension (Preston et al., 2003) and pre-eclampsia (Gonzalez-Quintero et al., 2003). Obese women with impaired endothelial dependent dilation (EDD) also have elevated EMP (Esposito et al., 2006). EMP have been proposed as a sensitive biomarker of disease activity (Ahn, 2005). They are more closely related to coronary endothelial function than soluble adhesion molecules (Koga et al., 2005). Ferreira et al. (2004) reported a postprandial increase in EMP in young healthy volunteers. However, Tushuizen et al. (2006) were unable to detect EMP in fasting or postprandial plasma.

Nitric oxide (NO) plays a key role in maintaining vascular function. In addition to mediating endothelial dependent dilation (EDD), NO appears to have anti-inflammatory properties and has been shown to reduce the expression of adhesion molecules on the vascular endothelium (Spiecker et al., 1998), inhibit leukocyte adhesion (Kubes et al., 1991) and inhibit platelet aggregation at the vessel wall (Radomski et al., 1987). Reactive oxygen species are generated postprandially and thought to scavenge NO. Bae et al. (2001a) have shown the postprandial increases in serum TG to be positively correlated with increases in oxidative stress. The postprandial changes in TG and oxidative stress were each negatively

correlated with the changes in EDD. Postprandial increases in serum TG have also been shown to be correlated with increases in EMP (Ferreira et al, 2004). As acute exercise has consistently been shown to reduce postprandial lipemia (Gill et al, 2004), the possibility exists that single bouts of exercise may also attenuate EMP.

Platelets are critical to the pathogenesis and progression of cardiovascular disease and in the events leading to acute coronary syndromes. In atheromas, activated platelets release growth factors that contribute to the proliferation of smooth muscle cells and lesion progression. Activated platelets can accumulate on vessel walls with the release products promoting vasoconstriction and inflammation in endothelial cells. Although a variety of methodologies have been employed to examine postprandial hemostasis, studies generally show increased activation of platelets and coagulation factors following high fat meals (Hyson et al., 2002, Larsen et al., 2000). Platelet reactivity is increased during and immediately after strenuous exercise (Ikarugi et al, 2003, Wang et al, 2006), potentially increasing the risk of acute coronary events. Platelet microparticles (PMP) may be viewed as a marker of platelet activation. Their shedding from parent platelets increases the anionic phospholipid surface in the circulation for the assembly of prothrombinase and tenase complexes (Horstman and Ahn, 1999). PMP also stimulate other cells involved in vascular homeostasis including endothelial cells and leukocytes (Barry et al, 1998).

The primary purpose of this study was to examine the influence of a high fat meal and acute exercise on endothelial function, employing EMP as a novel and sensitive biomarker. A

secondary objective was to examine the influence of postprandial lipemia and acute exercise on PMP

3.2 METHODOLOGY

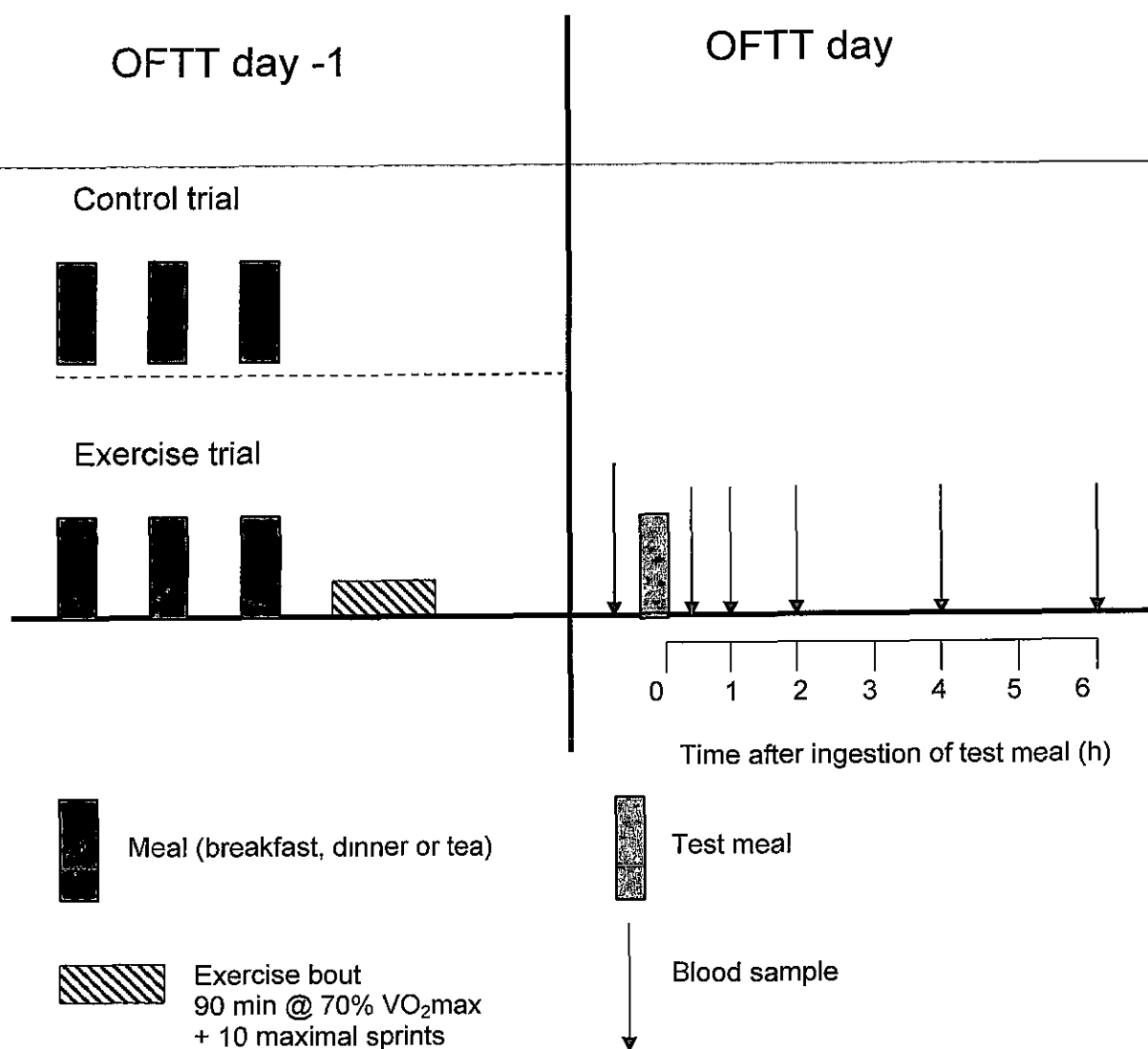
Subjects

Eight recreationally active men (mean \pm SD, age 26.9 ± 4.1 y, $\text{VO}_{2\text{peak}}$ 46.8 ± 4.9 $\text{ml kg}^{-1} \text{min}^{-1}$, BMI 26.0 ± 3.6 kg m^{-2} , % body fat $15.2 \pm 5.0\%$) volunteered for this study. Subjects were non-smokers, normolipidemic, free from cardiovascular disease and diabetes and not taking medication known to influence carbohydrate or lipid metabolism. Ethical approval was granted by the Dublin City University Ethics Committee and written informed consent was obtained prior to participation (Appendix A2).

Experimental design

Subjects visited the laboratory on four occasions. During a preliminary visit, they underwent a physical examination, had their body composition assessed and maximum oxygen uptake determined. They subsequently underwent two oral fat tolerance tests (OFTT) with a 6 h observation period, separated by 7 d approximately (Fig 3.1). On the evening prior to the OFTT, subjects either rested at home (CON trial) or completed a prolonged bout of cycle ergometry exercise (EX trial). The order of these trials was randomised.

Fig 3.1 Schematic representation of the experimental design



Exercise testing

Maximum oxygen uptake was determined using an incremental exercise test on a cycle ergometer (Monark, Vansbro, Sweden). The initial workload and increments were individualised so that the test was 8 - 12 min in duration. The experimental exercise bouts,

were conducted between 17.00 and 19.00 h on the evening prior to the OFTT. Subjects cycled at 70 rpm for 90 min at 70% $\text{VO}_{2\text{peak}}$, followed by ten 1 min sprints interspersed with 1 min of recovery to maximise glycogen depletion. Flywheel resistance was increased by 25% for the sprints and flywheel revolutions recorded every 20 sec in order to estimate energy expenditure

Expired air was collected continuously during the exercise bout for determination of VO_2 and VCO_2 via a breath by breath metabolic system (Vmax 229 Sensormedics). Energy expenditure and substrate oxidation during the continuous cycling was estimated using indirect calorimetry. Energy expenditure during the sprints was estimated from ACSM metabolic equation (American College of Sports Medicine, 2006) based on flywheel resistance and revolutions. Heart rate was recorded every 10 min by short range telemetry (Polar Electro Oy, Finland). Although water was consumed ad libitum following exercise, food intake was not permitted.

Oral fat tolerance tests

Subjects were required to abstain from alcohol and not to engage in exercise or heavy physical work for 3 days prior to each OFTT. They consumed their normal diet for 3 d prior to the first OFTT. Food items consumed along with portion size were recorded on sheets provided. This diet was then replicated in advance of the subsequent OFTT. Subjects arrived at the laboratory on the morning of each OFTT following an overnight (12 – 14 h) fast. A 20G intravenous catheter was then inserted into a forearm vein to facilitate blood collection. This catheter was kept patent during the postprandial follow-up period by flushing regularly.

with a 0.9% saline solution. The test meal consisted of croissants, butter, high fat ice-cream, chocolate and potato crisps with a macronutrient composition per 2m² body surface area of 97 g fat, 124 g carbohydrate and 1450 kcal. The meal was well tolerated by the subjects. Water intake was ad libitum during the first trial. This pattern of water intake was recorded

and repeated during subsequent trials. Subjects rested quietly in the laboratory during the 6 h observation period.

Blood sampling and storage

Blood samples were obtained with subjects in a seated position for 5 min with legs uncrossed in order to minimise plasma volume shifts. Serum vacutainers were allowed to stand for 30 min before centrifugation at 3000 rpm (1600g) for 15 min at 4°C. Sodium citrate vacutainers were pre-chilled in an ice bucket and samples returned to the ice bucket prior to centrifugation. Blood for microparticle analysis was collected, prepared and stored according to the double centrifugation method of Bernal-Mizrachi et al, (2004). Blood was collected in pre-chilled sodium citrate vacutainers and spun first at 160 g for 9 min to produce platelet rich plasma (PRP). This was harvested from the top leaving a 0.5 cm layer of plasma undisturbed close to cell debris. The PRP was centrifuged for a further 9 min at 1000 g to yield platelet poor plasma (PPP). PPP was aliquoted and stored at –80°C until analysis.

Biochemical analyses

Serum triglycerides, total cholesterol, HDL-cholesterol (HDL-C), free fatty acids (FFA) and glucose were determined using spectrophotometric assays, performed on an automated

bench-top clinical chemistry system (ACE®, Alfa Wassermann B V, Netherlands) using appropriate reagents, calibrators and controls (Randox Laboratories, UK) Serum insulin was determined by fluoroimmunoassay using a commercially available kit (Perkinelmer, Wellesley MA, USA). Serum concentrations of sVCAM-1, sICAM-1 and interleukin-6 (IL-6) were

determined in duplicate using a quantitative sandwich enzyme immunoassay technique and commercially available kits (R&D Systems, Minneapolis, USA) Haematocrit values, haemoglobin concentrations and counts of leukocytes were determined from an EDTA whole blood sample using an automated haematology analyser (AcTdiff2, Beckman Coulter, USA)

Microparticle counts were quantified by flow cytometry using CellQuest software (FACScan, Becton Dickinson) Microparticles were identified in PPP based on size and fluorescence, using 1.0 µm sizing beads and anti-CD144 PE, anti-CD146 PE, anti-CD31 FITC and anti-CD42b PE monoclonal antibodies EMP were defined as either CD144+, CD146+ or CD31+/42b- with PMP defined as CD42b+ For analyses based on single endothelial markers, 90 µl of PPP was incubated for 20 min with either 10 µl of anti-CD144-PE or 10 µl of anti-CD146 PE For the dual marker analysis, 85 µl of PPP was incubated for 20 min with 10 µl of anti-CD31 and 5 µl of anti-CD42b Samples were then diluted with 400 µl of PBS and analysed on the flow cytometer for 60 sec at medium speed. The flow rate was calibrated before each machine run using 15 µl of flow count beads diluted in 485 µl of PBS Fluorescence thresholds were set using PPP incubated with isotype-matched control antibodies Samples were analysed in duplicate and microparticle counts are expressed as microparticles per µl of plasma.

Samples for each subject were analysed in the same run. The intra-assay coefficients of variations were < 3% for all spectrophotometric analyses, 4% and 2% respectively for flow cytometry counts of EMP and PMP, and 3%, 8%, 8% and 20% respectively for the immunoassays of insulin, sVCAM-1, sICAM-1 and high sensitivity IL-6.

Data analysis

Postprandial responses to the test meal are reported as time-averaged postprandial values using the approach of Gill et al (2006). Time-averaged postprandial values represent the total area under the concentration vs time curve (AUC), calculated using the trapezium rule, divided by the length of the postprandial period (Appendix B). The significance of control and exercise trial differences for a range of fasting and summary postprandial parameters was determined using paired t-tests. When the significance of postprandial changes was of interest, a two way repeated measures ANOVA was performed (time x trial) with repeated measures on both factors, followed by the Bonferroni post-hoc test. The relations between selected variables were determined using Pearson correlations. Data are reported as mean \pm SEM unless otherwise stated. Significance was set at $p < 0.05$.

3.3 RESULTS

The VO_2 , RER and heart rate during the 90 min of continuous cycling were (mean \pm SD) $33.4 \pm 1.1 \text{ ml kg}^{-1} \text{ min}^{-1}$, 0.960 ± 0.005 and $151 \pm 3 \text{ b min}^{-1}$ respectively. Estimates of carbohydrate oxidation, fat oxidation and energy expenditure were (mean \pm SD) $352 \pm 21 \text{ g}$, $15 \pm 2 \text{ g}$ and $1463 \pm 78 \text{ kcal}$ respectively. As mean haemoglobin concentrations (13.9 ± 0.4

vs $14.0 \pm 0.3 \text{ g dL}^{-1}$) and haematocrit (41.8 ± 1.0 vs $41.2 \pm 1.2 \%$) were similar in the CON and EX trials respectively, values were not adjusted for changes in plasma volume

The influence of the test meal on a range of variables in the control and exercise trials is shown in fig 3.2–3.9. Fasting TG (table 3.1), postprandial TG (table 3.1) and postprandial TG increment TG (table 3.1) were lower ($p < 0.05$) in the EX trial. Fasting HDL-C (table 3.1), fasting FFA (table 3.1) and postprandial FFA (table 3.1) were higher ($p < 0.05$) in the exercise trial. The changes in postprandial insulin (table 3.1) did not reach statistical significance ($p = 0.11$) with fasting insulin similar in both trials (table 3.1). Postprandial glucose (table 3.1) was similar in both trials but fasting glucose (table 3.1) was lower ($p < 0.05$) in the exercise trial.

CD31+/42b-EMP (fig 3.6) increased postprandially in the CON and EX trials ($p < 0.05$). However, EMP values were similar ($p = 0.97$) between trials (table 3.2). EMP expressing CD144 and CD146 were not detectable in fasting or postprandial PPP. PMP (fig 3.7) decreased postprandially in the EX trial ($p < 0.05$) with the changes in the CON trial approaching significance ($p = 0.07$). Postprandial PMP were higher ($p < 0.05$) in the EX trial (table 3.2). There were no changes in sICAM-1 or sVCAM-1 postprandially. Postprandial sICAM-1 and sVCAM-1 values were similar between trials (table 3.2). IL-6 (fig 3.8) and leukocyte counts (fig 3.9) increased postprandially ($p < 0.05$). IL-6 values were similar in the CON and EX trials (table 3.2). Leukocytes were higher ($p < 0.05$) in the EX trial at 0 h but similar between trials at 6 h postprandially. Platelet counts did not change postprandially or

following exercise Neither EMP nor PMP counts were related to sICAM-1, sVCAM-1 or IL-6 (table 3.3)

Table 3.1 Fasting and time-averaged postprandial variables in the control and exercise trials

	CON trial	EX trial	P value
Fasting TG (mmol L ⁻¹)	0.95 ±0.13	0.63 ±0.07	0.028
Postprandial TG (mmol L ⁻¹)	1.98 ±0.29	1.18 ±0.13	0.026
Postprandial TG increment (mmol L ⁻¹)	1.02 ±0.52	0.54 ±0.17	0.031
Fasting insulin (mmol.L ⁻¹)	4.30 ±0.96	3.70 ±0.90	0.39
Postprandial insulin (mmol L ⁻¹)	19.45 ±4.19	14.60 ±1.89	0.11
Fasting cholesterol (mmol.L ⁻¹)	4.58 ±0.32	4.69 ±0.28	0.31
Fasting HDL-C (mmol L ⁻¹)	1.20 ±0.07	1.30 ±0.08	0.015
Fasting glucose (mmol L ⁻¹)	4.75 ±0.07	4.40 ±0.09	0.012
Postprandial glucose (mmol L ⁻¹)	4.76 ±0.12	4.72 ±0.08	0.815
Fasting FFA (mmol L ⁻¹)	0.66 ±0.07	0.73 ±0.06	0.20
Postprandial FFA (mmol L ⁻¹)	0.49 ±0.03	0.60 ±0.04	0.014

Values are mean ± SEM

Time-averaged postprandial values represent the total area under the concentration vs time curve, divided by the length of the postprandial period. Units are mmol L⁻¹

Table 3.2 Time-averaged postprandial values for markers of inflammation and coagulation in the control and exercise trials

	CON trial	EX trial	P value
EMP (counts $\times 10^3 \mu\text{L}^{-1}$)	2.51 \pm 0.14	2.52 \pm 0.31	0.97
PMP (counts $\times 10^3 \mu\text{L}^{-1}$)	8.43 \pm 1.50	15.31 \pm 2.13	0.04
sICAM-1 (ng mL $^{-1}$)	213.6 \pm 14.7	206.7 \pm 14.7	0.63
sVCAM-1 (ng mL $^{-1}$)	738.6 \pm 68.7	750.6 \pm 75.9	0.60
IL-6 (pg mL $^{-1}$)	2.14 \pm 0.63	2.44 \pm 0.51	0.57
Leukocyte counts (counts $\times 10^9 \text{L}^{-1}$)	6.09 \pm 0.51	6.73 \pm 0.62	0.17
Platelet counts (counts $\times 10^9 \text{L}^{-1}$)	215.8 \pm 36.9	223.5 \pm 43.0	0.34

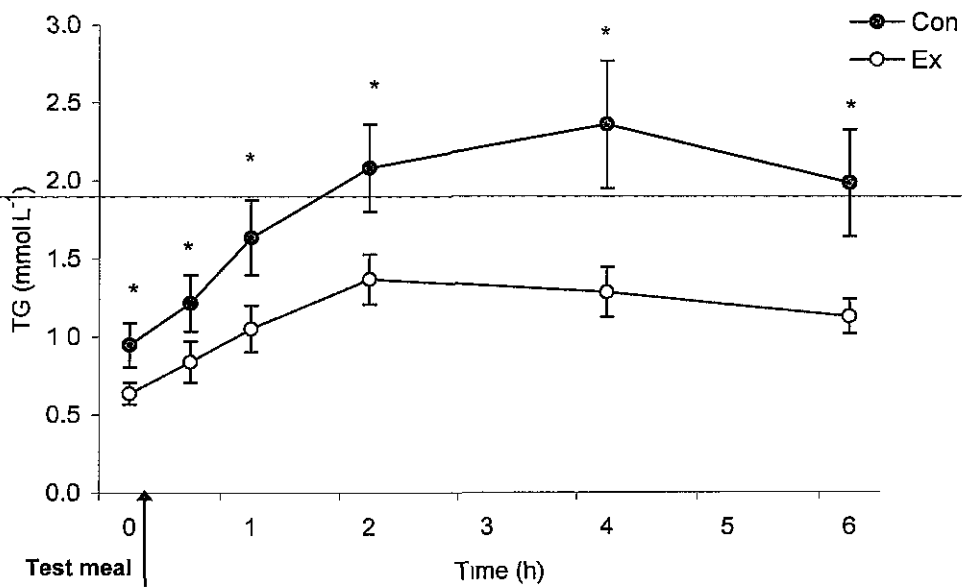
Values are mean \pm SEM

Time-averaged postprandial values represent the total area under the concentration vs time curve, divided by the length of the postprandial period. Units are mmol L $^{-1}$

Table 3.3 Correlation coefficients (r) of the associations of EMP and PMP with soluble adhesion molecules and IL-6

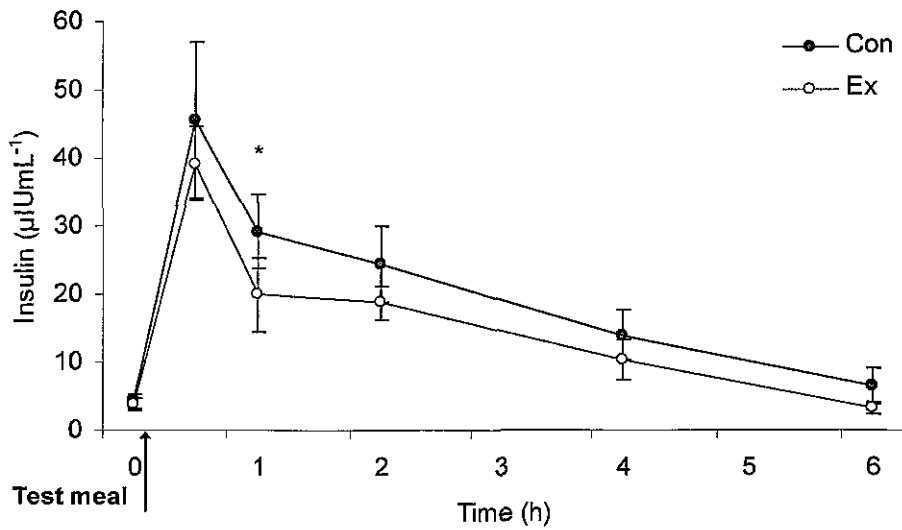
	sICAM-1	sVCAM-1	IL-6
EMP	0.20, p=0.63	-0.44, p=0.27	0.59, p=0.12
PMP	0.13, p=0.75	0.33, p=0.43	-0.05, p=0.91

Fig 3.2 Influence of acute exercise on the triglyceride response to a high fat mixed meal



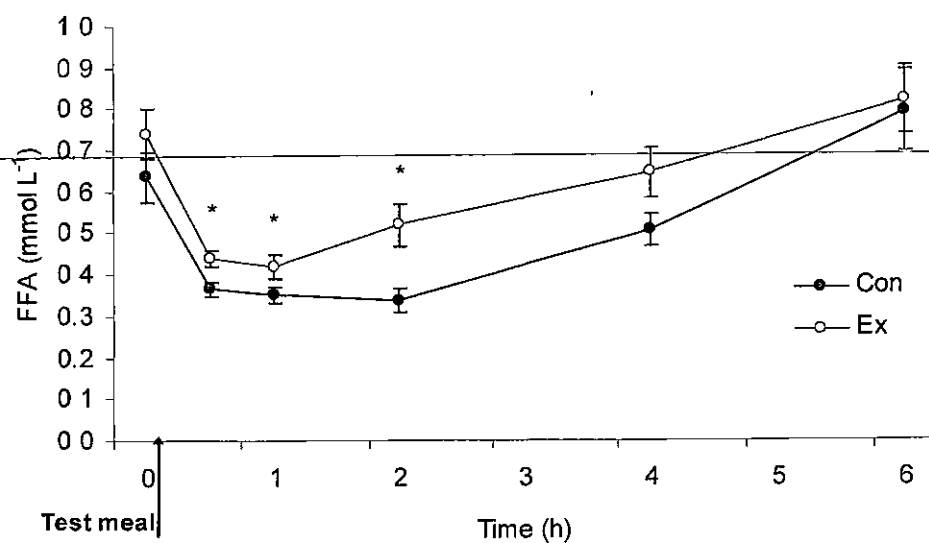
**p<0.05 compared to exercise trial*

Fig 3.3 Influence of acute exercise on the insulin response to a high fat mixed meal



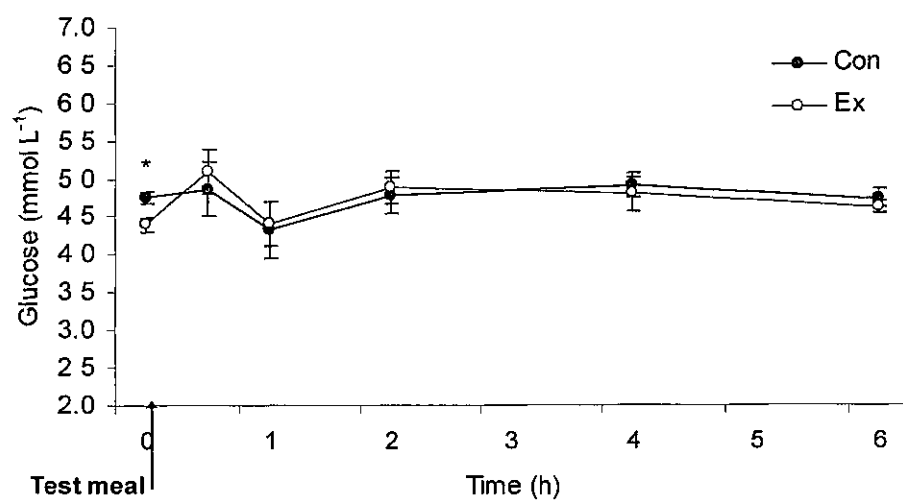
**p<0.05 compared to exercise trial*

Fig 3.4 Influence of acute exercise on the free fatty acid response to a high fat mixed meal



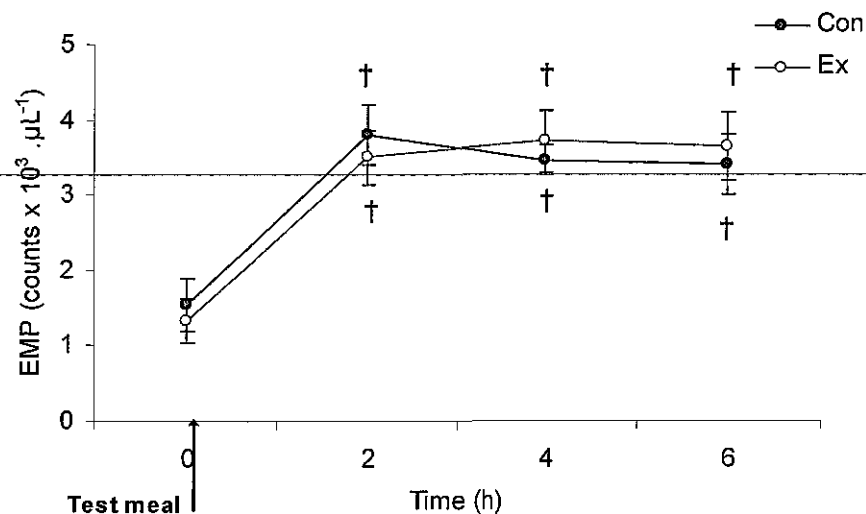
* $p < 0.05$ compared to control trial

Fig 3.5 Influence of acute exercise on the glucose response to a high fat mixed meal



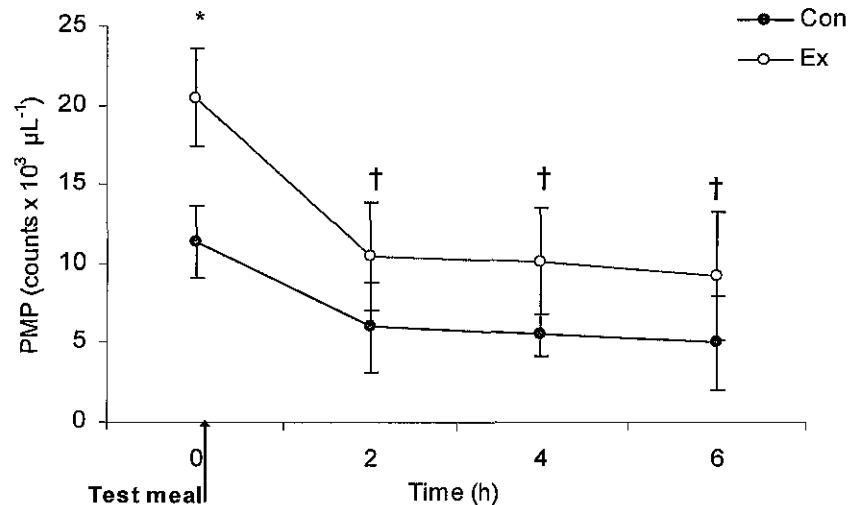
* $p < 0.05$ compared to exercise trial

Fig 3.6 Influence of acute exercise on the endothelial microparticle response to a high fat mixed meal



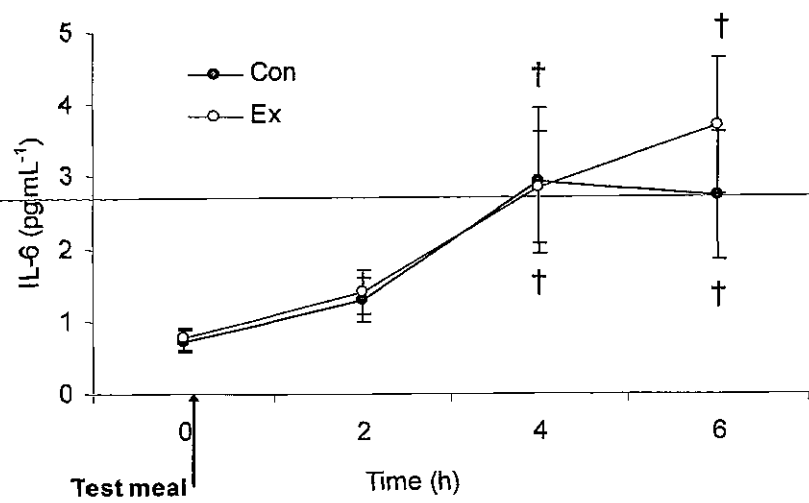
† $p < 0.05$ compared to 0 h value in same trial

Fig 3.7 Influence of acute exercise on the platelet microparticle response to a high fat mixed meal



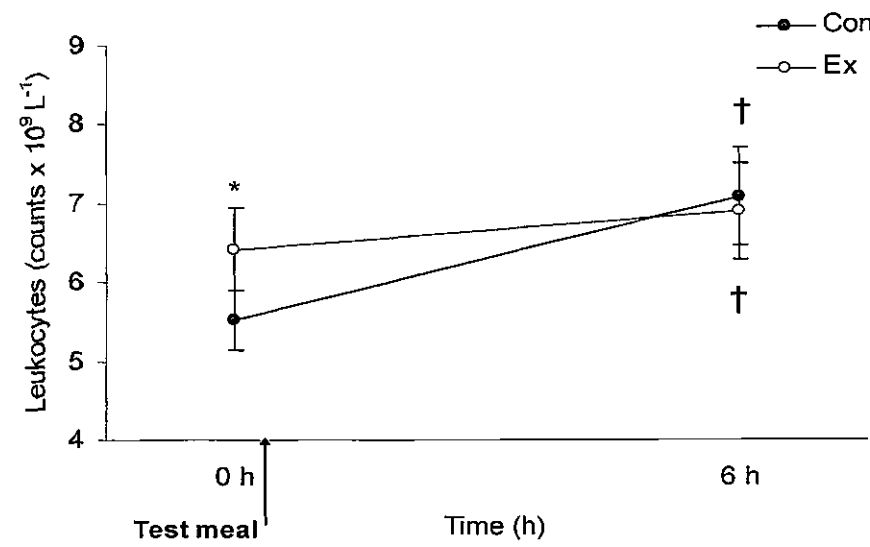
* $p < 0.05$ compared to control trial, † $p < 0.05$ compared to 0 h value in same trial

Fig 3.8 Influence of acute exercise on the IL-6 response to a high fat mixed meal



† $p < 0.05$ compared to 0 h value in same trial

Fig 3.9 Counts of leukocytes at the 0 h and 6h postprandial timepoints in the control and exercise trials



* $p < 0.05$ compared to control trial, † $p < 0.05$ compared to 0 h value in same trial

3.4 DISCUSSION

To our knowledge this is the first study that has examined the influence of a high fat meal, consumed with and without prior exercise, on EMP. EMP increased postprandially, indicative of endothelial cell activation, but this response was not attenuated by acute exercise, despite a considerable reduction in lipemia.

To our knowledge, only two previous studies have examined changes in EMP in the postprandial period following a single high fat meal, or two consecutive meals. Ferreira et al (2004) observed a postprandial increase in EMP, similar to that documented in this study, with the increases in serum TG related to the increases in EMP. In contrast, Tushuizen et al (2006) were not able to detect EMP in either fasting or postprandial plasma samples, but observed a postprandial increase in total microparticles (annexin V+ events). The key difference between these studies relates to methodologies used to quantify EMP and more fundamentally, to how microparticles are defined. In this study and that of Ferreira et al (2004), EMP were defined as CD31+/42b-, with no requirement for annexin V positivity. In contrast, only annexin V positive events, coupled with endothelial specific markers, were enumerated by Tushuizen et al (2006). Annexin V positivity is indicative of membrane asymmetry and the expression of negatively charged phospholipids on the outer microparticle membrane. It has been argued that this requirement excludes a large proportion of EMP that are formed in response to cell activation (Horstman et al, 2004). Greater consensus in relation to the definition and detection of microparticles would facilitate progress in this field. It should be noted however that the postprandial perturbations observed in this study are

relevant to clinical risk EMP defined as CD31+/42b- and assayed in platelet poor plasma have been shown to be elevated in a number of disease states including coronary artery disease (Bernal-Mizrachi et al , 2003), hypertension (Preston et al , 2003), obesity (Esposito et al , 2006), pre-eclampsia (Gonzalez-Qunitero et al , 2003), venous thromboembolism (Chirinos et al , 2005), thrombotic thrombocytopenic purpura (Jimenez et al , 2001) and multiple sclerosis (Minagar et al., 2001) EMP assayed using this methodology have also been shown to distinguish high and low risk coronary lesions (Bernal-Mizrachi et al , 2003)

EMP expressing CD144 and CD146 were not detectable either in the fasting or postprandial samples of our young healthy volunteers In contrast, CD144-EMP and CD146-EMP have been detected in the plasma of older (mean ages 56 - 66 years) apparently healthy subjects (Koga et al., 2005; Faure et al , 2006) This difference in age profile may account for the presence of EMP in plasma bearing proteins that have been shown to regulate the structural integrity of the endothelium, maintaining the cohesion of cell-cell junctions (Bardin et al., 2001, Petzelbauer et al , 2000)

The mechanisms mediating the postprandial increase in EMP are not fully understood However, oxidative stress has been proposed to be the pathogenic mechanism underpinning postprandial endothelial dysfunction The postprandial impairment of EDD (Plotnick et al., 1997) and the postprandial increase in soluble adhesion molecules (Nappo et al , 2002) are both preventable when high doses of anti-oxidant vitamins are administered with the test meal, or prior to a FFA infusion (Steer et al., 2003) It is unclear if the postprandial increase in EMP is also preventable with anti-oxidant vitamin supplementation

EMP not only reflect endothelial dysfunction but may play an active role in impairing EDD and in amplifying inflammation. EMP generated in vitro have been shown to impair EDD in rat aortic rings via an oxidative stress mechanism (Brodsky et al., 2004) and to facilitate transendothelial leukocyte migration (Jy et al., 2004). This raises an intriguing possibility

The protective influence of anti-oxidant vitamins on postprandial EDD could conceivably be mediated via a decrease in EMP. The mechanisms linking EMP, EDD and oxidative stress clearly warrant further attention.

The postprandial increase in EMP occurred in the absence of changes in soluble adhesion molecules. In addition, EMP did not correlate with either sICAM-1 or sVCAM-1. Preston et al. (2003) found blood pressure to correlate with EMP but not with sICAM-1 or sVCAM-1 in a group of hypertensive patients and normotensive controls, with EMP only weakly related to sVCAM-1 and not to sICAM-1. Koga et al. (2005) reported EDD of the coronary artery to be more strongly related to EMP than to sICAM-1. EMP appear to be a more sensitive marker of hypertriglyceridemia- or hypertension-induced endothelial dysfunction. As soluble adhesion molecules and EMP are released from the endothelium by different cellular processes (Horstman et al., 2004), each may be differentially regulated by risk factors for cardiovascular disease.

Despite the increase in EMP that accompanied postprandial lipemia, acute exercise did not attenuate EMP, sICAM-1 or sVCAM-1 in either the fasted or postprandial states. The importance of postprandial triglycerides and triglyceride-rich lipoproteins *per se* in activating endothelial cells is therefore unclear. In addition to their influence on serum TG, high fat

meals and acute exercise result in other perturbations in a range of metabolic, hormonal and inflammatory factors. In this study, the post-exercise decrease in lipemia was accompanied by an increase in HDL-C. Anti-inflammatory and anti-oxidant properties have been attributed to the HDL particle. It has been shown to increase EDD (Speiker et al, 2002), block the expression of adhesion molecules on the endothelium (Barter et al, 2002) and to inhibit LDL oxidation (Parthasarathy et al, 1990). FFA were higher in the exercise trial at all timepoints. FFA infusions are known to impair EDD (Steer et al, 2003). There was some evidence of an inflammatory response to the exhaustive exercise employed in this study, with leukocytes higher at 0 h in the exercise trial. EDD has also been shown to be impaired during acute inflammation (Hingorani et al, 2000). Although not measured in this study, systemic markers of oxidative stress have been shown to be elevated for 24 h after prolonged exhaustive exercise (Thompson et al, 2003). Thus, the multiple changes that accompany exhaustive exercise may exert inhibitory and stimulatory influences on endothelial cell activation status. Direct comparisons of postprandial hypertriglyceridemia and post-exercise hypotriglyceridemia must be considered with this in mind.

Only a few studies have examined the influence of acute exercise on postprandial endothelial function, with inconclusive findings. In a study of lean and obese middle-aged men, Gill et al. (2004) found EDD to be enhanced in the fasted state by prior exercise. However, in another study involving endurance-trained individuals, there was no change in EDD or in soluble adhesion molecules in either the fasted or postprandial states, during 7 d of detraining, despite a 53% increase in postprandial lipemia (Gill et al, 2003). In their review,

Moyna and Thompson (2004) have suggested that exercise training only improves EDD in individuals with abnormal function at baseline including the elderly and patients with cardiovascular disease. Future studies examining the influence of acute and chronic exercise, may only find EMP to be attenuated in these clinical cohorts.

Although not the primary objective of the study, we were also able to document changes in PMP, indicative of platelet activation, during the postprandial period and following acute exercise. The use of PMP as indicators of platelet activation has a number of distinct advantages. The PMP assay is simple and economical, utilises platelet poor plasma, and samples can be frozen for analysis at a later time (Horstman and Ahn, 1999). However, Preston et al (2003) observed only a weak relationship between platelet CD62 expression and PMP-CD42b counts. PMP decreased postprandially during the exercise trial in this study with the decrease in the control trial approaching significance. The implications of these postprandial changes are unclear. The majority of previous studies examining the influence of high fat meals on hemostasis have documented increases in the percentage of platelets expressing activation markers (Hyson et al, 2002, Brojerssen et al, 1998), the activation of coagulation factors (Larsen et al, 2000, Miller et al, 2002, Olsen et al, 2002; Kapur et al, 1996, Silveira et al, 1996) and in intravascular fibrin formation (Elmas et al., 2007).

PMP were higher in the exercise trial. The exercise bout used in this study was both vigorous and exhausting and the increase in PMP, suggestive of increased platelet activation, is not surprising. Numerous other studies reviewed elsewhere (Wang et al, 2006) indicate increases in platelet activation and aggregation following intense exercise. Whereas the

majority of these studies focus on platelet function during and for a short period after acute exercise, PMP counts in this study were determined from blood samples collected 12-18 h post-exercise. However, it is not possible to assess the risks associated with any increases in platelet activation, as no indices of fibrinolytic activity were assessed. Further work is needed, employing a variety of antigenic markers, to determine the utility of PMP when investigating the influence of postprandial lipemia and acute exercise on thrombus formation and the progression of atherosclerosis.

In summary, postprandial lipemia is accompanied by an increase in EMP, but not soluble adhesion molecules. It is therefore likely that EMP are a more sensitive marker of endothelial function than soluble adhesion molecules. A prolonged bout of acute exercise can decrease postprandial lipemia. However, this exercise attenuation of lipemia is not accompanied by a decrease in EMP, at least in young healthy volunteers.

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Chapter Four

INFLUENCE OF ACUTE EXERCISE ON POSTPRANDIAL LIPEMIA AND SKELETAL MUSCLE LIPOPROTEIN LIPASE ACTIVITY WITH AND WITHOUT CARBOHYDRATE REFEEDING

4.1 INTRODUCTION

Acute exercise has consistently been shown to reduce postprandial lipemia (Gill et al., 2002a). This effect is frequently attributed to an increase in the rate of removal of triglycerides (TG) from the circulation, mediated by an increase in skeletal muscle lipoprotein lipase (LPL) activity. Studies have shown increases in LPL activity (Lithell et al., 1979, Taskinen et al., 1980, Lithell et al., 1981, Lithell et al., 1984, Kiens et al., 1989, Seip et al., 1995, Kiens and Richter, 1998), LPL protein (Seip et al., 1997) and in the clearance rate of an intralipid infusion (Dufaux et al., 1981, Sady et al., 1986, Annuzzi et al., 1987) after prolonged strenuous exercise. However, studies have also shown reductions in postprandial lipemia following prolonged exercise of moderate intensity, in the absence of increases in muscle LPL activity, post-heparin LPL activity or intralipid clearance rate (Herd et al., 2001, Gill et al., 2003, Katsanos et al., 2004; Gill et al., 2001). The precise role of muscle LPL activity in mediating the exercise attenuation of postprandial lipemia remains unresolved.

In contrast, high carbohydrate (CHO) diets have been shown to increase plasma triglycerides (Abassi et al., 2000), decrease muscle LPL activity and decrease TG clearance rate (Lithell et al., 1982; Lithell et al., 1985). Kiens et al. (1989) reported a decrease in muscle LPL activity during euglycaemic hyperinsulinemic conditions with the decrease in LPL activity highly correlated with the insulin-induced increase in leg glucose uptake. Acute exercise and CHO feeding appear to have opposite influences on plasma triglycerides and possibly on muscle LPL activity.

Studies of acute exercise may not represent real life conditions, as they are not always designed to take account of the compensatory changes in energy and carbohydrate (CHO) intake that occur during exercise training (Dela et al , 1991) Ong et al. (1995) observed an increase in muscle LPL activity in rats following exercise training, an effect blunted by feeding after the last exercise bout. The mechanisms for this blunted response are unclear however, and the changes in serum TG were not reported. There is some evidence in humans that muscle glycogen can influence LPL activity. When muscle glycogen was manipulated through a combination of exercise and diet, the changes in muscle glycogen were closely related to the changes in LPL activity (Jacobs et al , 1982). The importance of muscle glycogen as a determinant of muscle LPL activity and postprandial lipemia warrants further attention. Although exercise has been shown to moderate the influence of high CHO diets on postprandial lipemia (Koutsari et al , 2001), it is not known if carbohydrate feeding moderates the influence of acute exercise on postprandial lipemia. These questions are not simply of academic importance. Should the exercise attenuation of postprandial lipemia be shown to be dependent on glycogen depletion during the exercise bout, there would be implications for exercise prescription.

The purpose of this study was to investigate the influence of acute exercise on postprandial lipemia and muscle LPL activity. Unlike previous studies this study involved two exercise trials so that the exercise effects could be determined in a glycogen depleted and glycogen repleted state. Prolonged exercise of vigorous intensity was employed to maximise glycogen depletion and any exercise effect on LPL activity.

4.2 METHODOLOGY

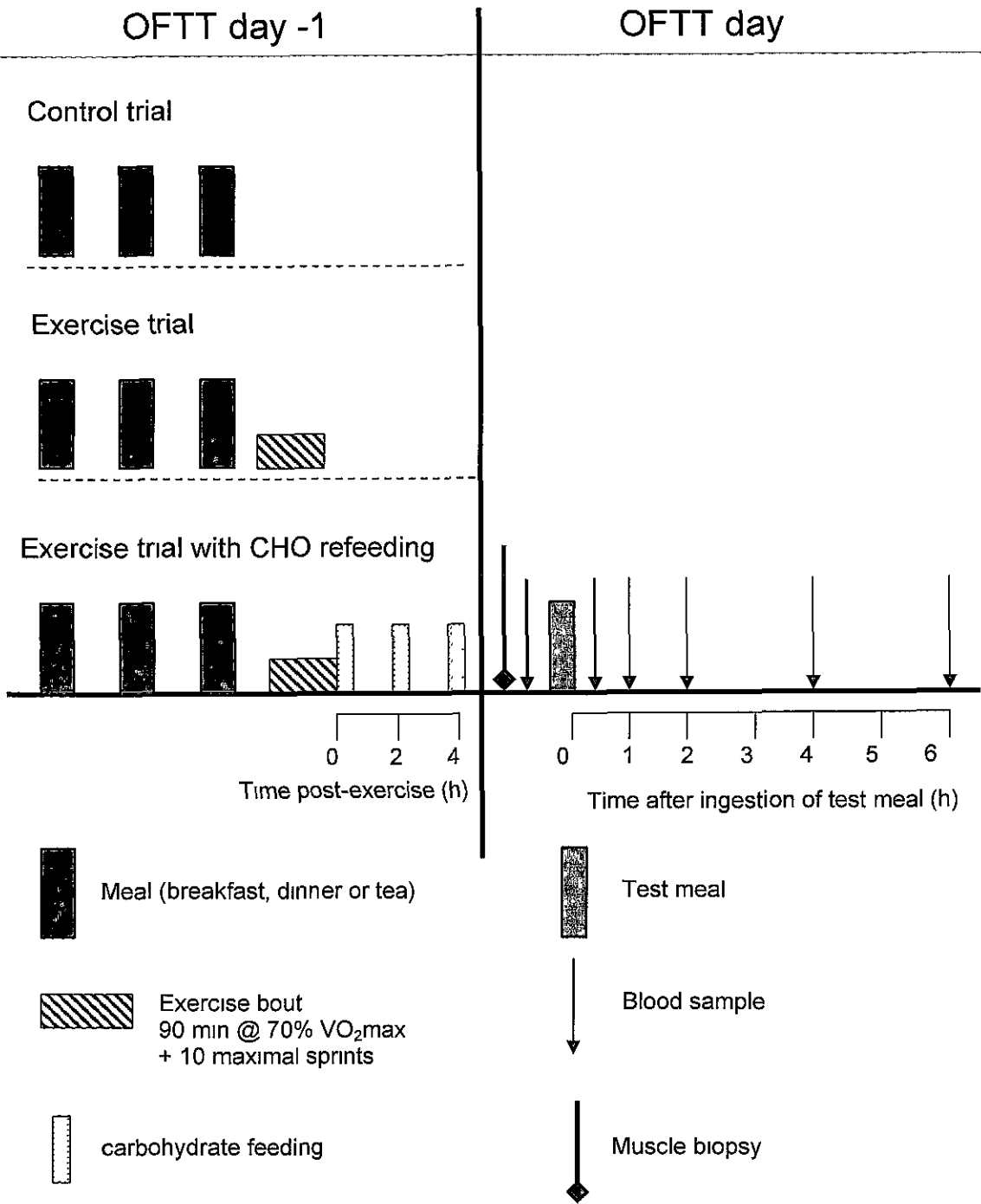
Subjects

Eight recreationally active men (mean \pm SD; age 26.9 ± 4.1 y, $\text{VO}_{2\text{peak}}$ 46.8 ± 4.9 $\text{ml kg}^{-1} \text{min}^{-1}$, BMI 26.0 ± 3.6 kg m^{-2} , % body fat $15.2 \pm 5.0\%$) volunteered for this study. Subjects were non-smokers, normolipidemic, free from cardiovascular disease and diabetes and not taking medication known to influence carbohydrate or lipid metabolism. Ethical approval was granted by the Dublin City University Ethics Committee and written informed consent was obtained prior to participation (Appendix A2).

Experimental design

Subjects visited the laboratory on six occasions. During a preliminary visit, they underwent a physical examination, had their body composition assessed and maximum oxygen uptake determined. They subsequently underwent three 6 h oral fat tolerance tests (OFTT), separated by 7 d approximately (Fig 4.1). One the evening prior to one OFTT, subjects rested quietly at home (CON trial). On the evening prior to two OFTTs, subjects completed cycle ergometry exercise in the laboratory. Following one of these exercise bouts, glucose was consumed to replace the CHO oxidised during exercise (EX-CHO trial). Following the other exercise bout (EX trial), only water was permitted (EX trial). The order of these trials was randomised.

Fig 4.1 Schematic representation of the experimental design



Exercise testing

Maximum oxygen uptake was determined using an incremental exercise test on a cycle ergometer (Monark, Vansbro, Sweden). The initial workload and increments were individualised so that the test was 8–12 min in duration. The experimental exercise bouts, were conducted between 17.00 and 19.00 h on the evening prior to the OFTT. Subjects cycled at 70 rpm for 90 min at 70% $\text{VO}_{2\text{peak}}$, followed by ten 1 min sprints interspersed with 1 min of resting recovery. Flywheel resistance was increased by 25% for the sprints and flywheel revolutions recorded every 20 sec in order to estimate energy expenditure. Subjects performed these sprints at a mean of $93 \pm 3\%$ $\text{VO}_{2\text{max}}$.

Expired air was collected continuously during the exercise bout via a breath by breath metabolic system (Vmax 229, Sensormedics, Yorba Linda, CA). Energy expenditure and substrate oxidation during the continuous cycling was estimated using indirect calorimetry (Weir, 1949; Peronnet and Massicotte, 1991) and energy expenditure during the sprints was estimated from the ACSM metabolic equation (American College of Sports Medicine, 2006) based on flywheel resistance and revolutions. Heart rate was recorded continuously using short range telemetry (Polar Electro Oy, Finland).

Oral fat tolerance tests

For 3 days prior to each OFTT, subjects were required to abstain from alcohol and not to engage in exercise or heavy physical work. On the day prior to each OFTT, diet was strictly controlled with subjects consuming 3 meals provided by the laboratory. These meals

consisted of breakfast cereal, milk, toast, butter, chocolate biscuits and ham and cheese sandwiches. This pre-OFTT diet was individualised to provide an energy content equal to 1.4 times BMR, with 56% as CHO, 30% as fat and 30% as protein. BMR was estimated from the Harris-Benedict equation (1919). On the two days prior to this, subjects consumed their

normal diet. Food items consumed along with portion size were recorded on sheets provided, prior to the first OFTT. This diet was then replicated in advance of subsequent OFTTs.

Following one of the exercise bouts (EX-CHO trial), carbohydrate refeeding was undertaken to replenish muscle and liver glycogen stores. Subjects consumed 105% of the carbohydrate oxidised during exercise ($4.4 \pm 0.4 \text{ g.kg}^{-1} \text{ BM}$). This was divided into three equal boluses and consumed immediately, 2 h and 4 h post exercise. The carbohydrate was delivered in the form of an 18% carbohydrate drink and 85% glucose confectionary. Carbohydrate refeeding was complete 4 h post exercise and at least 10 hours prior to the subsequent OFTT. Water was consumed at equivalent time-points in the CON and EX trials.

Subjects travelled to the laboratory on the morning of each OFTT by motorised transport and in a fasted state. A muscle biopsy (100-140 mg) was obtained from the midway point of the vastus lateralis immediately prior to each OFTT using a Bergstrom needle, with manual suction applied. Biopsies were obtained from alternate legs for different trials after local anaesthesia with 2% lidocaine. Samples were trimmed of any visible fat, snap frozen in liquid nitrogen and stored at -80°C until analysis. A 20G intravenous catheter was inserted into a forearm vein. This catheter was kept patent during the 6 h postprandial follow-up period by flushing regularly with a 0.9% saline solution. The test meal consisted of

croissants, butter, high fat ice-cream, chocolate and potato crisps with a macronutrient composition per 2m² body surface area of 97 g fat, 124 g carbohydrate and 1450 kcal. The meal was well tolerated. Water intake was ad libitum during the first trial. This pattern of water intake was recorded and repeated during subsequent trials. Subjects rested quietly in

the laboratory during the observation period with blood sampled at 30 min, 1, 2, 4 and 6 h postprandially.

Blood samples were obtained with subjects in a seated position for 5 min with legs uncrossed in order to minimise plasma volume shifts. Serum was allowed to stand for 30 min before centrifugation at 3000 rpm (1600 g) for 15 min at 4°C.

Serum analyses

Serum triglycerides, HDL-cholesterol, 3-hydroxybutyrate, free fatty acids and glucose were determined using spectrophotometric assays. The spectrophotometric analyses were performed on an automated bench-top clinical chemistry system (ACE[®], Alfa Wassermann B.V., Netherlands) using appropriate reagents, calibrators and controls (Randox Laboratories, UK). Serum insulin was determined by fluoroimmunoassay using a commercially available kit (PerkinElmer, Wellesley MA, USA). Haematocrit values and haemoglobin concentrations were determined from an EDTA whole blood sample using an automated haematology analyser (AcTdiff2, Beckman Coulter, Fullerton, CA). The intra-assay coefficients of variations were < 3% for the spectrophotometric assays and the fluoroimmunoassay.

Muscle glycogen

Frozen muscle samples were freeze-dried and dissected free of connective tissue. Glycogen concentrations were determined in duplicate by a standard enzymatic technique with fluorimetric detection (Passonneau and Lauderdale, 1974). Briefly, 2 mg freeze-dried muscle was allowed to thaw to -15°C . Muscle was incubated in 0.5 ml of 2N hydrochloric acid for 2 h at 100°C . Samples were then reconstituted to original weight with dH_2O before being neutralised with 1.5 ml of 0.67N NaOH. 1 ml of reagent mix containing Tris base, HCl, MgCl_2 , DTT, ATP, NADP, HK and G-6-PDH was added to samples and glycogen content determined by fluorimeter. The co-efficient of variation for this assay was 3.6%.

Lipoprotein lipase activity

For preparation of tissue homogenates for the LPL activity assay, muscle biopsy samples were ground with glass mortar and pestle on ice. The homogenisation buffer contained 0.05 M Tris HCl (pH 8.1), aprotinin ($2\text{ }\mu\text{g mL}^{-1}$), leupeptin ($10\text{ }\mu\text{g mL}^{-1}$), benzamidine (1 mM), pepstatin ($1\text{ }\mu\text{g mL}^{-1}$), EDTA (5 mM), BSA (1 mg mL^{-1}), phenylmethylsulfonyl fluoride (0.4 mM) and heparin (5 U mL^{-1} , 0.025 mg mL^{-1}). The samples were homogenised at a concentration of $10\text{ mg } 350\text{ }\mu\text{L}^{-1}$ buffer.

LPL enzyme activity was measured with a [^3H] triolein containing substrate. LPL activity was measured by the rate of hydrolysis of the [^3H] triolein containing substrate emulsified with lecithin, in the presence of pooled heat-inactivated human serum as the source of apolipoprotein C-II, and fatty acid-free albumin. Assays were performed at 37°C for

100 min. Serial dilutions of representative samples verified that the LPL assay was linear with time and amount of enzyme in the range that assays were performed

Data analysis

Summary postprandial responses to the test meal are reported as time-averaged postprandial values (Appendix B) using the approach of Gill et al (2006). Time-averaged postprandial values represent the total area under the concentration vs time curve (AUC), calculated using the trapezium rule, divided by the length of the postprandial period. The TG increment (Appendix B) represents the AUC above baseline, divided by the length of the postprandial period.

The significance of trial differences for the summary postprandial variables was determined using a one-way repeated measures ANOVA followed by a Fisher Least Significant Difference post-hoc test. The significance of between-trial differences at individual timepoints was determined using a two-way ANOVA (trial x time), followed by a priori contrasts to examine trial differences only. Associations between selected variables were determined using Pearson correlations unless otherwise stated. Data are reported as mean \pm SEM unless otherwise stated. Significance was set at $p < 0.05$.

4.3 RESULTS

The VO_2 (33.4 ± 1.1 vs $33.2 \pm 1.0 \text{ ml.kg}^{-1} \text{ min}^{-1}$), RER (0.96 ± 0.005 vs 0.96 ± 0.004) and heart rate (151 ± 3 vs $150 \pm 4 \text{ b.min}^{-1}$) during the 90 min of continuous cycling did not differ in the EX and EX-CHO trials respectively. Estimates of carbohydrate oxidation ($352 \pm$

21 vs 347 ± 17 g), fat oxidation (15 ± 2 vs 16 ± 2 g) and energy expenditure (1463 ± 78 vs 1450 ± 66 kcal) in the EX and EX-CHO trial exercise bouts (continuous cycling and sprints) were also similar. As mean haemoglobin ($p=0.92$) and haematocrit ($p=0.69$) were similar in the CON (13.9 ± 0.4 g.dL⁻¹, 41.8 ± 1.0 %), EX (14.0 ± 0.3 g.dL⁻¹, 41.2 ± 1.1 %) and EX-CHO (14.0 ± 0.2 g dL⁻¹, 41.5 ± 0.7 %) trials respectively, serum concentrations were not adjusted for changes in plasma volume. Muscle glycogen was $40\% \pm 4.3$ ($p<0.05$) and $94\% \pm 3.4$ ($p=0.24$) of control values on the morning of the EX and EX-CHO trials respectively (fig 4.2)

Fasting TG were closely related to postprandial TG in the CON ($r=0.92$, $p<0.05$), EX ($r=0.98$, $p<0.05$) and EX-CHO trials ($r=0.76$, $p<0.05$). Fasting TG, postprandial TG and postprandial TG increment were 33%, 40% and 47% lower respectively ($p<0.05$) in the EX trial compared to the CON trial (figs 4.3 & 4.4). In the EX-CHO trial fasting TG and postprandial TG were higher ($p<0.05$) than in the EX trial, and were not different to CON trial values. In the EX-CHO trial, postprandial TG increment did not differ from the EX trial or CON trial values (fig 4.4)

There was an inverse relationship ($p<0.05$) between muscle LPL activity and fasting TG, postprandial TG and postprandial TG increment in the CON trial (fig 4.5). LPL activity was not related to any index of lipemia in the EX or EX-CHO trials (fig 4.5). Correlations approaching significance were observed between $\text{VO}_{2\text{max}}$ (ml kg⁻¹ min⁻¹) and all indices of lipemia in the EX trial but not in the CON or EX-CHO trials (fig 4.6).

Muscle LPL activity was not significantly different across trials whether values are expressed as a percentage of CON trial values (fig 4.7) or in nmol FFA min⁻¹ g⁻¹ (fig 4.8)

There was a strong relationship between LPL activity in the EX and EX-CHO trials ($r=0.93$, $p<0.01$) when values are expressed as a % of CON values (fig 4.9). Analysis of individual data (table 4.1) indicates that three subjects experienced little change in muscle LPL activity with exercise ($<25\%$ change from CON trial values), three experienced considerable increases ($>100\%$ increase from CON trial) and two experienced considerable decreases ($>50\%$ decrease from CON trial).

The % change in muscle LPL activity following exercise refers to the % difference between EX and CON trial values ($EX - CON$) unless otherwise stated. The % change in muscle LPL activity following exercise was inversely related to initial CON trial LPL activity (fig 4.10). The % change in muscle LPL activity following exercise was also related to the change in fasting TG ($p=0.055$), postprandial TG ($p=0.048$) and postprandial TG increment ($p=0.061$) (fig 4.11). Similarly, the % change in LPL activity between the CON and EX-CHO trials was inversely related ($r= -0.88$, $p<0.05$) to the change in postprandial TG. The % change in LPL activity and the change in muscle glycogen were not related between the CON and EX trials or between the EX and EX-CHO trials (fig 4.12).

The influence of the test meal on serum insulin, glucose, FFA and 3-hydroxybutyrate in the CON, EX and EX-CHO trials is shown in fig 4.13. Fasting and postprandial insulin were not significantly different across trials ($p=0.16$) with differences between the CON trial and EX trial approaching significance ($p=0.11$) (table 4.2). Small differences existed in fasting and postprandial glucose across trials. Fasting glucose was similar in the CON and EX-CHO trials but lower ($p<0.05$) in the EX trial. Postprandial glucose was higher ($p<0.05$) in the EX-

CHO trial than in the EX and CON trials (table 4.2). Fasting HDL-C was higher ($p<0.05$) in the EX trial than in the CON and EX-CHO trials (table 4.2). Postprandial FFA and 3-hydroxybutyrate were higher ($p<0.05$) in the EX trial than in the CON and EX-CHO trial

Table 4.1 Individual values for SM-LPL activity ($\text{nmol FFA min}^{-1} \text{g}^{-1}$) in the CON, EX and EX-CHO trials

	CON	EX	EX-CHO	Effect of Exercise without CHO refeeding	Effect of Exercise with CHO refeeding
Subject 1	12.73	12.95	10.60	\leftrightarrow	\leftrightarrow
Subject 5	21.43	23.67	19.95	\leftrightarrow	\leftrightarrow
Subject 3	22.39		16.89		\leftrightarrow
Subject 4	4.25	9.31	15.77	\uparrow	\uparrow
Subject 8	16.23	39.51	42.07	\uparrow	\uparrow
Subject 2	14.08	28.94		\uparrow	
Subject 6	49.22	11.93	13.30	\downarrow	\downarrow
Subject 7	36.50	20.31	13.33	\downarrow	\downarrow

changes greater than 25% are noted with $\uparrow \downarrow$ arrows

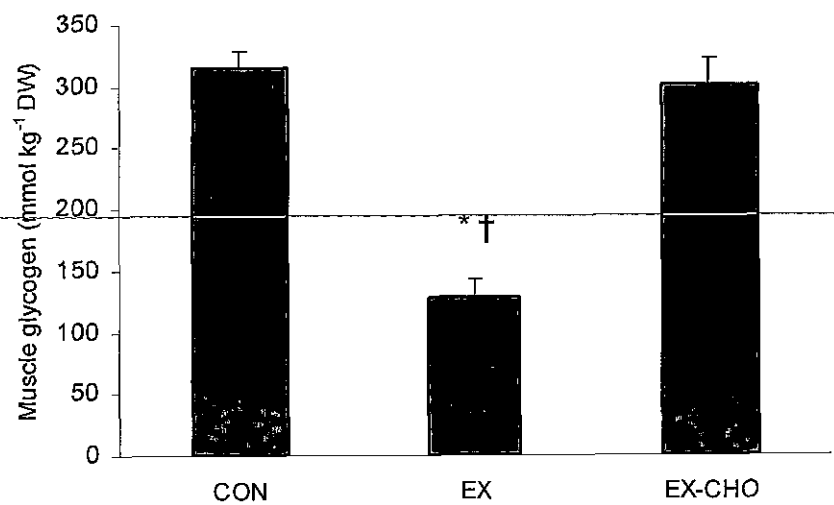
Table 4.2 Fasting and time-averaged postprandial values for a range of study variables in the CON, EX and EX-CHO trials

	CON trial	EX trial	EX-CHO trial
Fasting insulin (mmol L^{-1})	4.30 ± 0.96 ab	3.70 ± 0.90 a	5.19 ± 1.30 b
Postprandial insulin (mmol L^{-1})	19.5 ± 4.2	14.6 ± 1.9	18.4 ± 3.4
Fasting glucose (mmol L^{-1})	4.75 ± 0.07 a	4.40 ± 0.09 b	4.75 ± 0.11 a
Postprandial glucose (mmol L^{-1})	4.76 ± 0.12 a	4.72 ± 0.08 a	5.11 ± 0.12 b
Fasting HDL-C (mmol L^{-1})	1.20 ± 0.07 a	1.30 ± 0.08 b	1.20 ± 0.07 a
Postprandial FFA (mmol L^{-1})	0.49 ± 0.03 a	0.60 ± 0.04 b	0.49 ± 0.04 a
Postprandial 3-hydroxybutyrate (mmol L^{-1})	0.093 ± 0.03 a	0.209 ± 0.03 b	0.063 ± 0.01 a

Values are mean \pm SEM.

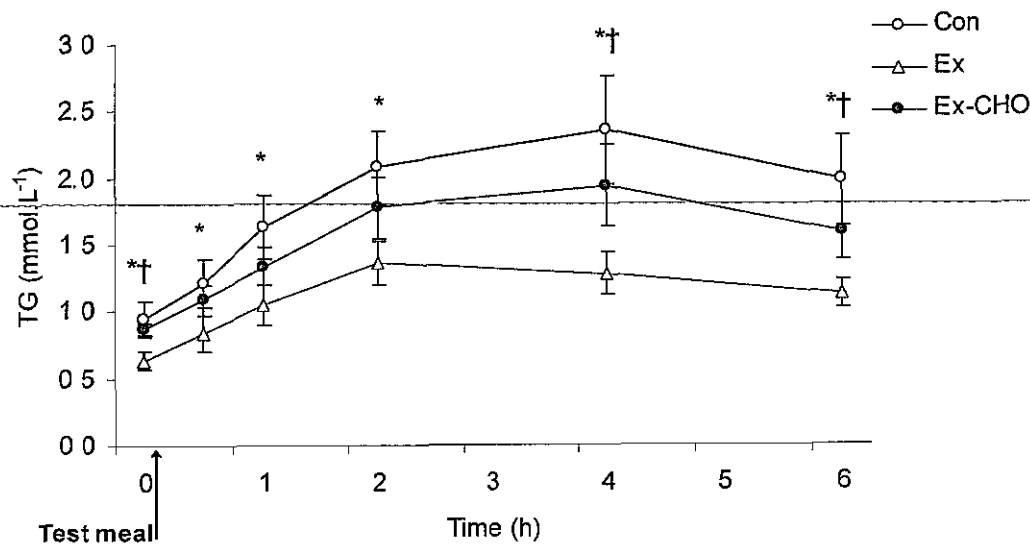
a, ab, b Different letters denote values that are significantly different ($p<0.05$). Values denoted as ab are not significantly different to values denoted a or b

Fig 4.2 Muscle glycogen concentrations in the CON, EX and EX-CHO trials



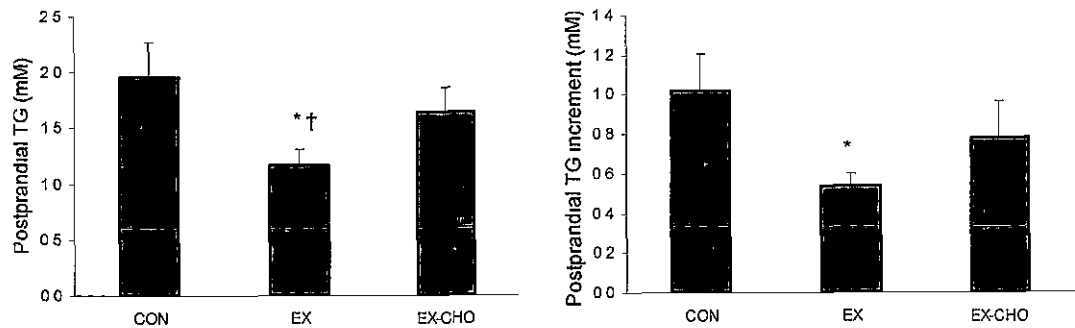
* CON vs EX trial $p < 0.05$, † EX vs EX-CHO trial, $p < 0.05$

Fig 4.3 Serum TG response to a high fat meal in the CON, EX and EX-CHO trials



* CON vs EX trial $p<0.05$, † EX vs Ex-CHO trial

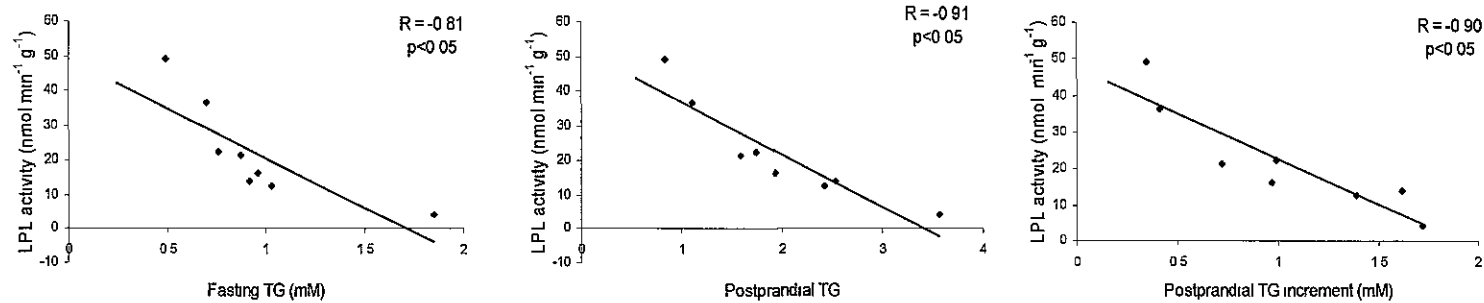
Fig 4.4 Time-averaged postprandial TG and time-averaged postprandial TG increment in the CON, EX and EX-CHO trials



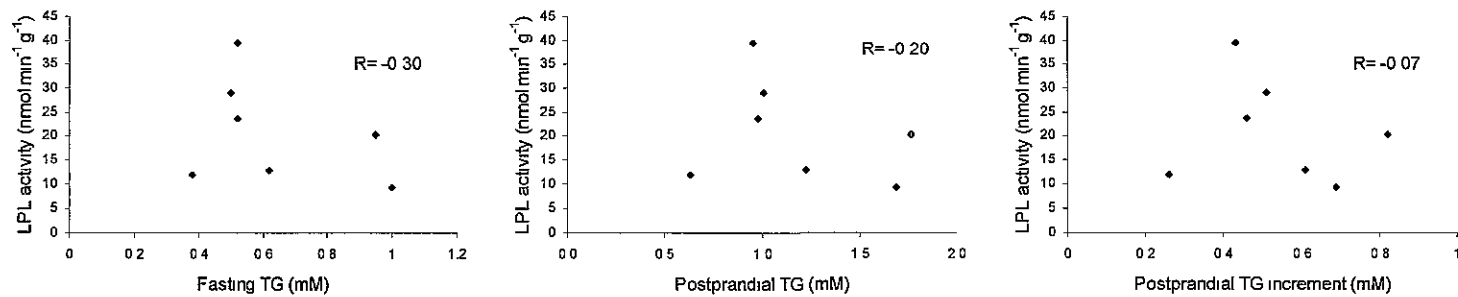
* CON vs EX trial $p<0.05$; † EX vs Ex-CHO trial

Fig 4.5 Relationship between LPL activity and fasting TG, postprandial TG and postprandial TG increment in the CON, EX and EX-CHO trials

CON trial



EX trial



EX-CHO trial

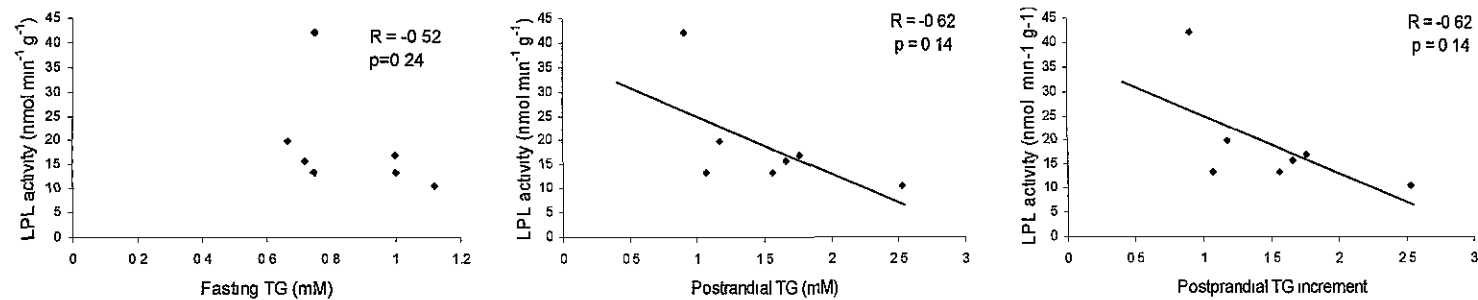


Fig 4.6 Relationship between $\text{VO}_{2\text{max}}$ ($\text{ml kg}^{-1} \text{min}^{-1}$) and (A) fasting TG, (B) postprandial TG and (C) postprandial TG increment in the EX trial

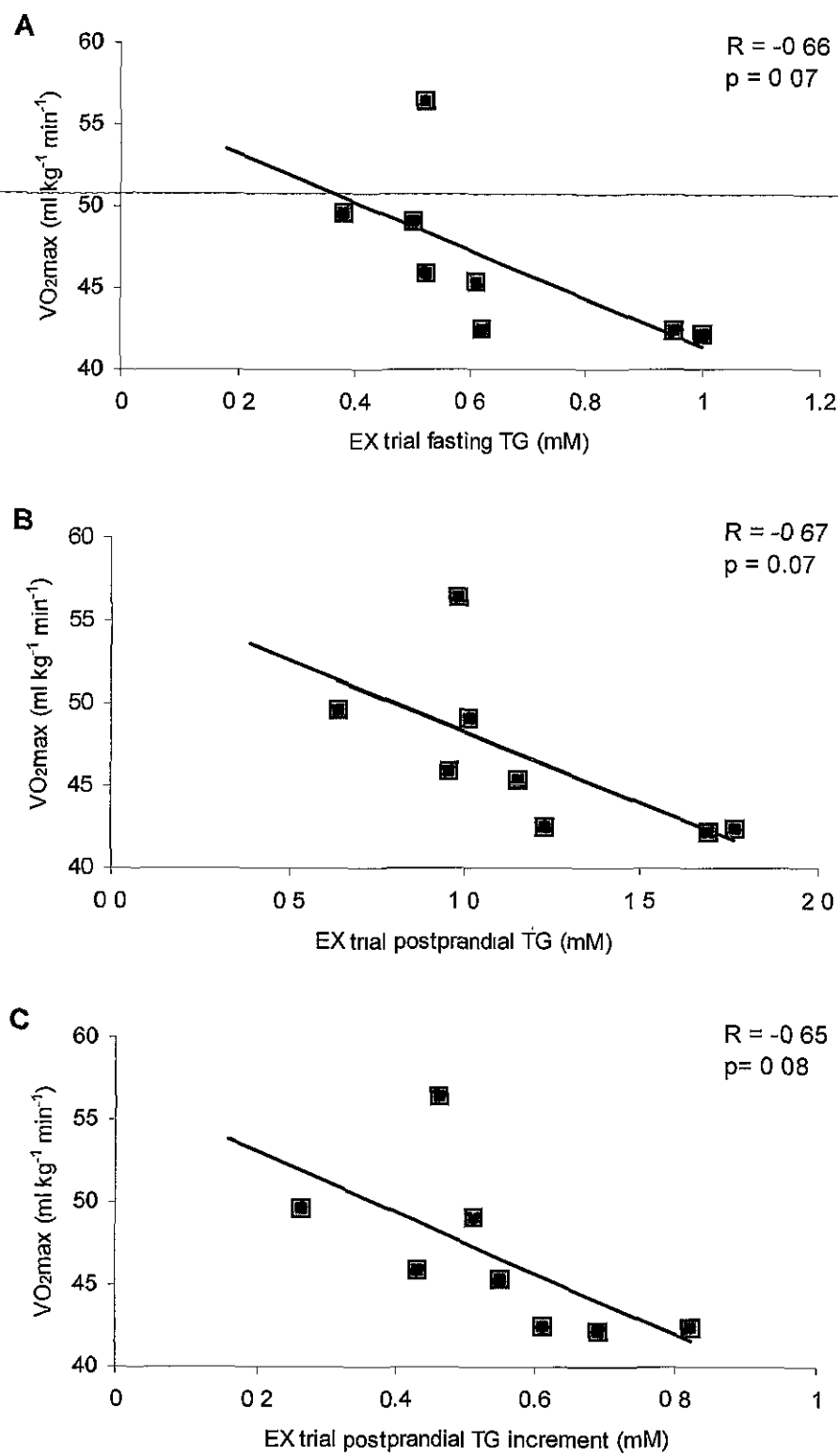


Fig 4.7 Percentage change in LPL activity from CON trial values in the EX and EX-CHO trials

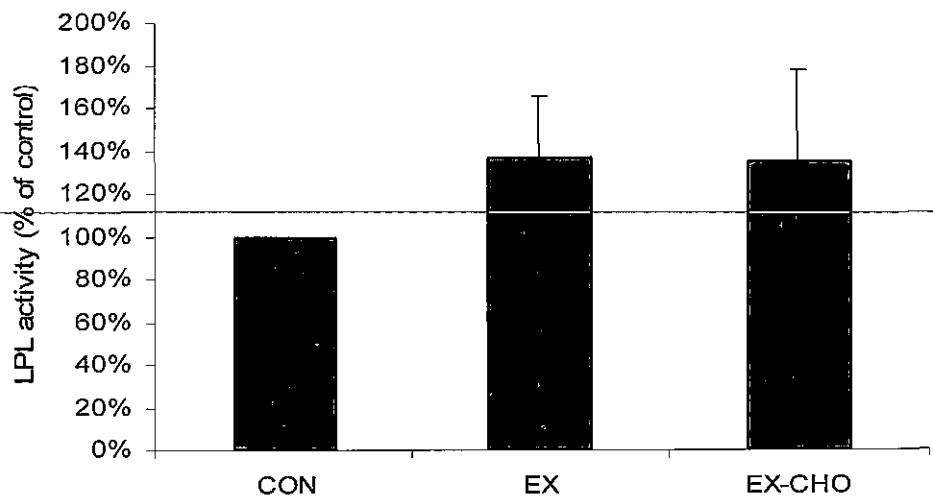


Fig 4.8 LPL activity ($\text{nmol min}^{-1} \text{g}^{-1}$) in the CON, EX and EX-CHO trials

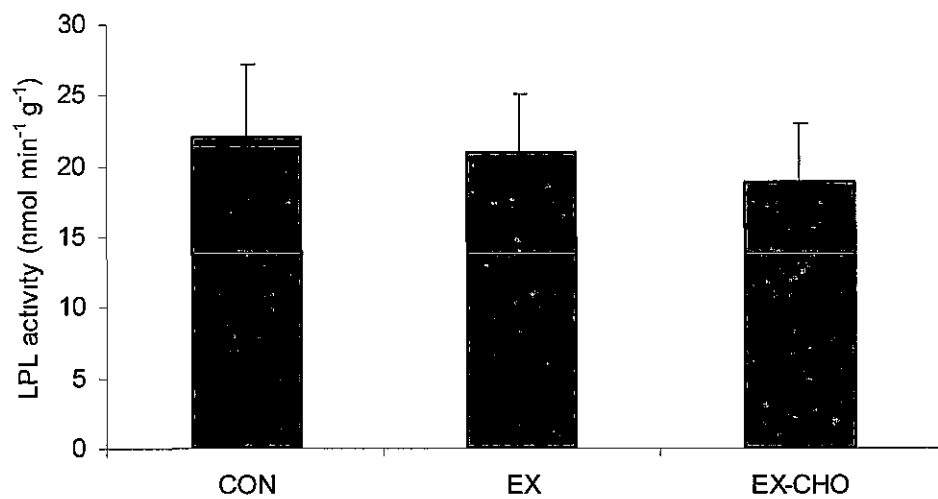


Fig 4.9 Relationship between LPL activity in EX and EX-CHO trials (expressed relative to CON trial values)

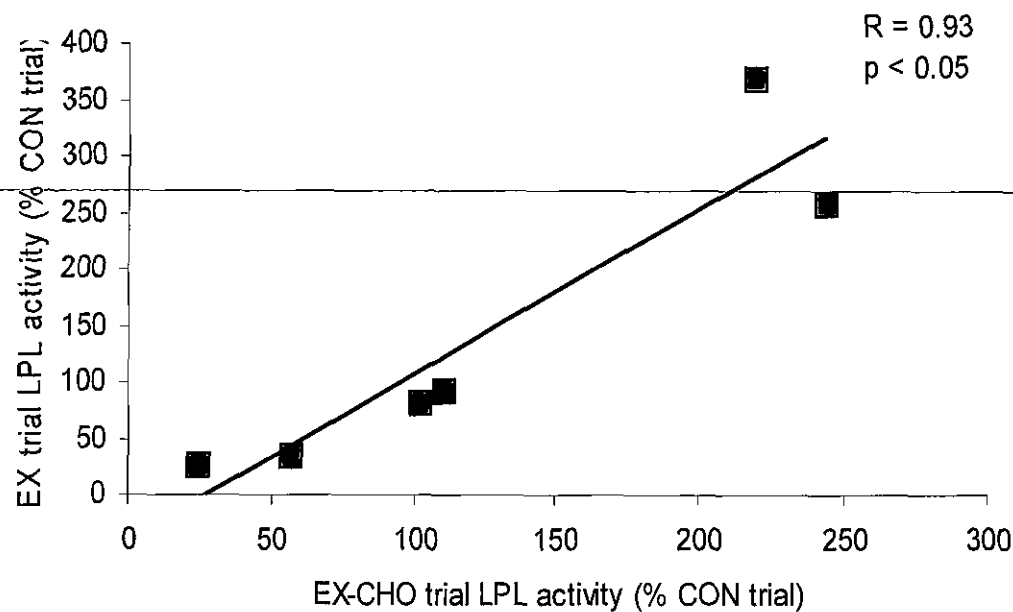


Fig 4.10 Percentage change in LPL activity following exercise vs CON trial values

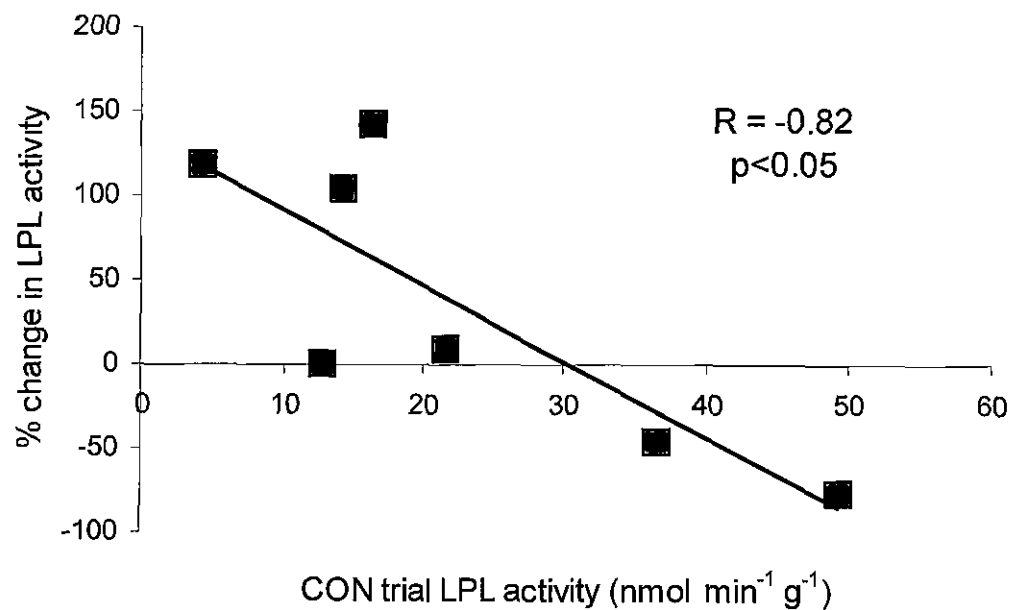


Fig 4.11 **Percentage change in LPL activity following exercise vs change in**
(A) fasting TG, (B) postprandial TG and (C) postprandial TG increment

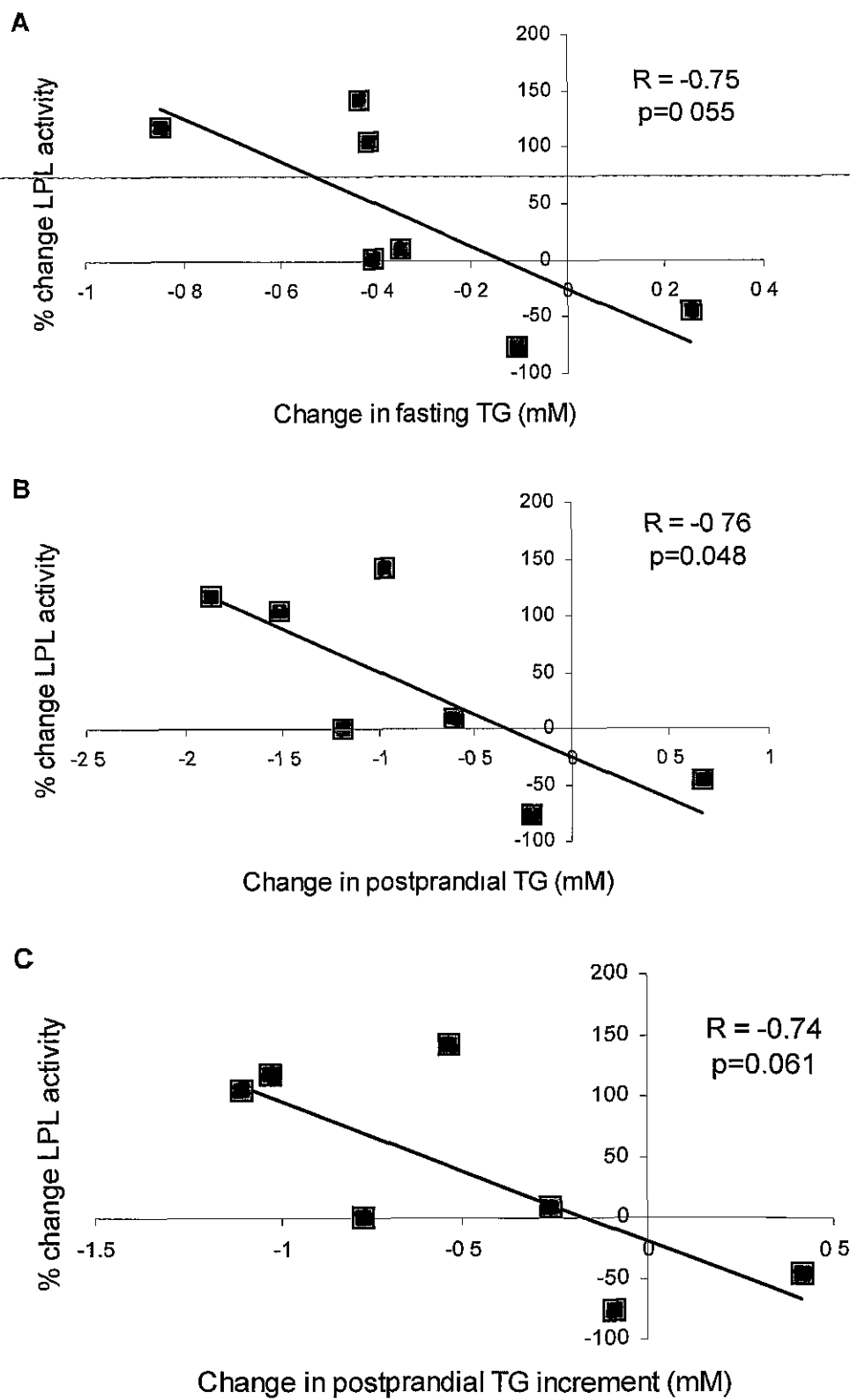


Fig 4.12 Associations of the % change in muscle LPL activity vs the change in muscle glycogen between (A) the CON and EX trials and (B) the EX and EX-CHO trials

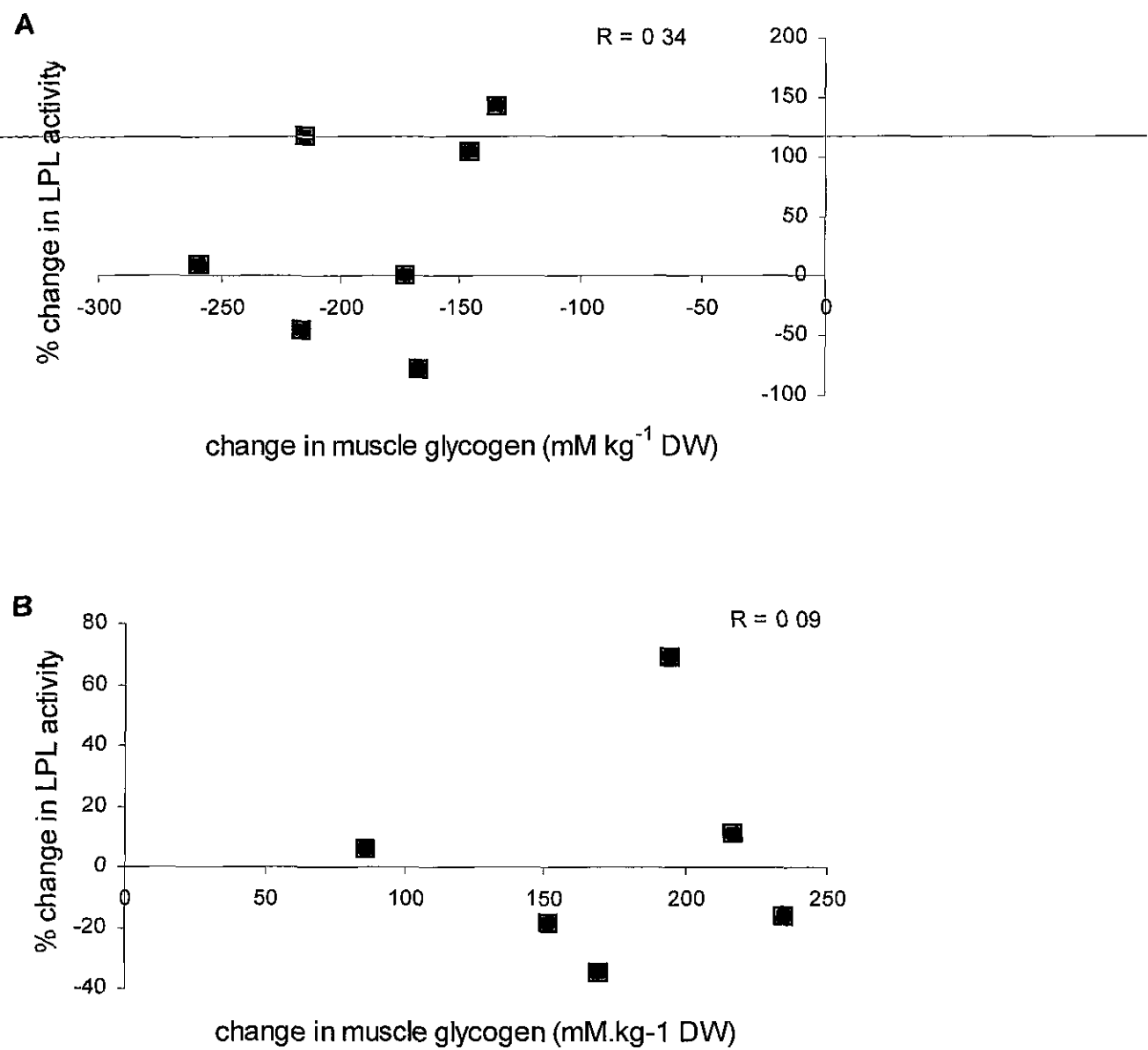
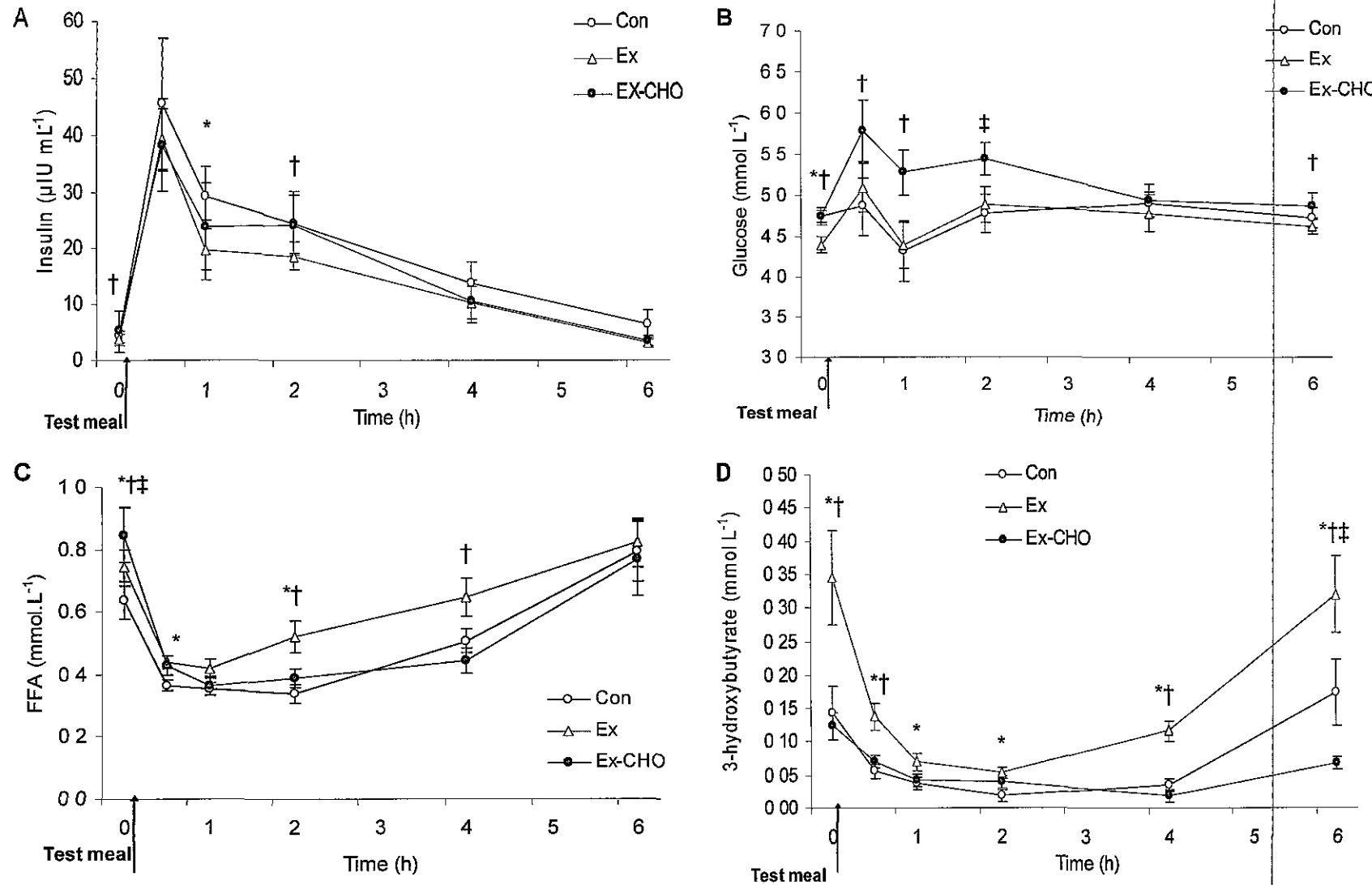


Fig 4.13 Serum (A) insulin, (B) glucose, (C) FFA and (D) 3-hydroxybutyrate response to a high fat meal in the CON, EX and EX-CHO trials



* CON vs EX trial $p < 0.05$; † EX vs EX-CHO trial, $p < 0.05$, ‡ CON vs EX-CHO trial, $p < 0.05$

4.4 DISCUSSION

This study is novel insofar as it examined the influence of acute exercise on postprandial lipemia and muscle LPL activity in the glycogen depleted and glycogen repleted state. The exercise protocol resulted in considerable glycogen depletion and the carbohydrate refeeding protocol successfully repleted muscle glycogen. Acute exercise reduced postprandial lipemia, though this effect was largely eliminated with carbohydrate refeeding and glycogen repletion. In contrast, exercise had no significant effect on muscle LPL activity, and carbohydrate refeeding did not moderate the LPL activity response to exercise. However, considerable inter-individual variation was observed in the LPL activity response to exercise.

Unlike previous studies, the present study involved two exercise trials so that the influence of acute exercise could be examined in the presence and in the absence of a post-exercise CHO refeeding regime to replete muscle glycogen. Regardless of refeeding, it is clear that there was no significant increase in muscle LPL activity when measured ~12 h post-exercise, whether values are expressed in $\text{nmol min}^{-1} \text{g}^{-1}$ or relative to CON values. Although the majority of studies report an increase in LPL activity or LPL protein following acute exercise, this increase is sometimes modest, qualified or limited to certain post-exercise timepoints. Herd et al. (2001) found muscle LPL activity to be unchanged 16 h post-exercise. Perreault et al. (2004) demonstrated an increase in muscle LPL activity following exercise in men but not in women. One legged knee extension exercise resulted in an increase in muscle LPL activity at 4 h but not at 8 h post-exercise (Kiens et al., 1989). Seip et al. (1995)

reported a modest, yet significant 35% increase in muscle LPL activity (similar to the non-significant % change in the present study), accounted for by changes in the heparin non-releasable fraction only. Whereas Seip et al (1997), documented increases in LPL protein at 8 h but not at 20 h post-exercise, Magkos et al (2006) found LPL protein to unchanged on the

morning after a prolonged exercise bout. Although prolonged bouts of acute exercise have consistently been shown to reduce serum TG, with lowest values occurring ~18 h post-exercise (Thompson et al, 1980, Kiens and Richter, 1998), the exercise effect on muscle LPL activity at similar timepoints is clearly not as robust. In their review, Gill and Hardman (2003) suggest that the importance of LPL-mediated TG clearance in explaining the exercise attenuation of postprandial lipemia may be influenced by the energy expended during the exercise session. A number of the earlier studies that documented post-exercise increases in muscle LPL activity involved prolonged bouts of competitive endurance exercise (Lithell et al, 1979) and military manoeuvres (Lithell et al, 1981, Lithell et al., 1984). The exercise protocol employed in the present study, finishing with ten maximal sprints, was also exhausting. The total energy expenditure approached 1500 kcal. The absence of a significant increase in LPL activity should not therefore be attributed to a suboptimal exercise stimulus.

The results of the present study suggest that muscle LPL activity is a key determinant of serum TG and an important mediator of the reduction in TG that follows acute exercise. Muscle LPL activity was strongly related to fasting TG, postprandial TG and the postprandial TG increment in the unexercised state (CON trial). The correlation with postprandial TG increment in particular indicates an important role for muscle LPL in clearing exogenous TG

from the circulation following a high fat challenge. Pulawa et al. (2007) also identified skeletal muscle LPL activity as a predictor of plasma TG. Overexpression of human LPL in skeletal muscle in mice resulted in a dose-dependent decrease in plasma TG. However, no correlations were evident in the EX or the EX-CHO trials. Interestingly, all indices of lipemia were related to VO_2max in the EX trial but not in the CON or EX-CHO trials. It appears that LPL activity is a key determinant of serum TG in the unexercised state, though overridden by other factors, possibly associated with aerobic fitness, in the exercised state.

Although exercise did not significantly increase LPL activity, a strong inverse correlation was observed between the change in LPL activity and the change in all indices of lipemia. Similarly, muscle LPL activity was not significantly different to CON values in the EX-CHO trial, yet a strong inverse correlation was observed between the change in enzyme activity between trials and the change in postprandial lipemia. Others have reported inverse relationships between the change in postprandial lipemia and the change in muscle LPL activity (Herd et al., 2001) and post-heparin LPL activity (Gill et al., 2003), in the absence of significant exercise-stimulated increases in LPL activity. These data suggest that with or without CHO refeeding, the exercise effect on LPL activity is an important determinant of the change in postprandial lipemia. An increase in LPL activity may not be a permissive factor for a reduction in lipemia but where an increase in enzyme activity occurs, the attenuation of lipemia will be of greater magnitude.

Considerable inter-individual variation was evident in the LPL activity response to exercise. There appeared to be a clear increase in LPL activity in 3 subjects and a clear

decrease in another 2 subjects. The similarity of the LPL activity responses in the EX and EX-CHO trials suggests that this response is repeatable and that the inter-individual variation observed was not due to measurement error or poor experimental control. Similar inter-individual variation was reported by Herd et al. (2001) though this was not confirmed with an

additional exercise trial. This heterogeneity in the LPL activity response to acute exercise warrants further attention. All muscle fibres may not respond similarly to exercise. Hamilton et al. (1998) observed an exercise-stimulated increase in LPL activity in fast glycolytic rat muscle, but not in fast oxidative or slow muscle. In the present study, those with the lowest CON trial values for LPL activity experienced the greatest percentage increase in enzyme activity. As muscle LPL activity has been shown to be highly correlated with the percentage of slow fibres, (Jacobs et al., 1982), it is plausible that our high responders (low initial values) had a preponderance of fast fibres. Lithell et al. (1979) reported considerable increases in LPL activity in less well-trained individuals following a competitive cross-country skiing event, with only small increases evident in the well-trained athletes. Pre-race IMTG stores were higher in slow fibres and a strong inverse correlation was observed between pre-race IMTG stores and the increase in LPL activity. Taken together, these studies suggest that individuals of low training status, with low initial muscle LPL activity, low IMTG stores and a predominance of fast fibres may have the lowest initial LPL activity and experience the greatest increase in activity following a prolonged bout of exercise. Kiens and Richter (1998) suggested that IMTG are an important source of fuel post-exercise during glycogen repletion, but are not utilised during exercise. It is plausible that in slow fibres of trained individuals

(highest IMTG), the supply of FFA to the mitochondria may be adequate to meet basal post-exercise energy needs. In contrast, increases in LPL activity may be necessary in fast fibres so that FFA can be more efficiently sourced from triglyceride-rich lipoproteins in the circulation. This theory needs to be tested by determining the LPL activity response to prolonged acute exercise in conjunction with muscle fibre typing and individual fibre analysis. Furthermore, this theory cannot account for the post-exercise decrease in LPL activity observed in two subjects.

To our knowledge this is the first study to compare the effects of CHO refeeding vs no refeeding on the LPL activity response to exercise in human skeletal muscle. Post-exercise dietary conditions have been shown to influence the LPL mRNA response to acute exercise in humans. Increases in LPL mRNA have been demonstrated if a low CHO diet but not a high CHO diet is consumed during the recovery period (Pilegaard et al., 2005). However, given that much of the influence of acute exercise and feeding on enzyme activity is post-translational (Ong et al., 1995, Simsolo et al., 1993, Bey and Hamilton, 2003), these changes in mRNA may not reflect changes in enzyme activity. Data from Ong et al. (1995) and Kiens and Richter (1998) suggest that post-exercise increases in LPL activity are reversed with carbohydrate feeding and in conjunction with muscle glycogen repletion. According to both groups, the availability of carbohydrate lessens the needs for FFA in the post-exercise period, allowing LPL activity to return to baseline more quickly. However, data from the present study do not support a role for carbohydrate refeeding or muscle glycogen status in moderating the LPLA response to acute exercise. The changes in LPL activity from CON trial values were

similar in the EX and EX-CHO trials. In addition there was no correlation between LPL activity and muscle glycogen in the EX trial expressed relative to CON values or between LPL activity and muscle glycogen in the EX-CHO trial expressed relative to EX trial values. These data suggest that any influence (positive or negative) of acute exercise on muscle LPL activity in humans is mediated by contractile mechanisms rather than by substrate depletion

The pronounced changes that occurred in postprandial lipemia across trials in the absence of significant changes in LPL activity, suggest that the effect of exercise may be mediated in part by non-LPL mechanisms. Differences in postprandial lipemia brought about by carbohydrate refeeding also appear to be mediated by non-LPL mechanism. These alternative mechanisms are open to speculation. Any influence of exercise on serum TG concentrations must be mediated by changes in TG uptake by muscle and adipose tissue or by changes in hepatic TG synthesis and secretion. It is unlikely that adipose tissue LPL activity contributed to the observed changes in postprandial lipemia as it has been shown to be unchanged following acute exercise in humans (Seip et al , 1995) and exercise training in rats (Ong et al , 1995).

Altered hepatic TG synthesis and VLDL-TG secretion must therefore be considered as another potential mechanism explaining the changes in postprandial lipemia in the EX and EX-CHO trials. In their tracer study, Magkos et al (2006) found VLDL-TG secretion to be unchanged on the morning following acute exercise, attributing the reduction in fasting TG to clearance alone. However, in three studies with rats, exercise training has been shown to reduce VLDL-TG secretion by 32-50% with similar reductions in serum triglycerides (Simonelli

and Eaton, 1978, Mondon et al , 1984, Fukuda et al , 1991). In one study (Fukuda et al , 1991), the decrease in hepatic TG secretion was accompanied by an increase in ketones, indicative of increased hepatic fatty acid metabolism. The authors attributed the hypotriglyceridemic effects of exercise to altered hepatic partitioning of long chain fatty acids, with the available fatty acids being directed towards oxidation rather than esterification in order to preserve depleted glycogen stores. The restoration of liver glycogen with carbohydrate refeeding would be expected to reverse any changes in hepatic fatty acid partitioning. Although muscle glycogen resynthesis appears to be prioritised over liver glycogen resynthesis during the first 2 h of post-exercise CHO refeeding (Maehlum et al., 1978), considerable liver glycogen repletion undoubtedly occurred in the EX-CHO trial between the end of the exercise bout and the start of the OFTT. In the present study, postprandial lipemia was partially restored in EX-CHO trial as glycogen stores were repleted. In addition, the ketone 3-hydroxybutyrate was markedly higher during the EX trial at all postprandial timepoints, but similar to CON values when the exercise was followed by refeeding. Thus, limited indirect evidence can be found in this study to support the hypothesis.

The findings of this study indicate that the attenuation of postprandial lipemia following acute exercise is related to the associated energy deficit, in agreement with Gill et al., (2002b) and Tsetsonis et al , (1996). More specifically, changes in muscle and/or liver glycogen appear to mediate the effects of acute exercise not only on postprandial TG concentrations but also on postprandial FFA, postprandial 3-hydroxybutyrate and fasting

HDL-C Postprandial insulin was not significantly influenced by acute exercise with or without carbohydrate refeeding in this study Malkova et al (1999) concluded that the nature of the substrate used during exercise, and consequently the magnitude of substrate depletion, did not influence the attenuating effect of prior exercise on postprandial lipemia However, the differences in glycogen stores achieved through manipulation in their study were small, in comparison to the EX and EX-CHO trial differences achieved in the present study The importance of glycogen status identified in this study has implications when conducting and interpreting research studies involving acute exercise Previous studies of postprandial lipemia have generally employed a prolonged exercise bout on the day preceding an oral fat tolerance test, but have not controlled post-exercise nutrient intake It is likely that major differences in post-exercise carbohydrate intake would exacerbate inter-individual and inter-study variation in the response to exercise These results also lend support to the argument (Dela et al , 1991) that the benefits of acute exercise may be overstated when studies do not take into account compensatory changes in dietary intake

In spite of the novel experimental design and novel findings, this study is not without limitations. No measure of leg blood flow was employed Malkova et al (2000) documented a 34% decrease in postprandial lipemia on the day following a prolonged bout of exercise, coinciding with a 37% increase in postprandial leg blood flow A post-exercise increase in TG clearance could occur in the absence of an increase in LPL activity, mediated by an increase (insulin-stimulated or otherwise) in leg muscle blood flow The determination of LPL activity from biopsied samples of muscle measures the maximal capacity of muscle to hydrolyse TG

ex vivo. Enzyme activity may be modulated *in vivo* by a range of stimulatory and inhibitory factors. Thus changes in muscle LPL activity *ex vivo* do not necessarily represent changes in the clearance of TG by muscle *in vivo*. Using a tracer methodology, Magkos et al (2006) reported an increase in VLDL-TG clearance following acute exercise, in the absence of any increase in muscle LPL mass. Due to a scarcity of muscle, it was not possible to determine heparin-releasable LPL activity separately from total muscle LPL activity. In theory, heparin-releasable LPL activity represents the extracellular fraction bound to the capillary endothelium and may more accurately reflect the capacity of muscle to clear TG from the circulation. Finally, it is unclear if LPL activity changed during the postprandial period as each experimental trial involved only one muscle biopsy. Kiens et al (1989) have shown muscle LPL activity to decrease during euglycemic hyperinsulinemic conditions, particularly in insulin sensitive individuals.

In conclusion, it is likely that multiple mechanisms mediate the exercise attenuation of postprandial lipemia. Our data suggest that unlike postprandial lipemia, muscle LPL activity is not significantly altered by acute exercise. Our data also suggest that unlike postprandial lipemia, the LPL response to exercise is not to be influenced by CHO refeeding. Although the changes in LPL activity brought about by acute exercise clearly influence the magnitude of the reduction in lipemia, it is likely that hypotriglyceridemic influence of exercise is also mediated by factors associated with glycogen depletion. The search for generalised mechanistic theories may also be oversimplistic however. The literature contains many examples of inconclusive or apparently conflicting studies. Large inter-individual differences

were observed in the LPL activity response to exercise. Attempts to further elucidate the role of muscle LPL activity and glycogen-related factors in mediating the exercise attenuation of lipemia may prove difficult, without first identifying the individual factors that interact with these main effects

4.5 REFERENCES

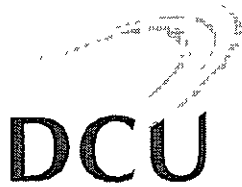
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APPENDIX A1



DUBLIN CITY UNIVERSITY RESEARCH - INFORMED CONSENT FORM

I. Project Title:

Influence Of Acute Exercise On Postprandial Lipaemia And Endothelial Dysfunction In Men Of Varying Body Mass Index

II. Introduction to this study:

The way the body processes the fat we eat is one of the factors that influences our risk of developing heart disease. After a single fatty meal, the level of fat in the bloodstream increases at first and then decreases again over an 8 hour period. Individuals who are not capable of processing fatty meals efficiently have a high concentration of fat in their bloodstream over a prolonged period. They are at higher risk of heart disease.

III. I am being asked to participate in this research study. The study has the following purposes:

1. To measure the rise and fall of fat in the bloodstream after a fatty meal
2. To examine the effect of a single exercise session on the way we process the fat we eat

IV. This research study will take place at the Centre for Sport Science and Health, Dublin City University

V. This is what will happen during the research study:

1. During the first preliminary visit I will have a brief medical examination and perform an exercise test to determine my fitness level. This will involve exercising at increasing speeds on a treadmill until I reach my maximal exercise capacity. For this, and all subsequent exercise tests, I will be fitted with a mouthpiece connected to a machine to measure the composition of gases in my breath. I will also have my weight, height and body fat measured using fat calipers.
2. On the second preliminary visit, I will perform another exercise test (not to exhaustion on this occasion) to determine the speed that corresponds to 70-75% of my maximum exercise capacity.
3. The main part of the study will involve the collection of blood samples following consumption of two high fat meals. Before each fat meal, I will have a small plastic tube, called a catheter, inserted into a vein in my arm to facilitate the taking of blood samples. I will eat a fatty meal and then have a blood sample taken at regular intervals for 8 hours. During this time I will be free to read and work quietly. The total amount of blood taken during this visit will be approximately 100 ml or 7 tablespoons.
4. On the evening before one of the fatty meals, I will go to DCU to walk at 70-75% of my maximum fitness until I burn between 600 and 700 calories. Depending on my fitness level this will take between 50 and 90 minutes. On the evening before the other fatty meal test, I will rest quietly at home.
5. For 2 days before each fatty meal test I will not be able to do any exercise or strenuous physical work (e.g. gardening). I will also not be able to drink alcohol.

- 6 For 2 days before the first fatty meal test, I will record the food I eat in a diary I will follow the same diet for the 2 days before the second fatty meal test
- 7 I will be asked to give my best effort during the maximal and submaximal exercise tests However, I will let the researchers know if I experience significant chest pain or other discomfort during any of the exercise sessions If I wish I can stop exercising at any time during any of the exercise sessions. .

VI. Sometimes there are side effects from having blood drawn and performing exercise tests. These side effects are often called risks, and for this project, the risks are:

- 1 I may feel a slight pain when the catheter is inserted and I may develop a bruise where the blood sample is obtained The pain and bruising is usually mild and a person trained in blood drawing will obtain my blood. The amount of blood drawn is not harmful, however, if I have a history of anemia, I should inform the investigator
2. Exercise testing carries with it a very small risk of abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients Because I will be asked to give a maximum effort, I may experience some muscle soreness in my arms and legs following each maximal exercise test It should be noted that if the experimental protocol is adhered to that the likelihood of these risks occurring is minimal

VII. There may be benefits from my participation in this study. These are:

- 1 After the study I will receive a copy of my personal results which include aerobic fitness level, body fat, triglyceride and cholesterol measurements as well as a summary of the overall results
- 2 I will be provided with information regarding how efficiently my body processes the fat I eat
3. I will be paid €25 for each fatty meal test that I take part in
4. No other benefits have been promised me

VIII. My confidentiality will be guarded:

Dublin City University will protect all the information about me and my part in this study My identity or personal information, will not be revealed, published or used in future studies The study findings will form the basis for preparation of a postgraduate thesis, academic publications, conference papers and other scientific

IX. If I have questions about the research project, I am free to call Dr. Niall Moyna at 01-7008802

- X. Taking part in this study is my decision** If I do agree to take part in the study, I may withdraw at any point including during an exercise test There will be no penalty if I withdraw before I have completed all stages of the study However, once I have completed the study I will not be allowed to have my personal information and results removed from the database

XI. Signature:

I have read and understood the information in this form My questions and concerns have been answered by the researchers, and I have a copy of this consent form Therefore, I consent to take part in this research project entitled. *"Influence Of Acute Exercise On Postprandial Lipaemia And Endothelial Dysfunction In Men Of Varying Body Mass Index."*

Signed: _____

Date: _____

Witness: _____



APPENDIX A2

DUBLIN CITY UNIVERSITY RESEARCH - INFORMED CONSENT FORM

I. **Project Title:** Effect of Acute Exercise and Muscle Glycogen on Postprandial Lipemia

II. **Principle Investigator:** Dr. Niall M Moyna

III. **Introduction to this study:**

Exercise is known to reduce the amount of fat in your blood after you eat a high fat meal. However, the reasons for this are not fully understood. One possibility is that the reduction in the amount of fat in your blood following exercise may be due to changes in the amount of related carbohydrate that are stored in your muscles. This study will examine the effects of exercise on the way the body processes fatty meals, with and without an attempt to refill the carbohydrate stores that were depleted during the exercise.

IV. **I am being asked to participate in this research study. The study has the following purposes:**

1. To examine the effects of exercise on the way the body processes fatty meals, with and without an attempt to refill the carbohydrate that were used during the exercise.

V. **This research study will take place at the Centre for Sport Science and Health, Dublin City University**

VI. **This is what will happen during the research study:**

1. During the first preliminary visit I will have a brief medical examination and perform an exercise test to determine my fitness level. This will involve exercising at increasing speeds on a treadmill until I reach my maximal exercise capacity. For this, and all subsequent exercise tests, I will be fitted with a mouthpiece connected to a machine to measure the composition of gases in my breath. I will also have my weight, height and body fat measured using fat calipers.
2. On the second preliminary visit, I will perform another exercise test to determine the speed that corresponds to 70-75% of my maximum exercise capacity.
3. The main part of the study will involve the collection of blood samples following consumption of high fat meals on 3 different occasions. Before each of the 3 fatty meal tests, I will have a small plastic tube, called a catheter, inserted into a vein in my arm to facilitate the taking of blood samples. I will also have a small piece of muscle removed from my thigh (60-100mg) to measure how much carbohydrate is in my muscle. This will be done by a medical doctor, using a local anesthetic. I will eat a fatty meal and then have blood samples taken at regular intervals for 6 hours. During this time I will be free to read and work quietly. The total amount of blood taken during each visit will be approximately 100 ml or 7 tablespoons.
4. The day before the three fatty meal tests will be spent at DCU where I will follow a strict diet. On the day before two of the fatty meal tests, I will cycle at 70-75% of my maximum fitness for 90 minutes followed by 10 one minute sprints. On the day before the other fatty meal test, I will rest quietly.
5. For 3 days before each fatty meal test I will not be able to do any exercise or strenuous physical work (e.g. gardening). I will also not be able to drink alcohol. I will have to follow exactly a diet agreed in advance.
6. I will be asked to give my best effort during exercise tests. However, I will let the researchers know if I experience significant chest pain or other discomfort during any of the exercise sessions. If I wish I can stop exercising at any time during any of the exercise sessions.

VII. Sometimes there are side effects from having blood drawn, muscle biopsied and performing exercise tests. These side effects are often called risks, and for this project, the risks are:

1. I may feel a slight pain when the catheter is inserted and I may develop a bruise where the blood sample is obtained. The pain and bruising is usually mild and a person trained in blood drawing will obtain my blood. The amount of blood drawn is not harmful, however, if I have a history of anemia, I should inform the investigator.
2. I may feel moderate pressure during insertion of the needle into the muscle and removal of muscle. Some minor aching and slight localized cramping may occur, but these symptoms usually go away when the needle is withdrawn. After the biopsy, the muscle is likely to be moderately sore for about 24 hours, similar to muscle soreness following unusually vigorous exercise or a muscle injury. Some bruising may also occur. A medical doctor will perform the biopsy.

Exercise testing carries with it a very small risk of abnormal heart rhythms, heart attack, or death in less than one in 30,000 patients. Because I will be asked to give a maximum effort, I may experience some muscle soreness in my arms and legs following each maximal exercise test.

VIII. There may be benefits from my participation in this study. These are:

1. After the study I will receive a copy of my personal results which include aerobic fitness and body fat levels along with blood concentrations of triglycerides, cholesterol, glucose and haemoglobin. This will allow me to compare my results to values for normal healthy individuals.
2. I will be paid €100 for my participation in the study.
3. No other benefits have been promised me.

IX. My confidentiality will be guarded:

Dublin City University will protect all the information about me and my part in this study. My identity or personal information, will not be revealed, published or used in future studies. The study findings will form the basis for preparation of a postgraduate thesis, academic publications, conference papers and other scientific publications. Confidentiality of information provided however, can only be protected within the limitations of the law. It is possible for data to be subject to subpoena, freedom of information claim or mandated reporting by some professions.

The original documentation, blood and muscle samples will be stored for 5 years. Thereafter, the blood and muscle samples will be disposed, and all documents will be shredded.

X. My confidentiality will be guarded: If I am in a dependent relationship with any of the researchers my involvement in the project will not affect ongoing assessment/grades/management or treatment of health at DCU.

XI. Dublin City University will not pay medical expenses or pay for pain and suffering, travel, lost wages, or other indirect costs of taking part in this project. If I have questions about the research project, I am free to call Dr. Niall Moyna at 01-7008802

XII. If I have questions about the research in general, I am free to contact the Secretary, Research Ethics Committee, Office of the Vice-President for Research, Dublin City University, ph 01-7008000, fax 01-7008002

XIII. My participation in this study is my own decision If I agree to take part in the study, I can stop at any time. There will be no penalty if I withdraw before I have completed the study.

XIV. Signature: I have read and understood the information in this form. My questions and concerns have been answered by the researchers, and I have a copy of this consent form. Therefore, I consent to take part in this research project entitled, Effect of Acute Exercise and Muscle Glycogen on Postprandial Lipemia.

Signed: _____

Date: _____ **Witness:** _____

Signature

APPENDIX B

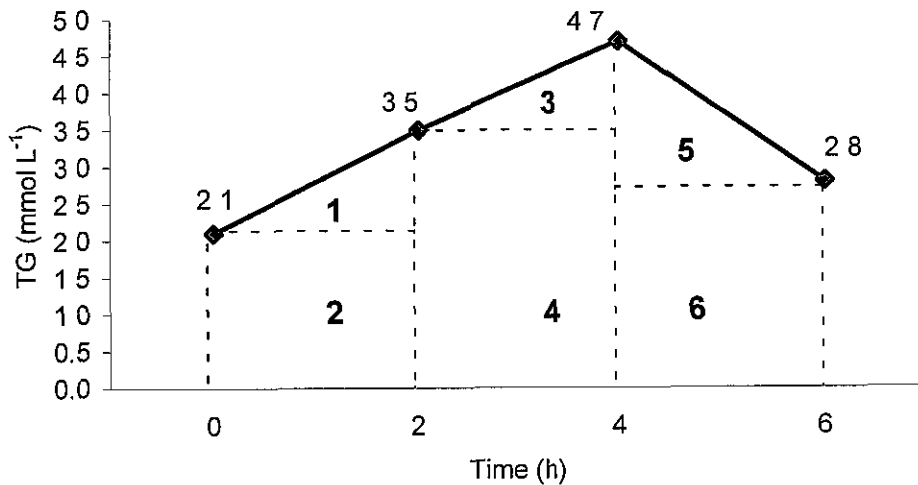
Summary Postprandial Calculations

1 Area under concentration vs postprandial time curve (AUC)

AUC is calculated using the trapezium rule, by dividing this area into rectangles and triangles as shown below (areas 1-6)

Area of triangle = $\frac{1}{2}$ base x height

Area of rectangle = base x height



$$\text{AUC} = \text{Area 1} + \text{Area 2} + \text{Area 3} + \text{Area 4} + \text{Area 5} + \text{Area 6}$$

$$= \left[\left(\frac{3.5 - 2.1}{2} \right) \times (2 - 0) \right] + \left[(2.1 \times (2 - 0)) \right] + \left[\left(\frac{4.7 - 3.5}{2} \right) \times (4 - 2) \right] + \left[(3.5 \times (4 - 2)) \right] + \left[\left(\frac{4.7 - 2.8}{2} \right) \times (6 - 4) \right] + \left[(2.8 \times (6 - 4)) \right]$$

$$= 21.3 \text{ mmol L}^{-1} \text{ h}$$

2 Time-averaged postprandial concentration

$$= \text{AUC} / \text{length of postprandial period (6 h)}$$

$$= 21.3 \text{ mmol L}^{-1} \text{ h} / 6 \text{ h}$$

$$= 3.55 \text{ mmol L}^{-1}$$

3 Postprandial increment

$$= \text{Time-averaged postprandial concentration} - \text{Fasting concentration}$$

$$= 3.55 \text{ mmol L}^{-1} - 2.10 \text{ mmol L}^{-1}$$

$$= 1.45 \text{ mmol L}^{-1}$$